Research Article

Mechanical brushing, social interaction and gut microbiota analysis: Gut-brain axis directed gut microbiota in healthy calves before and after mechanical brushing

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Abstract

Mechanical brushes are well-recognized grooming tools widely adopted to improve the well-being of dairy cows and calves, and automated brushing has been reported to interact with social networks and behaviour in several species. Building on these observations, the present study hypothesized that calves that naturally investigate and use mechanical brushes may undergo shifts in gut microbiota composition, potentially mediated by social interaction and the associated skin–oral network. This field study was conducted under commercial farm conditions in apparently healthy calves aged 30–70 days that were socially bonded to their pen mates and used a mechanical brush at least daily. Infectious enteropathogens were excluded using point-of-care rapid diagnostic test kits. Faecal samples were collected rectally before brushing and after three weeks of mechanical brush use. Following extraction of faecal DNA, the V4 and V4–V7 regions of the 16S rRNA gene were amplified and sequenced on the Illumina iSeq100 platform (2 × 150 bp), and data were processed using QIIME 2 and Kraken2 with taxonomic assignment against the SILVA database. Before brushing, the microbial community was dominated by *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). After three weeks, the relative abundances of *Y. enterocolitica* and *P. tolaasii* fell to 10.1% and 5.75%, respectively, while taxa absent at baseline — *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%) — became established. Increased proportions of *Phocaeicola vulgatus, [Ruminococcus] torque*s and *Pedobacter sp. HDW13* were also recorded, whereas *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were no longer detected. The reduction in potential pathogens, most notably *Y. enterocolitica,* is of particular interest given its role in enteric disease of cattle and its zoonotic importance. To the best of the author's knowledge, this is the first report associating mechanical brushing with gut microbiota alterations in calves, providing a rationale for enrichment-based interventions targeting gut health and welfare.

Keywords: Calf, environmental enrichment, gut microbiota, mechanical brushing, social interaction, 16S rRNA sequencing

Introduction

Given elevated scaling of dairy farms worldwide, dairy industrial processing exhibited a significant nutritional source for humans (Reynolds et al., 2013). Regarding past back ground of close era, human consumption of dairy products have gained elevated concern linked to welfare of dairy cows, and therefore calves, through its almost linked to quality of milk, raw milk delivery, dairy-related stuff and economic beneficiary (Barkema et al., 2015). High-welfare dairy farming facilities exhibited a pivotal participation within in the feasible progress for dairy production

(Crossley et al., 2021).

Relative researches participated out on weaned calves (Horvath & Miller-Cushon, 2019; Velasquez-Munoz et al., 2019; Zobel et al., 2017), presented that the elementary ambition modified the repetition and timing of mechanical brush intervention are elaborately interconnected with several issues [i.e. relevant temperature, encompassing noise concentration, and attainability for resources (Li et al., 2024).

Given mechanical brushing interacts with social network and behaviours among pigs (Högberg et al., 2025), horses (Lansade et al., 2022), the present investigator hypothesized that calves naturally investigating and exhibiting mechanical brushing behavior could cause switching in gut microbiota, probably related to social interacion. Besides another aim was to analyze whether alterations among gut microbiota could reflect skin-oral network.

Material and Method

Study demographics

This study was performed as prospective observational type, at field conditions. One of the involvement criteria, as was also reported above, was social network bounds previously described (Alıç Ural et al., 2022). Even if a calf was socialy bounded to the other relevant mates, and even prefer mechanical brush (Fig. 1), was participated. A total of 14 calves were composed of, of both sexes (9 female, 5 male) at the age of 30 to 70 days of age.

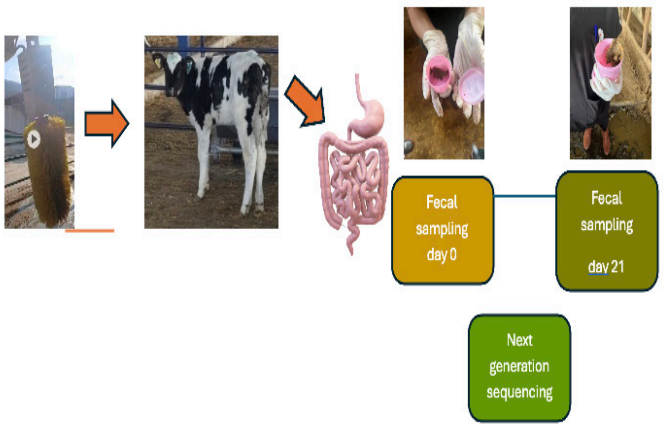


Figure 1. Schematic representation of present study, also evidencing hypothesis and methodology.

Ethical Approval

This study was ethically approved by the Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University-HADYEK (Approval No: 64583101/2024/21).

Animals and Experimental Design

The animal material of the study consisted of 16 calves presented to with complaints of diarrhea. Infectious causes of diarrhea including *E. coli*, Rotavirus, Coronavirus, *Cryptosporidium spp.*, and *Clostridium perfringens* were excluded using point-of-care rapid diagnostic test kits (Anigen, Rapid BoviD-5 Ag Test Kit, Korea). Inclusion criteria were i) usage and preference of mechanical brushing, ii) social networking and usage of other objects available at farm, iii) at least daily interference with mechanical brush (not solely single usage) and iv) evidence of healthy conformation.

Fecal sampling

Fecal microbiota analytes were collected directly from rectal route by use of singe, sterile sampling containers, as shown at the bottom, along with several steps of methodological intervention.



Figure 2. Organization and team work at field conditions showing macro appearence of methodological steps for relevant fecal sampling.

Microbiota Analysis and 16S rRNA Sequencing

DNA was throughly extracted among fecal samples using a commercially available fecal DNA extraction

kit with regard to the manufacturer's instructions. The V4 and V4–V7 regions of the 16S rRNA gene were amplified by use of indexed primers. PCR amplification, purification, and library preparation were performed according to standard protocols.

Sequencing was conducted using the Illumina iSeq100 platform with paired end reads (2 × 150 bp). Raw sequencing data were processed using bioinformatics pipelines including QIIME 2 and Kraken2 for taxonomic classification.

Bioinformatics and Statistical Analysis

Sequencing data were analyzed for microbial diversity and taxonomic composition. Alpha diversity indices and beta diversity analyses were performed, and taxonomic classification was conducted using the SILVA database. Non-parametric data were expressed as median (min–max). Statistical significance was set at $p < 0.05$.

Results

Gut microbiota analytes

Before treatment, the most dominant species in the composition of the microbiota were *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). *Mycobacterium canettii, Klebsiella michiganensis* and *Streptomyces sp. NBC_00536* were presented in lower proportions.

The microbiota composition was significantly different after mechanical brushing. The proportions of *Yersinia enterocolitica* (10.1%) and *Pseudomonas tolaasii* (5.75%) decreased and species not detected before treatment, such as *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%) appeared after treatment. Moreover, increased ratios of *Phocaeicola vulgatus*, [Ruminococcus] torques and *Pedobacter sp. HDW13* were observed. In contrast, *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were not detected in post-treatment samples.

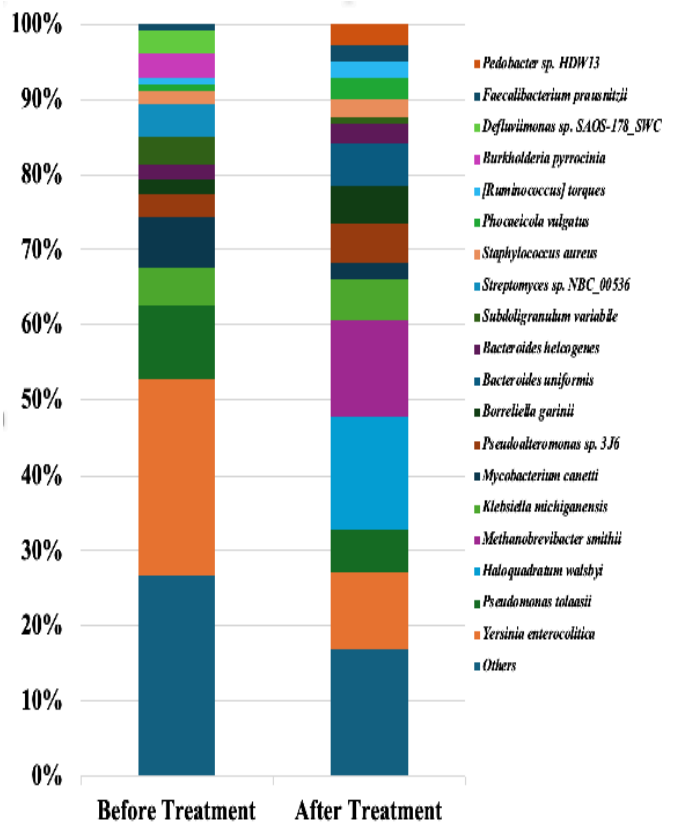


Figure 3. The basic structure of the bacterial community composition of two different sampling time, both at the species level, before and after mechanical brush treatment. Stacked bar graphs illustrate the relative abundances of bacterial species.

Discussion

In a previous research regarding dairy farming, social isolation of cattle was subjected. In that investigation it was aimed to detect even if environmental enrichment both by tactile (automated grooming brush) and visual (a mirror) stimulation might diminish stress. In a total of 18 cows exposed to 4 different isolation conditions of half an hour each; physiological and behavioural indicators of stress were analyzed. It was detected that the occurence of an automated grooming brush, a mirror or both within the isolation pen did not linked to decreased indicators of stress. In disaggreement with some other studies, diminished levels of stress among socially isolated heifers/cows (Mandel et al., 2019). In the present study altough the investigator did not analyzed stress biomarkers, the aim of her next and subsequent study would be thus aimed at this subject. Briefly, in the present study altered microbiome could be related to the animal

wellfare, and probably diminished stress by grooming via brushing. The present study was methodlogically involved social interaction, not isolation. Usage of mechanical brushing could alleviate stress and caused social interaction along with skin-oral network and communication. This could have helped microbiome shifting, as was shown in Fig. 3.

In a prior multidisciplinary study, social network analysis was performed in a group of 9 calves at the age of 10 to 16 weeks. Through full daily digital camera usage, interactions on each calf were detected and observed, field study lasted 4 weeks. At initial referral, only 2 was infected with giardiasis, whereas 3 other calves were also infected on the second week. All other elevant calves were additionaly infected at the end of trial, associated with close contact. In summary it was concluded that social networking played significant predisposing factor for giardiasis spread to those of calves enrolled (Alıç Ural et al., 2022). In the present study this social network modality was similarly adopted through usage of mechanical brushing to those of apperantly healthy calves. On the opposite direction and results, in comparison to that study (Alıç Ural et al., 2022), mechanical brush usage caused diminished relative abundaces of some selected and detected pathogens, detected at this study (Fig. 3).

In the present study prior to mechanical brush usage at day 0, the vast majority of bacterial species involved *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). Yersiniosis is one of the vast majority marked intestinal disease agent in association with *Y. enterocolitica*, influencing humans and and also cattle and relevant species (Chlebicz & Śliżewska, 2018; Saleh & Zenad, 2018). Several different social and environmental issues were linked to *Y. enterocolitica* spread, resulting with marked public health/economic problems (Curtis et al., 2014; Råsbäck et al., 2018). In a previous research 1020 samples [660 rectal swabs collected from live cattle] exhibited *Y. enterocolitica* isolation 3 out of 15 examined cattle herds. Isolation rates were as follows 14.7%, 7.4% and 5.5% of heifers, calves and adult cows, respectively (Łada et al., 2023). On the other hand altough Pseudomonadales was evident in healthy calves vs. diarrheic ones (Zhang et al., 2025), *Pseudomonas tolaasii* relative abundance was 9.94% prior to mechanical brush usage, where declined to 5.75%. Moreover mechanical brush usage caused diminished *Y. enterocolitica* abundance from 25.9% to 10.1% (Fig 3).

The microbiota composition was significantly altered following 3 weeks of mechanical brushing. Some species such as *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%), not previously detected previously, existed after mechanical brushing. Furthermore *Phocaeicola vulgatus*, *[Ruminococcus] torques and Pedobacter sp. HDW13* were existed, whereas *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were disappeared through 3 weeks of mechanical brushing (Fig 3).

Conclusion

In conclusion the present prospective observational study concludes that microbial alterations could have critical role within the wellfare of calves. This finding, altough was not supported by biomerkers of relevant, was based on personnal observations recorded (live weight gain, disease free status of at least stuıdy period etc.). Obtained findings provide insights for designing purposed interventions for modifying gut health among calves. Mechanical brushing for at least 3 weeks of usage, caused altered microbiome among calves enrolled herein.

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