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Short communication

Livestock-associated MRSA prevalence in veal calf production is associated with farm hygiene, use of antimicrobials, and age of the calves

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ABSTRACT

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is highly prevalent in pork and veal production chains. In this study, we used data from a cross-sectional survey on 2151 calves from 102 veal calf farms to identify potential risk factors, with the goal of reducing MRSA prevalence by developing intervention strategies. Overall, calves from rose veal farms had a lower risk of LA-MRSA carriage than calves from white veal farms. Data were analysed separately for white and rose veal calves, because management systems of the two production chains were largely different. Group treatment with antimicrobials appeared to be a risk factor for MRSA carriage in white veal calves in univariate analyses, but was not included in the final multiple regression model that included age of the calves and rodent control. Number of start treatment days was positively associated with LA-MRSA carriage in rose veal calves, and was the only risk factor selected for the final multiple regression model for this group. Interpretation of the results from this cross-sectional study is complicated by the strong correlation between antimicrobial use, LA-MRSA carriage and age of the calves. Other age-related factors may be more influential. However, taken together these findings emphasize the need for prudent use of antimicrobials, and point to improvement of farm hygiene as a control measure.

1. Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA; predominantly multilocus sequence type ST398) is highly prevalent in meat production animals worldwide (De Neeling et al., 2007; Smith et al., 2008; Graveland et al., 2010; Mulders et al., 2010) and has emerged in humans parallel with the increase in livestock (Morgan, 2008; Springer et al., 2009; Cuny et al., 2010). Moreover, MRSA was found in meat products (De Boer et al., 2009; Lozano et al., 2009; Pu et al., 2009; Weese et al., 2010), but this is supposed to play a minor role in the transmission of LA-MRSA to humans, if meat is cooked properly and hygienic measures are taken (Kluytmans, 2010). So far, LA-MRSA has not been shown to spread efficiently between humans (Voss et al., 2005; Van Rijen et al., 2008; Wassenberg et al., 2008; Van Cleef et al., 2010).

Because of the potential public health impact, it is necessary to reduce LA-MRSA prevalence in the primary meat production chain. Wenzel et al. (2007) noted that decision-making regarding MRSA was based on uncertainty and

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limited data, whereas intervention strategies should be evidence based. So far, it is known that LA-MRSA colonization in pigs is associated with the use of antimicrobials as group medication and the number of pigs present on the farm (Van Duijkeren et al., 2008; Broens et al., 2011). Recently it was shown that carrier prevalence in veal calves is also high, and antimicrobial use and farm hygiene were identified as risk factors (Graveland et al., 2010).

This paper describes a refined and subsector stratified analysis of risk factors, as a basis for determining causal factors of MRSA carriage which could lead to development of intervention strategies to control LA-MRSA in veal calves. The study focuses specifically on the animal data, including more potential risk factors. A previously conducted large cross-sectional study among 2151 calves on 102 veal calf farms provided a unique dataset, containing data on farm and animal characteristics, hygiene protocols, antimicrobial use, and LA-MRSA status of the animals.

2. Materials and methods

2.1. Background information on veal calf production chain

The veal production chain can be roughly divided into two categories with distinctly different management practices, based on the diet of the calves. White veal calves are fed calf milk replacer and limited roughage; whereas rose veal calves are fed calf milk replacer during the first weeks, and after that they are fed roughage and concentrates. White veal calves are also generally slaughtered at younger age, around 6–8 months, and rose veal calves around 8–12 months. In the Netherlands, calves from various European countries arrive on the fattening farm when they are around two weeks of age, individually housed until they are 6–8 weeks old. Hereafter, they are sorted based on their size and/or feed intake, and generally housed in groups of 4–10 animals. Calves may be relocated to other pens during their fattening period, based on their size and/or feed intake. This ensures the groups to be as homogenous as possible, which ensures the calves to be fed as balanced as possible. In white veal farming, usually an all in-all out system is used. Among rose veal farms, starters (calves stay on the farm until they are 12 weeks of age), fatteners (calves arrive on the farm at 12 weeks of age), or a combination of the two can be distinguished. Farms can have multiple stables, each consisting of one or more separate ("closed") areas, the compartments. Within one compartment housing conditions and management are similar for all calves. Each compartment is divided into pens, lined up in rows.

In general, the first weeks after arrival on the farm, calves are fed from buckets. When the calves are placed into groups, they are generally fed from a gully (on the floor), or from a trough. Sometimes a different feeding system is used, such as a teat. Calves have free access to drinking water from a water bowl, the feed trough, a nipple, or none (i.e. water is given when needed).

In this study, a group treatment is defined as a treatment with antimicrobials of all calves in a stable, not being a start treatment. A start treatment is defined as a (preventive) group treatment with antimicrobials, which the calves usually receive one day after arrival on the farm for 5–10 days. After 12 weeks of age, little antimicrobial group treatments are usually given. It should be noted that calves on rose fattener farms may have received start and/or group treatments on the starter farms they originated from, but this information is not available. This might cause misclassification to affect associations, and therefore these fattener farms are excluded in a more detailed analysis.

2.2. Data collection

Data were collected in a cross-sectional study among 2151 veal calves (Graveland et al., 2010). From May 2007 until March 2008, 102 Dutch veal calf farms were visited, randomly selected (based on willingness to participate) from 3000 farms in total. Nasal swabs were collected from a sample of the animals per farm (in general: square root of the number of calves on the farm, ranging from 10 to 43 animals), which were randomly chosen based on convenience and spread over the farm. The swabs were subsequently tested for MRSA carriage by bacterial culturing, confirmed by PCR, as previously described (Graveland et al., 2009, 2010). Farmers filled out a questionnaire together with a researcher on farm characteristics, hygiene protocols, housing and calf characteristics, and animal antimicrobial use. A Dutch study showed an increase over the course of the study in MRSA prevalence among pig farms (Broens et al., 2011), and in US beef calf farming, the month of arrival on the farm influenced the health of the calves (Moore et al., 2002). Therefore, sampling month and data on weather conditions (sun hours per day, daily precipitation, and mean temperature) on the day of sampling were included as explanatory mechanisms behind possible influence of the sampling time. Weather data were collected from the website of the Royal Netherlands Meteorological Institute (KNMI, 2009).

2.3. Statistical analysis

A generalized linear mixed model was used to analyse associations of the potential risk factors with the MRSA status of each calf. Analyses were performed using the GLIMMIX procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), assuming a binary distribution and logit link, and using the Satterthwaite approximation to estimate the denominator degrees of freedom for the tests of fixed effects (Fai and Cornelius, 1996). Analyses were adjusted for clustering of calves within compartments and within farms, by treating these as random effects. Age was categorized, based on general housing system and antimicrobial use. Data for rose and white veal farming were analysed separately, because of the large differences between farm management systems.

To avoid colinearity problems, we calculated Pearson's correlation coefficient for all variables that were selected for inclusion in the multiple regression model (i.e. P-value < 0.25 in the univariate analyses). For (pairs of) variables with a correlation coefficient > 0.40, only one variable was included in the multiple regression model, and the choice was based on the results of the univariate analyses and the biological plausibility. Multiple regression analysis was performed using (manual) stepwise-backward regression. Outcomes with a P-value < 0.05 were considered to be statistically significant.

3. Results

3.1. Antimicrobial use

Descriptive characteristics of the farms and animals included in the study can be found in Table 1, including the prevalence of MRSA carriage. We specifically considered whether calves had received a start treatment, the number of antimicrobial drugs used in the start treatment, the duration of the start treatment in days, whether calves had received a group treatment, the number of group treatments received by the calf, and the total duration of these group treatments in days.

Having received group treatments was significantly associated with MRSA carriage in the 2151 calves included in this study (both white and rose veal calves; unadjusted OR = 2.0, 95% CI: 1.1–3.4; $P = 0.02$), similar to an earlier report (Graveland et al., 2010). When data from rose veal fattener farms were excluded from this analysis, the group treated calves had even higher odds of being MRSA carrier, with unadjusted OR: 2.6 (95% CI: 1.3–5.0; $P = 0.005$). In an age stratified analysis group treated calves between 7 and 12 weeks of age showed a trend for 4 times higher odds of MRSA carriage ($P = 0.08$) than calves of the same age group that had not been group treated.

Variables that did not meet the criteria for inclusion in the multivariable analyses were sampling month, sun hours and mean temperature on the day of sampling, number of calves on the farm, number of calves per pen, whether the farm had multiple locations, presence of an all in-all out system, presence of a disinfection container at the entrance, number of times calves were relocated between pens, presence of other farm animals (distinguishing between pigs, cattle, and horses) and type of drinking system.

3.2. White veal farms

The variables that met the criteria for inclusion in the multiple regression model were the daily precipitation (in mm/day), rodent control, presence of companion animals, sex of the calves, age of the calf, feeding system and whether or not the calves had received group treatments.

The final regression model for calves from white veal farms (1182 calves/50 farms) showed that calves < 6 weeks of age ($n = 206$) were 2.5 times less likely to be MRSA carrier then calves > 12 weeks of age ($n = 740$), OR = 0.4 (95% CI: 0.2–1.0; $P < 0.05$). Performing rodent control (either by the farmer or a professional, $n = 44$ farms) was a significant risk factor for MRSA carriage in calves, resulting in an eightfold increase in the risk of MRSA carriage (OR: 8.1, 95% CI: 1.3–51; $P < 0.05$) when compared to no rodent control or by means of natural enemies ($n = 6$ farms). Within the white veal calves type of feeding system used was strongly associated with the age of the calves, and age was also strongly correlated with group treatment (treated vs. not treated: 16.9 vs. 6.7 weeks, $P < 0.0001$).

3.3. Rose veal farms (starter and combination)

The variables that met the criteria for inclusion in the multiple regression model were number of sun hours, presence of an all in-all out system, cleaning of the housing between production cycles, age of the calf, whether the calf had received a start treatment, number of start treatment days, number of group treatment days. Only the number of start treatment days was associated with MRSA carriage in the final regression model (475 calves/26 farms) obtained after stepwise backward regression, with an estimated OR of 1.3 (95% CI: 1.1–1.4) per day. Number of start treatment days was strongly correlated with cleaning of the stables, start treatment, and using an all in-all out system.

4. Discussion

In this study we identified potential risk factors for LA-MRSA carriage in veal calves. Overall, calves from rose veal farms had a lower risk of LA-MRSA carriage than calves from white veal farms. In the whole dataset, group treatments with antimicrobials appeared to be a risk factor for MRSA carriage, although interpretation of this result is not straightforward due to the strong association between antimicrobial use and age of the calves. Factors that were shown to have a positive association with LA-MRSA carriage in white veal calves in multiple regression analysis were rodent control and age of the calves. In calves from rose veal farms the number of start treatment days was positively associated with LA-MRSA carriage.

The results of the current and previous studies indicate that MRSA carriage might be related to age. A pilot study in pigs in the US, showed that older pigs had lower odds of MRSA carriage (Smith et al., 2008), whereas a Canadian longitudinal study showed that MRSA carriage risk in pigs reached an optimum around 50 days of age, after which it decreased (Zwambag et al., 2009). In a previous analysis of the current data, where age was analysed as a continuous variable by smoothing, MRSA prevalence in calves not treated with antimicrobials seemed to reach a plateau just after 30 weeks of age (Graveland et al., 2010). In the current study, age of the calves is considered a proxy of changes that occur during life: first, the way calves are being housed changes over time. Second, age is related to antimicrobial use, and third, we cannot exclude that age is a proxy of other biological changes over time, such as a change in feed and gut flora.

We found that calves younger than six weeks (individually housed) had a 2–3 times lower risk of being MRSA carrier than calves older than 12 weeks (group-housed). Group-housed calves can have more high risk contacts and thus a higher risk of becoming infected with MRSA, assuming MRSA transmits between calves. Calves generally are relocated between pens (regularly until the calves are about 14–15 weeks old), thus increasing the possibility of placing a MRSA carrier into a susceptible group of calves, and increasing the risk of MRSA transmission. Further, after arrival on the farm, calves are usually given a start

Table 1
Descriptive characteristics of veal calves from randomly selected Dutch veal farms in a cross-sectional study.

Variable	White veal	Rose veal (without fatteners)	Rose veal	All
# farms	50	26	44	102
# calves	1182	475	764	2151
MRSA prevalence – farm level	82%	73%	75%	78%
MRSA prevalence – animal level	31%	18%	21%	28%
Number of calves per farm (range (median))	90–2200 (625)	25–1700 (280)	25–1700 (310)	25–2200 (441)
Age of sampled calves (range weeks (median))	2–29 (15)	2–104 (13)[a]	2–104 (19)[a]	2–104 (16)
Only start treatment given (calves)	125 (11%)	122 (26%)		
Only group treatment given (calves)	35 (3%)	15 (3%)		
Both start and group treatment given (calves)	1022 (86%)	273 (57%)		
No start or group treatment given (calves)	0	65 (14%)		
Number of group treatments received (range (median))	0–7 (2)	0–6 (1)		

[a] Four calves from one farm were 104 weeks old, which is an exception. Without these calves, the maximum age of rose veal calves was 52 weeks.

treatment, and in the following weeks group treatments will be given. After 12 weeks of age, less group treatments are given because of decreased disease incidence, or because more often individual treatments are given. This might explain why the point estimate for the group of 7–12 week-old calves was the highest in the white veal model, albeit not significantly.

Group treatments (ignoring whether or not the calf had received a start treatment) were a significant risk factor for calves from white veal farms in the univariate analysis, with a similar effect for all calves taken together. Previously we reported a difference in MRSA prevalence between group treated and untreated calves, adjusted for number of calves per farm, farm hygiene, sector (white vs. rose veal), number of stables on farm, number of calves per pen, presence of other animals on the farm and rodent control (Graveland et al., 2010). This difference could mainly be attributed to the larger difference in MRSA carriage prevalence at higher age. In the current more detailed stratified analysis a strong difference in MRSA carriage was observed in white veal calves aged between 7 and 12 weeks, depending on whether or not they were group treated. These results together indicate that the use of antimicrobials is very likely modified by other factors that determine the effect on a population level, such as well practiced relocation of the calves and feeding management. The cross-sectional study design in combination with the strong correlation between group treatment and age of the calves makes it difficult to identify the independent contribution of each to MRSA carriage using multiple regression analysis. The associations between age, use of antimicrobials and MRSA prevalence suggest that group treatments with antimicrobials are a condition for MRSA to remain present on a farm after introduction, but that other factors determine transmission of MRSA. This can also explain why calves older than 12 weeks generally have a high risk of being a MRSA carrier while they do not regularly receive group treatments. It seems relevant to further explore interactions between age, relocation of animals, and use of antimicrobials in experimental designs and intervention studies.

Our results indicate that on white veal farms performing no rodent control at all (or with natural enemies) would be preferred over performing rodent control, either by the farmer or by a professional. However, reversed causality might explain these findings. Rodent control might be the result of poor hygiene and presence of rodents. MRSA has been shown to occur in rats on livestock farms (Van de Giessen et al., 2009). Furthermore, poor hygiene may cause health effects in calves which in turn might lead to a higher risk of MRSA carriage, due to a higher antimicrobial use. This result seems to be in line with the significant protective effect of cleaning of the stables in the univariate rose veal data analysis, another farm hygiene factor (data not shown; a similar point estimate was found in the univariate analysis on white veal data). However, it is also very likely that farms where rodent control is performed have a higher hygiene standard, which would contradict the results.

Because of the sampling design, larger farms will have a larger influence in the analyses. However, we have run various models with various dependent variables (e.g. proportion of positive calves per farm, farm status with different cut-off values for considering the farm positive, farm level variables only), and the results are consistent with the findings shown here, indicating results are robust.

5. Conclusions

Despite the large amount of data in this study, the association between the use of antimicrobials and LA-MRSA carriage in calves is difficult to establish in a multiple regression analysis, as other age related factors are correlated and may be more influential. This needs further exploration and study in experimental designs or intervention studies, but the findings do further emphasize the need for prudent use of antimicrobials. Increasing farm hygiene should be incorporated into control programmes, and will not only be helpful in controlling MRSA but also many other pathogens. Risk factors such as the type of veal and age of the calves provide important information on potential additional risk factors but are less easily incorporated into control programmes.

Conflict of interest

None declared.

Authors' contributions

MB carried out the statistical analyses and interpretation of the data, and drafted the manuscript. HG participated in the conception and design of the study, collected the data, and contributed to the critical revision of the manuscript. LP contributed to the statistical analyses and interpretation of the data, and critically revised the manuscript. JW and DH participated in the conception and design of the study, and contributed to the interpretation of the data and the critical revision of the manuscript. All authors read and approved the final manuscript.

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