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Characterization of mucosa-associated bacterial communities in abomasal ulcers by pyrosequencing

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ABSTRACT

Abomasal ulcers are important pathological alterations of the gastrointestinal tract in cattle and are exceptionally hard to diagnose *in vivo*. The microbiome of the abomasum in cattle with or without ulcers has hardly been studied to date, and if so, the studies used culture-dependent methods. In the present study, the bacterial communities associated with abomasal ulcers of slaughter cows, bulls, and calves in Austria were described using 16S rRNA gene pyrosequencing. Sequences were clustered into 10,459 operational taxonomic units (OTUs), affiliating to 28 phyla with *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Tenericutes* dominating (96.4% of all reads). The most abundant genera belonged to *Helicobacter*, *Acetobacter*, *Lactobacillus*, and novel *Mycoplasma*-like phylotypes. Significant differences between the microbial communities of healthy and ulcerated calves compared to cows and bulls could be observed. However, only few statistically significant differences in the abundances of certain OTUs between healthy and ulcerated abomasal mucosa were found. Additionally, near full-length 16S rRNA gene sequences of the most abundant phylotypes were obtained by cloning and Sanger sequencing ($n = 88$). In conclusion, our results allow the first deep insights into the composition of abomasal mucosal bacterial communities in cattle and describe a hitherto unknown high diversity and species richness of abomasal bacteria in cattle. Our results suggest that bacteria may have only limited involvement in the etiology of abomasal ulcers. However, future research will be needed to verify the contribution of bacteria to abomasal ulcer formation as presence or absence of bacteria does not necessarily correlate with etiology of disease.

1. Introduction

Abomasal ulcers are an important cause of indigestion in cattle of all breeds and ages and production systems (Smith, 2009). Until now, the exact etiological agents of abomasal ulcer formation are not completely understood. Different factors like stress, proliferation of bacteria within the gut, abrasion of the abomasal mucosa due to roughage,

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and mineral deficiencies have been associated with abomasal ulceration (Eddy, 2004; Jelinski et al., 1996; Mills et al., 1990).

Physiologically, the mucosa of the abomasum is protected by a mucous layer, bicarbonate buffer, and high rate of blood circulation in the submucosal tissue. However, if the balance between ulcerogenic factors and protective mechanisms is disturbed, the acidic contents of the abomasum damage the lining and cause ulceration (Smith, 2009).

In cattle, ulcers occur in several forms and have been classified according to Fox (1980) and Whitlock (1980) as lesions of four types: (i) a superficial erosion of the mucous membrane, (ii) a deeper lesion eroding larger blood vessels leading to substantial hemorrhage and (iii) perforating the abomasa wall leading to local or (iv) generalized peritonitis, respectively. Braun et al. (1991) introduced a more detailed subtyping of type one ulcers ranging from a discoloration of the epithelium (type 1a) to distinct craters (type 1d) without reaching the submucosal layer, which are therefore not classified as type two lesions. All four types can cause severe illness or are potentially fatal. However, the consequences of the disease are highly dependent on the severity of the lesions. Generally, the diagnosis of ulcers is difficult because affected cattle show typically non-specific clinical signs. An accurate clinical diagnosis is only possible with bleeding ulcers (Eddy, 2004).

The prevalence of abomasal ulcers varies significantly due to differences in the examined population, case definitions and different means of diagnosis. Clinically apparent abomasal ulcers were reported with a prevalence of 0.2% in young beef calves on herd level (Katchuik, 1992) and were found in 2.2% of adult dairy cattle (Smith et al., 1983). Jensen et al. (1976) showed that 1.6% of necropsied yearling feedlot cattle had abomasal ulcers with fatal perforations or hemorrhages. Studies in slaughtered animals are more sensitive in detecting low-grade ulcers and demonstrate that between 67% and 87% of veal calves are affected (Welchman and Baust, 1987; Wiepkema et al., 1987). Overall, abomasal ulceration can cause considerable economic losses for the cattle industry and represents a significant challenge for animal welfare.

Previous studies suggested several microbiological agents to be associated with the formation of gastric ulcers in cattle. Gitter and Austwick (1957) found fungal hyphae in abomasal ulcers while Roeder et al. (1987) and Mills et al. (1990) reported *Clostridium perfringens* to be the major bacterial agent in abomasal ulcers. Isolation of *Campylobacter* supports an association with abomasal ulceration. However, the relationship between *Campylobacter* and neonatal abomasal ulceration is undetermined (Jelinski et al., 1995). In humans, *Helicobacter pylori* is known as an important etiological agent of peptic ulcers (Kuipers et al., 1995). However, *H. pylori* were not isolated in any sample of ulcerated or healthy abomasa of cattle (Jelinski et al., 1995; Valgaeren et al., 2013).

Knowing the composition of the bacterial abomasum microbiota may provide additional insights into the ecology of species related to cattle disorders. However, the task of describing the microbial composition involved in the etiology of abomasal ulcers by traditional microbiological methods is seriously hampered by the fact that the majority of the microorganisms present in the environment are not cultivable under standard laboratory conditions (Handelsman, 2004). Sequencing technologies such as Roche/454-pyrosequencing or particularly Illumina MiSeq technology are fundamentally changing the way in which microbial communities can be studied. To the best of our knowledge, no study exists to date where the microbiome of abomasal ulcers was captured entirely and compared to tissue samples from healthy animals. It was the objective of this study to utilize 16S rRNA gene-targeted pyrosequencing technology to characterize the bacterial communities associated with abomasal ulcers of slaughter cows, bulls and calves in Austria to verify the following hypotheses: Cattle with abomasal ulcers have a distinct microbiome of the abomasal mucosa which is different from healthy cattle and might contain infective agents involved in the pathogenesis of abomasal ulcers; the microbiome of adult cattle differs from that in calves.

2. Materials and methods

2.1. Animals and sampling

The samples for the study were obtained at two different abattoirs in Austria on five different days over the course of four months. A total of 215 fattening bulls, cows and calves were examined immediately after slaughter. Of these 215 animals, 42 were randomly selected for microbiome analyses.

The animals were stunned and hung by the hind legs, exsanguinated, and skinned. Then the abdomen was opened; the gastrointestinal tract (GIT) was removed entirely from the carcass and moved to a separate room, where the parts of the GIT were separated for further processing or disposal. The abomasa were opened along the greater curvature including the pylorus and evaluated for abnormal contents such as gravel or sand. The pH of the abomasal content was measured, and subsequently the abomasum was cleaned under running water until the mucosa was completely cleaned from digesta. Additionally, the mucosa was individually rigorously rinsed with sterile ice-cold phosphate buffered saline times to remove remains of free-floating bacteria. Abomasal mucosa samples for microbiological analysis were taken from the corpus and the pyloric region of bulls, cows and calves (Table 1 and Fig. S1).

2.2. Ulcer documentation

The abomasal mucosa was examined for ulcers, which were typed according to a modified version of the system by Fox (1980) and Whitlock (1980), and subtyped according to Braun et al. (1991) as shown in Table 1. The size, location and number of ulcers were recorded, and each ulcer was photographed.

2.3. Abomasal mucosa sampling for microbiological analysis

Initially, ulcers were excised with a margin of healthy mucosa immediately after slaughter at the abattoir. The