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Distinct bacterial metacommunities inhabit the upper and lower respiratory tracts of healthy feedlot cattle and those diagnosed with bronchopneumonia

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ABSTRACT

Specific nasopharyngeal bacterial communities can provide colonization resistance against respiratory pathogens in cattle. However, the role of bacterial communities of the lower airways in respiratory health remains largely unknown. Therefore, our objective was to compare nasopharyngeal and tracheal bacterial communities between healthy feedlot cattle and those with bronchopneumonia (BP). Deep nasal swabs and trans-tracheal aspiration samples were collected from steers with (n = 60) and without (n = 60) BP at 4 feedlots in Western Canada. After DNA extraction, 16S rRNA gene (V4) was amplified and sequenced. Alpha-diversity analysis revealed a lower bacterial diversity in the nasopharynx and trachea of steers with BP compared to healthy pen-mates. Bacterial communities present within the airways clustered into 4 distinct metacommunities that were associated with sampling locations and health status. Metacommunity 1, enriched with *Mycoplasma bovis*, *Mannheimia haemolytica* and *Pasteurella multocida*, was dominant in the nasopharynx and trachea of steers with BP. In contrast, metacommunity 3, enriched with *Mycoplasma dispar, Lactococcus lactis* and *Lactobacillus casei*, was mostly present in the trachea of healthy steers. Metacommunity 4, enriched with *Corynebacterium, Jeotgalicoccus, Psychrobacter* and *Planomicrobium,* was present in the nasopharynx only. Metacommunity 2, enriched with *Histophilus somni*, *Moraxella* and *L. lactis,* was present in both healthy and sick steers, but was primarily detected in one feedlot. We concluded that distinct bacterial metacommunities inhabited the nasopharynx and trachea of healthy feedlot cattle and those with BP. Because *L. lactis* and *L. casei* can inhibit *M. haemolytica* growth *in vitro*, their presence in healthy steers may have provided colonization resistance against bacterial respiratory pathogens.

1. Introduction

Bacterial bronchopneumonia (BP) is one of the beef industry's most important health problems (USDA, 2013). Beef cattle of all ages can be affected with BP; however, they are most likely to be affected during the first 50 days after entry into a feedlot (Babcock et al., 2010) as they are exposed to a wide range of viral and bacterial respiratory pathogens (due to comingling), concurrent with various stressors (*e.g.* weaning and transportation) that can suppress their immune system (Caswell, 2014).

Important bacterial pathogens associated with BP in feedlot cattle include *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* (Klima et al., 2014; Timsit et al., 2017a). For these pathogens, colonization of the respiratory tract is a necessary first step before causing infection (Noyes et al., 2015). Therefore, inhibition of this first step of pathogenesis by resident bacterial communities (*i.e.* microbiota), a process called colonization resistance, may be of upmost importance to respiratory health (Timsit et al., 2016a). Furthermore, if a pathogen has colonized the respiratory tract mucosal surface, it may be beneficial to both the bacterial community and the host if these pathogens are supressed, preventing their overgrowth, inflammation and subsequent local or systemic spread (Timsit et al., 2016a).

In the last few years, there is increasing evidence for the role of resident bacterial communities in the nasopharynx in preventing respiratory pathogens from establishing an infection (Corbeil et al., 1985; Holman et al., 2015a; Zeineldin et al., 2017). For example, at feedlot entry, bacterial communities in the nasopharynx of cattle that remained healthy during the feeding period were more diverse than in those that developed respiratory disease (Holman et al., 2015a). As a more diverse flora resists colonization by bacterial respiratory pathogens (Pettigrew

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et al., 2012), this may explain why these cattle remained healthy. Furthermore, cattle that remained healthy had a greater relative abundance of *Lactobacillaceae* and *Bacillaceae* (Holman et al., 2015a), capable of inhibiting growth of *M. haemolytica, P. multocida* and *H. somni in vitro* (Amat et al., 2017; Corbeil et al., 1985).

The role of bacterial communities in the lower respiratory tract in respiratory health is largely unknown. Indeed, to date, most studies on respiratory bacterial communities have only studied the nasopharynx (Holman et al., 2015a,b; Holman et al., 2017, 2018; Timsit et al., 2016b). Therefore, the objective of this study was to compare nasopharyngeal and tracheal bacterial communities between healthy feedlot cattle and those diagnosed with BP. Our hypothesis was that both nasopharyngeal and tracheal bacterial communities differ between these two cattle populations, with a higher diversity, a lower relative abundance of bacterial pathogens and a higher relative abundance of known inhibitors of bacterial respiratory pathogens in healthy cattle compared to cattle with BP.

2. Methods

2.1. Ethics approval

This study was conducted in strict accordance with recommendations of the Canadian Council of Animal Care (Olfert et al., 1993). The research protocol was reviewed and approved by the University of Calgary Veterinary Sciences Animal Care Committee (AC15-0109).

2.2. Animals

Mixed-breed beef steers at high-risk of developing BP (*i.e.* recently weaned, co-mingled and auction-market derived) were studied between November 2015 and January 2016 at four commercial feedlots in Southern Alberta, Canada (one-time capacity ranging from 5000 to 25,000). At on-arrival processing, all steers received a subcutaneous injection of a long-acting macrolide to control BP (tulathromycin, Draxxin, 2.5 mg/kg, Zoetis, Kirkland, QC, Canada), were weighed and vaccinated against infectious bovine herpes virus-1, bovine viral diarrhea virus (types I and II), bovine parainfluenza-3, bovine respiratory syncytial virus, *Mannheimia haemolytica*, *Histophilus somni* and clostridial pathogens. They were also dewormed with pour-on ivermectin solution.

Steers were housed in large outdoor dirt-floor pens containing ~250-300 steers. They were fed twice daily, a concentrate barley-based receiving/growing diet formulated to meet or exceed nutrient requirements. This diet contained 25 ppm of monensin (Rumensin 200, Elanco, Guelph, ON, Canada) and 35 ppm of chlortetracycline (Aureomycin 220, Zoetis). Each morning before feeding, bunks were visually evaluated and feed deliveries were adjusted to ensure that sufficient feed was available for *ad libitum* consumption.

At ~30 d after arrival, steers received another vaccination against infectious bovine herpes virus-1, bovine viral diarrhea virus (types I and II), bovine parainfluenza-3, bovine respiratory syncytial virus and a growth implant.

2.3. Study design

Experienced pen checkers observed steers daily for signs of clinical illness. Steers with one or more visual signs of BP (*e.g.* depression, nasal or ocular discharge, cough or dyspnea) were removed from the pens by pen checkers and, if not previously treated for BP or another disease during the feeding period, were clinically examined by an experienced veterinarian (ET). For every steer suspected of having BP, one or two apparently healthy steers (no visual signs of BP or other disease) were conveniently selected (*e.g.* close to the gate, close to the apparently sick animal, *etc.*) as pen-matched contemporary controls and similarly examined (if not previously treated for BP or another disease during the feeding period). Clinical examinations included assessment of visual signs of respiratory disease (nasal and ocular discharge, tachypnea, dyspnea, depression), determination of respiratory rate and rectal temperature and, a complete lung auscultation using a conventional stethoscope to detect abnormal lung sounds (*e.g.* increased bronchial sounds, crackles and wheezes). A blood sample (~4 mL; plain tube) was also collected by jugular puncture from each steer to determine presence of inflammation *via* determination of serum haptoglobin concentration, as described (Timsit et al., 2011).

Steers with at least one visual BP sign, a rectal temperature ≥ 40 °C, abnormal lung sounds detected at auscultation and no prior treatment against BP or other diseases during the feeding period (*i.e.* 1st BP occurrence) were sampled by deep nasal swab (DNS) and trans-tracheal aspiration (TTA). Cattle selected as pen-matched controls that had no visual BP signs, a rectal temperature < 40 °C, no abnormal lung sounds detected at veterinary auscultation and no history of treatment against BP or another disease during the feeding period were also sampled by DNS and TTA.

2.4. Case definition

Steers with ≥ one visual BP sign (including depression, nasal and/or ocular discharge, cough or dyspnea), a rectal temperature ≥ 40 °C, abnormal lung sounds detected at auscultation, no prior treatment against BP or other diseases during the feeding period (*i.e.* 1st BP occurrence) and a serum haptoglobin concentration ≥ 0.25 g/L were defined as BP cases.

Steers with no visual sign of BP or other disease, a rectal temperature < 40 °C, abnormal lung sounds detected at auscultation, no prior treatment against BP or other diseases during the feeding period (*i.e.* 1st BP occurrence) and a serum haptoglobin concentration < 0.25 g/L were defined as control. Control steers that became sick within 30 days after enrolment were removed from the study.

2.5. Sampling procedures

Trans-tracheal aspirations were performed as described (Timsit et al., 2013). Briefly, an area of skin covering the ventral aspect of the middle third of the trachea was shaved and a surgical skin preparation was done. Local anaesthesia of the skin overlying the trachea was done by subcutaneous injection of 3 ml of lidocaine 2%. A catheter (75 cm long, OD 2 mm; Centracath, Vygon, Ecouen, France) was used. The trochar part of the catheter was passed between two tracheal rings, the flexible part was advanced ventrally ~40 cm, and 50 mL of sterile saline (0.9% NaCl) was introduced in the catheter using a 50 mL syringe. Immediately after injection, gentle suction was provided by withdrawing the plunger. On average, 5–10 mL of fluid were recovered, immediately placed into empty sterile tubes and transported in a container (temperature, 4 °C).

Deep nasal swabs were collected as described (Timsit et al., 2013) using long guarded swabs (27 cm) with a rayon bud (MW 124, Medical Wire & Equipment, Corsham, UK). One DNS were collected from each steer. Immediately after collection, DNS samples were placed into Amies transport media and transported in a container (temperature, 4 °C).

2.6. Sample processing and DNA extraction

Within 24 h after collection, samples were processed at the Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada. During this processing, rayon tips were removed from DNS and placed back into the Amies media, followed by centrifugation (13,000 × *g* for 5 min) to pellet the bacteria and tip. Afterwards, resulting pellets were stored at -80 °C pending DNA extraction. For TTA samples, 1–2 mL subsample of aspiration was centrifuged (2000 × *g*, 5 min) and the resulting pellet re-suspended in