A potential treatment approach for subclinical mastitis in dairy cows: auriculotherapy of the auricular branch of the vagus nerve

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Abstract

This research aims to test the hypothesis that neural therapy (NT) on the auricular branch of the vagus nerve (ABVN) in dairy cows diagnosed with subclinical mastitis (SCM) results in a reduction of the somatic cell count. Therapeutic options for SCM are mostly based on use of antibiotics and often lead to unsatisfactory results. An alternative therapy targeting the anti-inflammatory properties of the vagus nerve showing good efficacy, economic viability and without major side effects would be of considerable interest. Auriculotherapy (AT) was performed using three repeated infiltrations of 8.0 mg (0.4 ml) procaine hydrochloride (2%) at the location of the ear tag associated with the auricular acupuncture point (AAP) of the udder. Some 85 clinically healthy cows from nine dairy farms were sampled for evaluation of quarter somatic cell count (QSCC) on four days (d0, d2, d4 and d6). Quarters with a QSCC > 100 000 cells/ml on d0 were included in the analysis. Over the study period, a total of 784 quarters were analysed, 385 control (CON) quarters from 40 cows and 399 treated (TRE) quarters from 45 cows. Results showed that AT of the ABVN resulted in a significant reduction of the QSCC after three treatments. The effect was independent of bacteriological culture results of the quarter milk samples. The bacteriological cure rate, however, was not influenced by AT. To our knowledge this is the first report of AT reducing QSCC in dairy cows with SCM. Before AT can be regarded as an alternative therapeutic approach, further research should focus on possible long-term effects of AT on the reduction of SCC, any bacterial elimination and the neural pathways of AT in dairy cows with SCM.

Keywords: Auriculotherapy; non-antibiotic therapy; somatic cell count; subclinical mastitis; vagus nerve

Research Article

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According to the International Dairy Federation (IDF) recommendations subclinical mastitis in dairy cows is defined as a disease without clinical inflammatory signs of the udder and a SCC > 100 000 cells/ml with the presence or absence of pathogens. Subclinical mastitis is commonly detected by measuring SCC, but there is no standardized approach in terms of SCC cut-off values and evaluation on basis of individual composite SCC or QSCC. As a consequence, the reported prevalence of SCM varies widely between 13 and 53% (Fox et al., 1995), since the chosen criteria vary with country (Hiitiö et al., 2003). Excessive use and misuse of antibiotics in agriculture, humans and animal husbandry accelerate the occurrence of antimicrobial resistance and have a negative impact on the environment worldwide (Sandgren et al., 2008). Pathogen induced SCM is a major cause for the worldwide consumption of antibiotics (Krömker and Leimbach, 2017). Due to the disadvantages of antibiotic treatment, recent research has explored the use of non-drug strategies, e.g. feeding of probiotics or plant extracts and immunoprophylaxis to improve udder health in SCM (Wu et al., 2015, 2018; Seroussi et al., 2018; Catozzi et al., 2019).

Since the late 1990s, a number of studies have investigated the immunoregulatory role of the vagus nerve in different inflammatory disorders (Maier et al., 1998; Bonaz et al., 2017). The direct stimulation of the vagus nerve branches leads to a significant improvement of...
Neural therapy on the auricular branch of the vagus nerve by neural therapy has an anti-inflammatory effect on the subclinical inflamed udder and results in decreased SCC.

Material and methods

The study procedures were discussed and approved by the Ethics Committee of the University of Veterinary Medicine, Vienna, in accordance with Good Scientific Practice guidelines and the national authority according to §8 of Law for Animal Experiments, Tierversuchsgesetz-TVG (BMWF-68.205/0033-WF/V/3b/2017) of Austria.

Animals and study design

Nine conventional Simmental dairy farms in Lower Austria, which obtained monthly milk recording data from the regional Dairy Herd Improvement Service (Landeskontrollverband), participated in this study. The farm inclusion criteria were that at least 80% of cows showed SCC < 150 000 cells/ml and that a maximum of 15% had SCC > 200 000 cells/ml milk on average over the year. Only cows with an individual composite SCC of > 200 000 cells/ml milk in the latest milk record data were enrolled to increase the probability for finding cows with QSCC > 100 000 cells/ml in at least one quarter. All selected cows were clinically healthy and received no treatment with antibiotics and/or nonsteroidal anti-inflammatory drugs in the current lactation. In our study, cows with QSCC > 100 000 cells/ml independent of the bacteriological result were defined as having SCM, which is in accordance with the definition of the IDF. The Excel randomization function determined the allocation of cows to control group (CON) without any treatment, and treatment group (TRE) with application of local anaesthetics. Documentation of data was performed using Microsoft Excel 2010 (Microsoft Office Cooperation, Redmond, WA, USA). In addition, data relating days in milk (DIM), parity, farms, season, position of ear tag, affected udder side and bacteriological analysis were documented.

Collection of quarter milk samples for determination of QSCC and bacteriological examination

Quarter milk samples were collected on days 0 (before treatment), 2, 4, and 6 for measurement of QSCC. Furthermore, aseptic quarter milk samples were taken on d0 and d6 for bacteriological examination. After sampling, all milk samples were immediately stored at 4°C and delivered to an analytical laboratory (Qualitätslabor Niederöstereich, Gmünd, Austria) on the same day. An automated somatic-cell counter (Fossomatic™ FC, Foss, DK-3400 Hillerød, Denmark) analysed the QSCC. Collection and microbiological procedures were defined and performed as outlined by the National Mastitis Council (Hogan and National Mastitis Council (U.S.), 1999).

Auriculotherapy with local anaesthetics

Neural therapy on the ABVN of the scar of the left ear was performed in TRE on d0, d2 and d4. For this purpose, each cow was restrained in the feeding fence and the head was tied to the right side of the animal using a halter. The whole ear, especially the area around the ear tag, was examined for pathological changes such as marked reddening, swelling, exudate and hyperalgesia.
by palpation. After clipping all long hair of the pinna, the area was cleaned and disinfected using 70% ethyl alcohol-soaked cotton balls (Ethanol 70%, Liquid Production GmbH, Flintsbach am Inn, Austria). Cows of TRE group were treated by intracutaneous infiltration of 0.1 ml procaine hydrochloride 2% (Procamidor® 20 mg/ml, Richterpharma AG, Wels, Austria) at each location clockwise at 12, 3, 6 and 9 around the ear tag (Fig. 2) using 1 ml syringes (Omnifix®-F, B. Braun Melsungen AG, Melsungen, Germany) and 0.6 × 30 mm needles (Henry Schein Inc. Melville, USA). Cows from CON group were handled identically, however, without injection of local anaesthetics.

**Statistical analysis**

All data were analysed with the statistical program SPSS (SPSS version 20; IBM Corporation, NY, USA). Because QSCC values were not normally distributed, data were log_{10} transformed and categorized for subsequent analysis. A general linear model with repeated measurements (one-way analysis of variance: ANOVA) was used to compare the change of QSCC over the sampling days (d0, d2, d4 and d6). The group variable CON and TRE was included in the model to exclude a potential confounding interaction. The change of QSCC over time within CON and TRE was compared using ANOVA and adjusted by Bonferroni post hoc test. The sampling days were defined as within-subject repeated measurements (one-way analysis of variance: ANOVA) and categorized for subsequent analysis. A general linear model with repeated measurements within groups showed a significant interaction between the groups and time revealing significant differences in QSCC over the time (P < 0.05). The analysis of repeated measurements within groups showed a significant change of QSCC over the time (d0 to d6; P = 0.001) in TRE, whereas within CON (P > 0.05) no differences were found (Fig. 3). The pairwise comparison between the sampling days resulted in a significant reduction of QSCC after the third treatment from d0 to d6 (P < 0.01) (Table S2 in online Supplementary File). Including independent variables (DIM, parity, farm, season, position of the ear tag, udder side, and bacteriological result) were included in the analysis of variance (ANCOVA). Moreover, effects on QSCC change in bacteriological positive samples on d0, AAP corresponding to position of ear tag (yes/no), side of udder quarter (left/right) were evaluated in CON and TRE. Bacteriological cure was defined as absence, on d6, of the bacteria identified on d0. Differences in bacteriological cure rate between CON and TRE were analysed by χ²-test. In general, values were considered significant when P ≤ 0.05.

### Results

Based on the monthly milk record data over all four seasons of the nine participating dairy farms, 99 cows were initially enrolled into the study. Finally, 85 dairy cows met the inclusion criteria, whereas 14 were excluded from further analyses because of clinical mastitis (n = 7) or QSCC < 100 000 cells/ml at all quarters on d0 (n = 7). The milk samplings on days d0, d2, d4 and d6 resulted in a total of 784 QSCC, corresponding to 385 QSCC of CON (40 cows) and 399 QSCC of TRE (45 cows). Cows were grouped according to parity into primiparous (n = 21), two lactation cows (n = 45). Moreover, the stage of lactation was then stratified into thirds, resulting in categories, SCC ≤ 100 (n = 16), 100–199 (n = 24) and ≥200 (n = 45) DIM. Since sampling in summer was performed at one farm only, the data of the season summer was restricted to those of the one specific farm. The position of the ear tag was categorized according to Fig. 1. None of the cows in the study showed signs of inflammation at the scar around the ear tags. Bacteriological examinations of milk samples were performed on days 0 and 6. Median QSCC on d0 was higher in TRE (447 000) than in CON (397 000). The changes of median QSCC over time demonstrated an increase from d0 to d2 and a decrease from then on in both groups (Table 1). Descriptive statistics of QSCC of previously mentioned categories (variables) in groups CON and TRE are shown in online Supplementary File Table S1.

#### Table 1. Median, first and third quartile of quarter somatic cell count (in 1.000 cells/ml milk) of control (CON) and treatment group (TRE) on days (d) 0, 2, 4 and 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Quarters (n)</th>
<th>d0</th>
<th>d2</th>
<th>d4</th>
<th>d6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (n = 40)</td>
<td>Quarters (n)</td>
<td>98</td>
<td>97</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>397</td>
<td>489</td>
<td>380</td>
<td>337</td>
</tr>
<tr>
<td></td>
<td>Quartile 1</td>
<td>191</td>
<td>215</td>
<td>263</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>Quartile 3</td>
<td>888</td>
<td>1301</td>
<td>1016</td>
<td>963</td>
</tr>
<tr>
<td>TRE (n = 45)</td>
<td>Quarters (n)</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>447</td>
<td>499</td>
<td>475</td>
<td>343</td>
</tr>
<tr>
<td></td>
<td>Quartile 1</td>
<td>186</td>
<td>215</td>
<td>227</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Quartile 3</td>
<td>1321</td>
<td>1593</td>
<td>1961</td>
<td>1025</td>
</tr>
</tbody>
</table>

**Effect of AT over time on the change of QSCC in TRE compared with CON**

The initial analysis included the groups (CON, TRE) as potential confounding variables into the model. The results indicated an interaction between the groups and time revealing significant differences in QSCC over the time (P < 0.05). The analysis of repeated measurements within groups showed a significant change of QSCC over the time (d0 to d6; P = 0.001) in TRE, whereas within CON (P > 0.05) no differences were found (Fig. 3).
of ear tag position on QSCC in TRE (Table S3 in online Supplementary File). Including the covariates into the pairwise comparison, however, resulted in a significant reduction of QSCC from d0 to d6 only in TRE ($P < 0.01$).

**Effect of AT over time on the change of QSCC in bacteriological positive samples of TRE compared with CON**

The bacteriological analysis of quarter milk samples on d0 and d6 revealed various bacteria, which were grouped into environmental (i.e. Enterobacteriaceae, *Streptococcus dysgalactiae*, Enterococcus, Acinetobacter), major contagious (i.e. *Streptococcus uberis, Streptococcus agalactiae*), and minor contagious species (i.e. Non-aureus *Staphylococcus* spp., *Corynebacterium* spp.). Major pathogens with higher importance, e.g. *Escherichia coli, Staphylococcus aureus*, and fungi (*Aspergillus fumigatus*) were listed separately. Bacteriological samples with more than one isolate were defined as multiple isolates. The proportion of the bacteriological findings in CON and TRE samples are presented in Table S4 in online Supplementary File. In total, 52.0% (51 of 98) samples in CON and 59.0% (59 of 100) of samples in TRE were bacteriological positive at d0. Minor contagious isolates were predominantly found in both groups. The bacteriological cure rate, defined as the absence of bacteria on d6 compared with d0, was not different between CON (27.5%, 14 of 51) and TRE (20.3%, 12 of 59) ($P = 0.38$). Using the group variable (CON, TRE) in the statistical model, an interaction between the groups and time was evident ($P < 0.05$). The multiple comparison (CON, TRE) in the statistical model, an interaction between the groups and time was evident ($P < 0.05$). The pairwise comparison of the sampling days revealed that the reduction of QSCC occurred from d2 to d6 ($P < 0.01$) (Table S7 in online Supplementary File). An increased QSCC on d2 after the first treatment was apparent, however, this pattern was also present in CON (Table S1 in online Supplementary File). Moreover, there was an effect on QSCC when treatment was performed at the ear tag position 9 (TRE: $P = 0.001$) demonstrating reduction of QSCC after the third treatment from d0 to d6 (Table S8 in online Supplementary File). Including the group variable in the analyses, however, showed no interaction between the groups and time of QSCC change ($P > 0.05$). Because of the low number of ear tags positions other than 9 and 10 no analyses on position-level were performed.

**Effect of the udder (quarter) side on the results of AT application**

The bovine ear chart indicates that the AAP for the left udder is on the left ear and the AAP for the right udder is on the right ear (Fig. S1 in online Supplementary File). In CON, 184 samples were taken from the left udder side and 201 from the right udder side in TRE, 191 samples were taken from the left udder side and 208 were taken from the right udder side. A significant reduction of QSCC was only found in TRE, but independent of the udder side (left: $P = 0.001$, right: $P < 0.05$).

**Discussion**

The aim of this study was to evaluate the effect of AT in dairy cows with SCM. Both CON and TRE contained a comparable number of quarters, so that the stochastic basis for statistical evaluations was given. Our findings support the hypothesis that there
is a positive effect of ABVN stimulation on the QSCC after the third treatment. Including several variables into the model indicated significant interactions of a few variables. The systematic effect, however, was not confounded because results revealed a group effect on QSCC reduction indicating significant differences between treated and control cows. The significant variable in the data set of TRE was the position of the ear tags. Analysing the ear tag position as dependent variable identified the ear tag position at the AAP as the significant factor on the reduction of QSCC. Among the controls, however, we assume a confounding effect of the different farms and seasons on QSCC as data from the summer season was generated on one farm only. Furthermore, we observed that under certain conditions, e.g. in bacteriologically negative samples or AT at the AAP (position 10), an increase of QSCC after the first treatment was obvious, but three consecutive treatments resulted in a significant reduction of QSCC. In the field, the application of AT in cows suffering from SCM was easy to achieve. Nevertheless, intracutaneous infiltration of LA into the ear can cause the animals some pain and since repeated applications are required for a successful therapy, defensive reactions need to be taken into consideration. Therefore, a tight fixation of the cow’s head to the feeding fence is crucial for the safety of both cow and veterinarian.

The underlying principle by which stimulation of the ABVN in cows leads to a decrease of SCC is uncertain. A neuronal response to inflammation, however, described by a cholinergic anti-inflammatory pathway via the vagal nerve was already reported (Tracey, 2002). Furthermore, the stimulation of the vagal nerve led to significantly decreased amounts of circulating TNF in the serum and, therefore, suppressed inflammation (Borovikova et al., 2000). This mechanism might be the underlying principle of the vagal acupuncture, reducing the QSCC in the udder of dairy cows suffering from SCM.

While AT at the ear tag position had no effect on the bacterial cure rate of infected quarters, this effect even in animals with bacteriological positive samples might again be explained by a general support of the immune system. This aspect, however, was not the aim of our study. Even in antibiotic therapy of mastitis, the bacterial cure rate is influenced by many different factors, such as animal- or farm-specific factors, application, dosage and duration of therapy. Furthermore, antibiotics often fail to eliminate bacteria completely (Krömker and Leimbach, 2017). Further research would be desirable to investigate the potential anti-inflammatory effect of AT on longer-term bacteriological cure in cows suffering from mastitis.

The study was designed to perform AT on the scar around the ear tag, assuming that most ear tags are located in the region of the AAP to the contrary was the contralateral ear tag, indicating that the area of vagal innervation at the cow’s ears is different compared with humans. Auricular therapy was successful both in bacteriological negative and positive samples. However, the bacterial cure rate was not affected.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S002202992100087X

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**References**


