Short communication

Nasal microbiota composition dynamics after occupational change in animal farmers suggest major shifts

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Abstract

Previous studies have suggested a significantly higher diversity in the nasal microbiota of pig farmers compared to people having no contact with farm animals. However, the fate of this nasal microbiota specificity after farmers stop being in contact with the pig farm environment is unknown. The aim of this study was to investigate the change in the nasal microbiota of pig-farmers after the change of occupation.

Methods: Anterior and posterior nasal swabs were collected from seven people during employment on pig farms, and again after a period of at least 50 days after leaving the pig farm. Illumina MiSeq sequencing of 16S rRNA was conducted to characterize the dynamics of the nasal microbiota. The microbiota of actively working pig farmers was compared to microbiota after they had stopped working (ex-pig-farmers) and to control groups (cow farmers and non-exposed individuals).

Results: Following a prolonged period without exposure to pigs, α-diversity of both anterior and posterior cavities dropped significantly. The composition of the microbiota of pig-farmers had a low inter-similarity with the non-exposed group while ex-pig-farmers were more similar to cow-farmers and the non-exposed group than to their own microbiota during pig farming.

Highlights

- The pig farmer's microbiota composition specificity is not permanent.
- Change of the nasal microbiota of pig-farmers, after they stop being exposed to pigs is observed.
- Alpha-diversity dropped significantly and microbiota becomes more similar to the microbiota of the control groups (cow-farmers and non-farmers).

1. Introduction

The nasal microbiota exists at the interface between the exterior environment and the interior of the human body and can undergo modification due to external environmental factors. It has been demonstrated that the nasal microbiota of humans can be influenced by by the level of particles matter pollution (1) the presence of pets (2) or farm animals (3). Indeed, we previously indicated that pig-farmers have a significantly more diverse nasal microbiota than cow-farmers or non-farmers (3, 4). We also showed that this microbiota is more similar to the nasal microbiota of the pigs and to the airborne microbiota of the pig house than to the nasal microbiota of non-farmers. Another study showed that people living and working on dairy farms have also a rich and distinct nasal microbiome compared to that of non-farmers (5).

However, the fate of this specific nasal microbiota when farmers cease to be exposed to the pig farm environment remains to be investigated. Indeed, despite demonstrations of colonization of the farmers' nasal cavities with animal associated microorganisms (6-8), the permanent or transient character of this colonization is unknown. The aim of this research is to study the change of the microbiota from anterior and posterior nasal cavities of pig-farmers, in terms of diversity and specificity, after they stop being exposed to pigs.

2. Materials and methods

Sampling was conducted in Switzerland between October 2014 and July 2015.

The volunteers were grouped by occupation into either office workers (non-farmers, n=19), cow-farmers without any previous occupational contact with pigs (n=12) or pig-farmers (pig-

farmers/ex-pig-farmers, n=7). For this study, the non-farmers and the cow-farmers were sampled once while pig-farmers were sampled twice. The latter included a time point while actively working on pig farms (pig-farmers) and after having stopped working with pigs (ex-pig-farmers) for at least 50 days (mean 70, min 51-max 102; see details in supplementary file, table S1). Anterior and posterior samples were taken using a dry cotton swab (Dryswab, MWE, UK) and a flocked nylon fiber swab, allowing to reach the posterior cavity (E-Swab, Copan, Italy) respectively. DNA extraction, amplification of the V4 region, Illumina MiSeq sequencing, were conducted as previously described by using DADA2 package version 1.5.0 to analyze the reads which request no rarefying of sequence reads (3). All calculations were performed in R version 3.1.2 (http://www.R-project.org) with the base and vegan package and all graphs were created using the ggplot2 package, if not stated otherwise.

Alpha- and Beta-diversity was assessed as previously described (3). Unweighted (Jaccard) and weighted (Ružička) distance matrices were clustered using the hierarchical clustering method unweighted pair group method with arithmetic mean (UPGMA; *hclust* function) to observe possible cluster changes. Cluster allocation was visualized using the *alluvial* function (alluvial package). The BioProject study accession number is PRJEB39411 for the pig farmer samples and the control samples are part of accession PRJEB26637.

3. **Results**

The mean age, the proportion of smokers, and sex in the 3 groups were not significantly different (One-way ANOVA, $p>0.05$). The 90 samples resulted in 3,264,192 sequencing reads. The mean number of reads per sample was 36,269 (± standard deviation 21,698) ranging from 3,692 to 120,642 reads. Reads were clustered into a total of 7057 SVs (Sequence Variants).

Richness expressed as the number of sequence variants (SV) and Shannon Diversity Index (SDI) of the nasal microbiota of the four different groups are represented in figure 1a and 1b.

In pig-farmers and cow-farmers, richness and SDI observed in anterior and posterior nasal cavities was significantly higher than in non-farmers ($p<0.001$ for all comparisons). Cow-farmers had significantly higher richness and SDI than ex-pig-farmers in their anterior nasal cavities ($p=0.002$ for richness; $p=0.02$ for SDI). Once individuals ceased to be exposed to pigs, richness and SDI of both anterior and posterior cavities dropped considerably ($p<0.004$ for richness comparisons; $p<0.03$ for SDI comparisons). The richness and SDI in the anterior cavity did not longer differ from those of non-farmers but, in contrast, showed significantly lower values than these of cow-farmers. Concerning the posterior cavity, only the richness remained significantly higher than that of non-farmers while SDI showed no significant differences between ex-pig-farmers and non-farmers. No differences were observed in the posterior cavity between ex-pig-farmers and cow-farmers for neither richness nor SDI values.

Clustering analyses showed significant differences for the four groups in the anterior and posterior cavities using Jaccard ($P=0.016$) or Ružička calculation models ($P=0.016$). (Fig 1c and 1d, S1).

Referring to the plot, pig-farmers showed the largest dissimilarity with the non-exposed group, while ex-pig-farmers and cow-farmers were between pig-farmers and non-farmers.

The alluvial plots highlight the change of cluster identity of the anterior (Fig 1e and 1f) and posterior (Fig 1g and 1h) nare of ex-pig-farmers. We observed that the majority of pig-farmers changed clusters after they stopped working with pigs. However, the results differ according to whether we use the Jaccard or the Ružička calculation models. By using the Jaccard index,

113 the clusters of the anterior nares of pig-farmers changed and two of them switched to a
114 cluster that was representative for non-farmers, whereas four of them joined a cluster of cow-
115 farmers. Using the Ružička index, six of the pig-farmers switched to a cluster common to cow-
116 farmers and non-farmers after they stopped being exposed to pigs (ex-pig-farmers). This
117 finding did not apply to the posterior nare microbiota. The majority of the pig-farmers (4/7)
118 underwent no change of cluster after they ceased working with pigs (Fig 1g and 1h).
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4. Discussion

Our main findings showed a decreased richness and SDI of both anterior and posterior cavities of ex-pig-farmers compared to the time point when they were exposed to pigs daily. These alpha-diversity values became more similar to those of the non-farmers.

Concerning the beta-diversity, we again observed that nasal microbiota of ex-pig-farmers became either more similar to the microbiota of non-farmers or more similar to the microbiota of cow-farmers. This can be explained by the fact that the majority of the ex-pig-farmers (5/7) became cow-farmers. However, as they were cow-farmers for only a short period (< 50 days) we speculate that the colonization with a microbiota commonly found in cow farmer had not yet been fully achieved. Interestingly, this finding was not true for the posterior nare microbiota, which seemed to be stable for a longer time. Unfortunately, we are not able to exactly quantify the duration of this stability, but it would be very interesting to know how long this animal microbial signature will remain detectable in ex-pig-farmers. Indeed, the potential health effects of harboring more diverse and/or different bacterial communities in the nasal microbiota are currently not known. This modification of the microbiota could be beneficial if it is associated with a protective effect against allergic/atopic diseases, according to the hygiene hypothesis (9). But, this modification could also be worrying if it leads to the colonization of human by antimicrobial resistant bacteria.

It has been widely documented that livestock-associated Methicillin resistant *Staphylococcus aureus* (MRSA) colonize the nasal cavities of farmers (10). Two recent studies showed that MRSA was detected in almost all persons immediately after a sporadic short-term exposure to pigs colonized with MRSA, but 94% of them were negative when a second sample was

collected maximum 24 hours later (11, 12). Therefore, a single short-term exposure to animals led to a transient contamination rather than a true colonization by MRSA. In contrast, another study showed that a cessation of pig exposure for 7-14 days (holidays) did not clear the nasal MRSA colonization (13). Apart from MRSA, a pig farmer microbiome could harbor additional resistance genes and therefore act as a reservoir of the pig farm resistome. It is important to know if such changes in the microbiome are long lasting to evaluate possible public health issues. This is particularly important if farmers acquire pathogenic species which are antibiotic resistant. As our sample size is small, and the density of sampling time points of the included individuals precludes a quantitative timeline for changes to the microbiota, the results need to be interpreted cautiously. Indeed, other factors such as life style (14) and seasons (4) can also shape the microbiome. However, these first results showed that specific nasal microbiota of pig-farmers changed to a non-pig farmer nasal microbiota after cessation of pig farming using a longitudinal design which is a particular strength of the study. Further studies are needed to confirm this finding and clarify the key roles of animals and air on the modification of the microbiome.

5. Conclusion

It has been described that work-related microbial and nonmicrobial exposures may modify the worker microbiome (1, 3). Here, we demonstrated that this modification is not permanent for pig farmer's microbiota composition. The nasal microbiota of ex-pig-farmers no longer showed the characteristics of a regular pig farmer’s microbiota and became either more similar to the microbiota of the non-exposed group or the microbiota of cow-farmers.

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Declaration

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The authors declare no conflict of interest relating to the material presented in this article

Ethical clearance for this study was obtained from the Human Research Ethics Committee of the Canton Vaud (243/14 and P_2017-00265).

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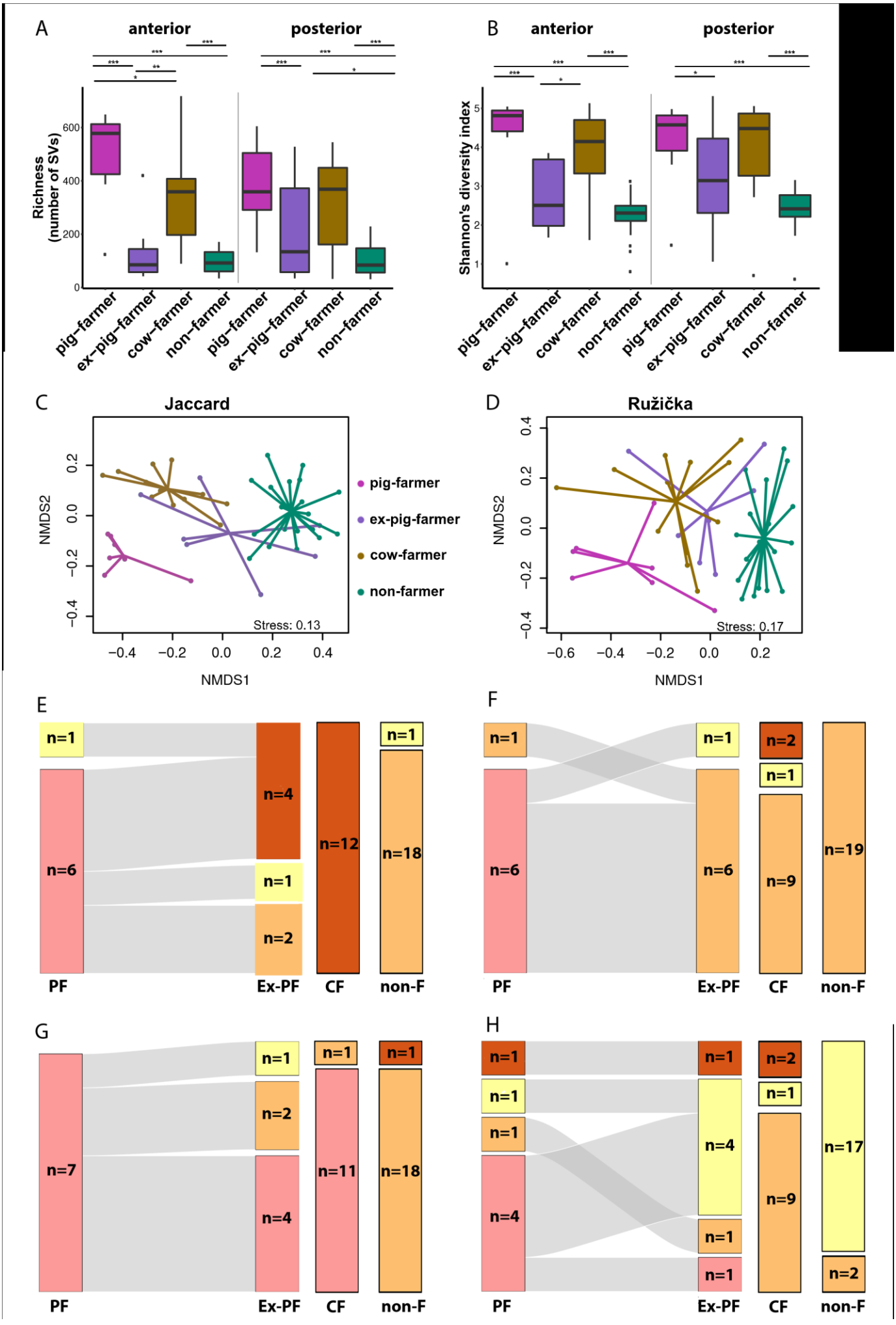
Figure legends

Figure . Alpha-diversity comparison of samples from individuals during (pig-farmer) and after pig exposure (ex-pig-farmer),cow-farmers and non-farmers. (A) Richness values for anterior and posterior samples; (B) SDI values for anterior and posterior samples. Beta-diversity comparison of anterior samples from individuals during (pig-farmer) and after pig exposure (ex-pig-farmer), cow-farmers and non-farmers. NMDS plot of unweighted (Jaccard) (C) and weighted (Ružička) (D). Alluvial plot showing cluster assignments of anterior nare, based on hierarchical clustering of unweighted (Jaccard) (E) and weighted (Ružička) (F) distances. Alluvial plot showing cluster assignments of posterior nare, based on hierarchical clustering of unweighted (Jaccard) (G) and weighted (Ružička) (H) distances. The number of samples in a cluster is indicated and each cluster is indicated with a different colour (four different colurs for each of the four different clusters).


A
anterior
posterior
Richness (number of SVs)
pig−farmer
ex−pig−farmer
cow−farmer
non−farmer
B
anterior
posterior
Shannon's diversity index
C
Jaccard
NMDS1
NMDS2
Stress: 0.13
pig-farmer
ex-pig-farmer
cow-farmer
non-farmer
D
Ružička
Stress: 0.17
E
n=1
n=6
n=4
n=1
n=2
n=12
n=1
n=18
PF
Ex-PF
CF
non-F
F
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n=6
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