PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Risk factors for livestock-associated MRSA persistent carriage and environmental exposure in veal calf farmers and their family members: an observational longitudinal study.
AUTHORS	Dorado-Garcia, Alejandro; Bos, Marian; Graveland, Haitske; van Cleef, Brigitte; Verstappen, Koen; kluytmans, jan; Wagenaar, Jaap; Heederik, Dick

VERSION 1 - REVIEW

REVIEWER	Dr Maureen Boost Associate Professor The Hong Kong Polytechnic University Hong Kong SAR No competing interests
REVIEW RETURNED	03-Jun-2013

THE STUDY	There are no details about selection of the farms included, do they vary in size, location. No mention of any inclusion or exclusion criteria. There is no characterization of the MRSA isolates - are they all indeed LA-MRSA ST types? Why were the cats not sampled if they were seen as potential sources of infection?
RESULTS & CONCLUSIONS	1. The supplemental table data is somewhat difficult to interpret The numbers listed in the sub-section "S.aureus in the nose" sometimes exceed the numbers listed under MRSA and MSSA - surely an individual must have one of these to appear in this column? I can understand the number in this third section being lower than the combined values of both the previous sub-sections as some subjects may be co-colonized. 2. In the text there is no mention of colonization rates in week 12 although sampling was performed, nor are these levels discussed. 3. It is assumed that all MRSA detected is LA-MRSA but there is no confirmation of this
GENERAL COMMENTS	Line 29: Carriage rate rather than prevalence as the whole population is not known Include the data fro week 12 carriage rates in the main paper and discuss

REVIEWER	Haenni Marisa, PhD Anses Lyon France

	No competing interest to declare
REVIEW RETURNED	03-Jul-2013

GENERAL COMMENTS	The authors estimated the risk factors for farmers and their family members to be persistent MRSA carriers. The manuscript is well-written and, even if results are not entirely new, they undoubtedly add information in the field of MRSA exposure. This manuscript thus deserves to be published, with some minor modifications or comments. Major comments: The authors are assessing the persistence of LA-MRSA. Were the recovered isolates characterized into more details in order to prove that they were ST398? Supplemental Table 1: the number of sampled employees is so small compared to farmers and their family that statistics are not really relevant. I suggest removing this category. Minor comments; Why did the author choose to sample the animals on day 0 and week 12 only, especially since you defined persistent carriage in humans at day 7? It could have been interesting to sample the free-ranging farm cats in order to study their role as MRSA carriers/reservoirs. How did the authors make the difference between free-ranging farm cats and pets? Is it really relevant to indicate a percentage (17.6%) pooling farmers and family members? The difference is so evident that the mean value may have no scientific meaning anymore. Do you have any information on the antibiotics (or families of molecules) that were used to treat calves? Supplemental Figure 1 does not add a lot of information and could thus be deleted.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

1. There are no details about selection of the farms included, do they vary in size, location. No mention of any inclusion or exclusion criteria.

This study is part of a larger interventional study. Farms were selected in comparable triplets regarding type of calves, production parameters and feed program. Inclusion criteria have been added to the section “Study design and population” (lines 133-137).
The study included farms of different sizes. The mean number of animals per farm was 803. Below the first quartile (Q1) farms varied from 180 to 600 animals, within the interquartile range (Q1-Q3) farms had from 618 to 849 animals, above the upper quartile (Q3) the farms had up to 1600 animals.

No significant linear relationships were found between farm size and MRSA animal prevalence in any of the 2 sampling moments. No association was found either between farm size and human MRSA persistent carriage as defined in the study. There was a significant relationship (PR=1.55; 95% CI=1.13-2.22 per 400 animals increase in farm size) between number of animals per farm and cross sectional human MRSA carriage on week 12, however this association disappeared when adjusting for number of working hours in the farm. We thus interpret the association between numbers of animals and carriage as an artefact and consider the time exposed to animals as the true underlying factor and thus did not include these findings in the paper.

2. There is no characterization of the MRSA isolates - are they all indeed LA-MRSA ST types? It is assumed that all MRSA detected is LA-MRSA but there is no confirmation of this.

This is a crucial comment and based on our previous studies (e.g. Graveland et al. Plos One, 2010) we expected to see predominantly ST398 among the MRSA isolates. Laboratory results were available after submission of the manuscript and only RT-PCR on C01 gene was done for rapid detection of ST398 MRSA. Results showed that, ST398 was present in 90.5% of the human MRSA isolates, in 97.9% of the MRSA positive animal pools and 90.9% of the MRSA positive EDCs. We looked at the not-ST398 MRSA positive subjects and none of them reported a visit to the hospital during the previous 12 months. Although we cannot exclude that these strains were acquired in a hospital earlier or during holidays, we considered all MRSA as acquired on the farm and other than ST398 livestock-associated sequence types might be involved in this study. A sensitivity analysis restricted to only ST398 has been done and similar associations were obtained as described in the paper.

The description of the lab procedures has been updated indicating the C01 gene detection (lines 200-201). A paragraph has been added to the “descriptive results section” (lines 258-260) and the restricted to ST398 sensitivity analysis have been added in the main text where applicable (lines 276-277 and 316-317). A paragraph regarding this question has been added in the discussion (lines 405-411).

3. Why were the cats not sampled if they were seen as potential sources of infection?

The cats were not sampled in this study because they were out of the scope of the research but the association was detected in our analysis. However a large cross sectional study from our group by Gravelandand co-authors conducted earlier on veal farms sampled 35 cats from 25 farms. 26 of them came frequently in the veal stables and only one of them was found to be MRSA positive with a spa type t011 which belongs to ST398. This might not give a direct explanation to the association but cats could certainly act as mechanical vectors of MRSA. This was published in 2009 in a report from the National Institute for Public Health and The Environment (RIVM), Bilthoven, The Netherlands. Some lines have been added in the discussion (lines 455-461) on these findings and a new reference has been added to the main text (line 597).

4. The supplemental table data is somewhat difficult to interpret. The numbers listed in the subsection "S.aureus in the nose" sometimes exceed the numbers listed under MRSA and MSSA - surely an individual must have one of these to appear in this column? I can understand the number in this third section being lower than the combined values of both the previous subsections as some subjects may be co-colonized.

In the supplemental table 1, S.aureus in nose can be higher than the sum of the previous 2 sections on MRSA and MSSA. Numbers of S.aureus in nose are the result of the sum of persistent carriers of MRSA and MSSA but also the ones that have MSSA or MRSA on different sampling moments. We consider the last ones as persistent carriers of S. aureus since the detection of MRSA in one of the 3

samplings does not discard MSSA presence. As discussed in the manuscript , the lab procedures can underestimate the presence of MSSA when MRSA is detected.

5. In the text there is no mention of colonization rates in week 12 although sampling was performed, nor are these levels discussed. Include the data for week 12 carriage rates in the main paper and discuss.

In the supplemental figure 1 (former supplemental figure 2), the cross sectional prevalence for human MRSA carriage on week 12 is displayed. In the “Descriptive results” section, the average cross-sectional nasal prevalence is stated for the 4 sampling moments together. One sentence has been added to the section “Contamination of the environment with MRSA” stating that there was a slight increase in prevalence among farmers on week 12 as compared to the previous sampling moment (lines 352-354). An explanation in the difference in prevalence estimation between day 0 and the rest of sampling moments has been also added to the discussion (lines 396-399). We understand that we can talk about slight increase in prevalence on week 12 comparing the same type of samples (dry cotton swabs for all sampling moments except day 0).

6. Line 29: Carriage rate rather than prevalence as the whole population is not known.

Rates commonly involve person time information in the denominator. We do not have person time type of information in this study. Results are repeated cross sectional measures that are presented as prevalences.

Reviewer #2:

1. The authors are assessing the persistence of LA-MRSA. Were the recovered isolates characterized into more details in order to prove that they were ST398?

This is a crucial comment and based on our previous studies (e.g. Graveland et al. Plos One, 2010) we expected to see predominantly ST398 among the MRSA isolates. Laboratory results were available after submission of the manuscript and only RT-PCR on C01 gene was done for rapid detection of ST398 MRSA. Results showed that, ST398 was present in 90.5% of the human MRSA isolates, in 97.9% of the MRSA positive animal pools and 90.9% of the MRSA positive EDCs. We looked at the not-ST398 MRSA positive subjects and none of them reported a visit to the hospital during the previous 12 months. Although we cannot exclude that these strains were acquired in a hospital earlier or during holidays, we considered all MRSA as acquired on the farm and other than ST398 livestock-associated sequence types might be involved in this study. A sensitivity analysis restricted to only ST398 has been done and similar associations were obtained as described in the paper.

The description of the lab procedures has been updated indicating the C01 gene detection (lines 200-201). A paragraph has been added to the “descriptive results section” (lines 258-260) and the restricted to ST398 sensitivity analysis have been added in the main text where applicable (lines 276-277 and 316-317). A paragraph regarding this question has been added in the discussion (lines 405-411).

2. Supplemental Table 1: the number of sampled employees is so small compared to farmers and their family that statistics are not really relevant. I suggest removing this category.

For the prevalence estimation in supplemental figure 2, the 4 employees are considered as farmers since they had similar level of animal contact. I included the 4 employees in the category of farmers in the supplemental table 1.

3. Why did the author choose to sample the animals on day 0 and week 12 only, especially since you defined persistent carriage in humans at day 7?

The logistics for sampling more than 3,000 animals 3 times in one week are quite complex and the information provided would not be as interesting as the one provided by humans. Humans are followed over time individually, however animal samples were taken in 10 pools of 6 animals each per farm and the purpose was not to follow the individual animal but to give a prevalence estimation to look into population dynamics.

4. It could have been interesting to sample the free-ranging farm cats in order to study their role as MRSA carriers/reservoirs.

The cats were not sampled in this study because they were out of the scope of the research but the association was detected in our analysis. However a large cross sectional study from our group by Graveland and co-authors conducted earlier on veal farms sampled 35 cats from 25 farms. 26 of them came frequently in the veal stables and only one of them was found to be MRSA positive with a spa type t011 which belongs to ST398. This might not give a direct explanation to the association but cats could certainly act as mechanical vectors of MRSA. This was published in 2009 in a report from the National Institute for Public Health and The Environment (RIVM), Bilthoven, The Netherlands. Some lines have been added in the discussion (lines 455-461) on these findings and a new reference has been added to the main text (line 597).

5. How did the authors make the difference between free-ranging farm cats and pets?

The difference between free ranging farm cats and cats as pets are made by 2 different variables. The item pets include cats, dogs or any other companion animal as pet.

6. Is it really relevant to indicate a percentage (17.6%) pooling farmers and family members? The difference is so evident that the mean value may have no scientific meaning anymore.

The percentage 17.6% has been removed from the text (line 243-246). It is just extra information pooling the whole population of the study but we agree that this figure is not really informative.

7. Do you have any information on the antibiotics (or families of molecules) that were used to treat calves?

This information was not available in this phase of the analysis but it will be available in the future for the analysis of the interventional part of the study.

8. Supplemental Figure 1 does not add a lot of information and could thus be deleted.

We agree on this and just the study design section provides enough information for an overview of the study. Thus this figure can be deleted and supplemental figures 2 and 3 are now numbered as 1 and 2 respectively).

9. Would you be willing to share your data? Cast your vote in our Online Poll.

The survey was completed and we would be willing to share the data depending on the purpose and general access.

VERSION 2 – REVIEW

REVIEWER	HAENNI Marisa, PhD Anses France
REVIEW RETURNED	06-Aug-2013

GENERAL COMMENTS	The authors gave point-by-point answer to all my comments. My major point, which was the specific identification of ST398 , were taken into account in an entirely satisfying manner. Thus, I consider that this manuscript is now publishable.