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Non-inferiority of nitric oxide releasing intranasal spray compared to sub-therapeutic antibiotics to reduce incidence of undifferentiated fever and bovine respiratory disease complex in low to moderate risk beef cattle arriving at a commercial feedlot

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ABSTRACT

Undifferentiated fever, or bovine respiratory disease complex (BRDc), is a challenging multi-factorial health issue caused by viral/bacterial pathogens and stressors linked to the transport and mixing of cattle, negatively impacting the cattle feedlot industry. Common practice during processing at feedlots is administration of antibiotic metaphylaxis to reduce the incidence of BRDc. Nitric oxide (NO) is a naturally occurring nano-molecule with a wide range of physiological attributes. This study evaluated the metaphylactic use of intranasal NO releasing spray (NORS) to control BRDc incidence in calves at low-moderate risk of developing BRDc, arriving at a commercial feedlot as compared to conventional antibiotic metaphylaxis. One thousand and eighty crossbred, multiple-sourced, commingled, commercial, weaned beef calves were screened, enrolled, randomized and treated upon arrival. Animals appearing sick were pulled (from their pen) by blinded pen keepers then assessed for BRDc symptoms; blood samples were taken for haptoglobin quantification and the animals were rescued with an antibiotic. After 35 days both groups showed no significant difference in BRDc incidence (5.2% of animals from NORS group and 3.2% from antibiotic group). Average daily weight gain of animals at day 150 for the NORS cohort was 1.17 kg compared to 1.18 kg for the antibiotic group ($p > 0.05$). There was no significant difference in mortality in the first 35 days ($p = 0.7552$), however, general mortality over 150 days trended higher in the antibiotic cohort. NORS treatment was shown to be safe, causing neither distress nor adverse effects on the animals. This large randomized controlled study in low-moderate BRDc incidence risk calves demonstrates that NORS treatment, as compared to conventional metaphylactic antibiotics, is non-inferior based on BRDc incidence and other metrics like weight and mortality. These data justify further studies in higher BRDc incidence risk populations to evaluate NORS as an alternative strategy to reduce sub-therapeutic metaphylaxis antibiotic use in beef cattle production.

1. Introduction

Undifferentiated fever, also known as bovine respiratory disease complex (BRDc), is a significant challenge for cattle producers and feedlot managers (Jericho and Kozub, 2004; Wittum et al., 1996). The negative economic impact of BRDc on the cattle industry is twofold; namely, increased health costs for treating the condition and second, reduced growth performance seen in affected animals. Several viruses and bacteria have been associated with BRDc. The main bacterial pathogen of BRDc is *Mannheimia haemolytica*, which produces a potent leukotoxin as its principal virulence factor (Highlander et al., 2000). When cattle are infected with any one of several bovine respiratory viruses, such as bovine herpesvirus 1 (BHV-1) and bovine respiratory syncytial virus (BRSV), they become highly susceptible to *M. haemolytica* challenge (Hodgson et al., 2005). Far greater numbers of *M. haemolytica* cells are required to cause pneumonia in the absence of viral infection, even when the bacterial cells are inoculated into the bronchus (McClenahan

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and Czuprynski, 2008). These observations indicate that viral infection impairs host defense mechanisms against *M. haemolytica*, or amplifies undesirable aspects of the host response to this bacterial pathogen. Interestingly, by themselves, these pathogens are rarely capable of causing disease in healthy cattle. However, when combined with predisposing environmental stresses, such as; early weaning, multi-sourcing, transport and commingling, these pathogens compromise the immune system and facilitate BRDc infection (Griebel et al., 2005). Because of these factors, calves are most susceptible to developing BRDc during the first month after arrival at the feedlot.

The current practice of low-dose metaphylactic antibiotic administration to all calves as a prophylactic treatment against BRDc upon arrival at the feedlot is becoming more difficult, in large part, due to the emergence of resistant microorganisms and consumer concerns about residual antibiotics in the final product (Rerat et al., 2012). Resistance to antibiotics in cattle populations themselves has also been observed (CIPARS report, 2013; Zaheer et al., 2013). The macrolides, tilmicosin (Mycotil®) and tulathromycin (Draxxin®), are frequently administered subcutaneously to high-risk cattle, either prophylactically, metaphylactically, or therapeutically to cattle suffering from the disease (CIPARS report, 2013). A recent study found a significant increase in the proportion of erythromycin resistant enterococci following macrolide treatment regardless of administration method (orally or by injection) (Zaheer et al., 2013). The Canadian Public Health Agency also recommends that antibiotics should be "limited to treating infection and not long-term mass medication for growth promotion or guarding against disease." (Doering, 2014). In addition, a majority of consumers are demanding reduced antibiotics in meat and are willing to pay more (ConsumerReports, 2012; British Columbia Cattlemen's Association, 2012).

Given the potential for antibiotic resistance, increasing regulatory and legal pressures on sub-therapeutic antibiotic use, and the consumer demand for reduced antibiotic use in meat, alternatives to antibiotics for the treatment of BRDc are urgently needed. Efficacious non-antibiotic based antimicrobial treatments that can stop both the early progression of the infection and spread of disease in a herd is highly desirable. Potential treatments, if proven cost effective and simple to administer, would protect the health of cattle and create a new "antibiotic free" product, which would protect food chain sustainability by preventing respiratory disease in animals raised in concentrated, high through-put industrial feedlots.

Nitric oxide (NO) is a naturally occurring endogenously produced nano-molecule (nitrosylite) in most mammals, shown to possess antimicrobial and immunological properties (Moncada et al., 1991) that may have utility in the treatment of BRDc. NO is a primary signaling molecule in biological systems that, in low concentrations, can promote the growth and activity of immune cells, while at higher concentrations NO covalently binds DNA, proteins and lipids, thereby inhibiting or killing pathogens (Schairer et al., 2012). NO overwhelms microbial nitrosative detoxification mechanisms and thus can nitrosylate multiple biochemical proteins essential for microbial reproduction and survival (Miller et al., 2007). NO has antimicrobial activity against bacteria, yeast, fungi, and viruses both in vitro and in vivo animal studies (Ghaffari et al., 2005; Miller et al., 2007, 2009, 2013; Regev-Shoshani et al., 2013a,c; Rimmelzwaan et al., 1999; Weller et al., 2001). NO possesses simple pharmacokinetics and an extremely short half-life (seconds) without residual nitrites and methemoglobin in humans (Young et al., 1996). NO was shown to be safe to deliver to humans at 160 ppm (Miller et al., 2012). It binds to hemoglobin creating methemoglobin and within hours is reduced to nitrites and nitrates that are excreted in the urine. In a previous study, we have shown that there is no nitrite residue in meat and tissue of slaughtered animals treated with NO. We have also shown that nitrite levels in serum return to baseline 30 min post NO treatment (Regev-Shoshani et al., 2014).

NO is a known mucolytic and vasodilator, as well as a smooth-muscle relaxant, and is therefore an effective short acting bronchodilator (Zapol et al., 1994). As such, the bronchodilator effect of NO could contribute to the removal of secretions and airway quiescence maintenance; it has also been reported to modulate ciliary beat frequency in the airways (Jain et al., 1993). Further, NO is a known nitrosylating agent and has been shown to disrupt cysteine bonds by binding to the sulfur moieties decreasing mucous viscosity (Landino et al., 2007). Together, NO administered to the airways during pulmonary infections potentially thins secretions, restores the mucociliary apparatus, and augments removal of secretions. During infection, NO not only has the potential to improve oxygenation, but its direct vasodilatory effect (Borland and Higenbottam, 1989) can improve blood flow to the entire respiratory tract. This increased blood flow would bring nutrients and white blood cells while increasing the local temperature, which would all be beneficial for resolution of infection.

We have previously shown in a small efficacy study that NO reduces incidence of BRDc, and in other studies demonstrated its safety and bioavailability in beef calves (Regev-Shoshani et al., 2013b, 2014). In this large randomized and controlled study, we have used our previously described nitric oxide releasing intranasal spray (NORS) that releases NO gas and can be administered in less than 5 s (Regev-Shoshani et al., 2014). We evaluated the relative efficacy of this spray as compared to a conventional antibiotic in preventing BRDc in calves upon arrival at a feedlot.

2. Materials and methods

2.1. Animals and management

Commingled, multi-sourced, mixed-breed beef calves were purchased from auction markets for this study. All studies were conducted at Cattleland Feedyards Ltd., a 25,000 head, full service feedlot, located near Strathmore, Alberta, Canada, with all management practices adhering to the Canadian Council of Animal Care guidelines (Canadian Council on Animal Care, 1993) and Canadian Beef Cattle Code of Practice guidelines (Agriculture Canada, 1991). In addition, the research protocols were reviewed and an Experimental Studies Certificate issued by the Veterinary Drug Directorate of Health Canada. Animals identified for the study were approximately 6 months old with an estimated initial mean body weight between 180 and 290 kg. All animals were transported via commercial cattle liners to the feedlot within 10 days starting November 4, 2013. These calves were chosen in order to provide study groups with an anticipated high risk incidence rate of BRDc ranging between 30 and 40%, which is representative of the beef industry in Canada for these "assembled" herds of cattle typically acquired at auction marts. After a 24 h acclimatization period, the calves were screened, enrolled, randomized, processed, weighed, and temperature measured with blood samples taken by a veterinarian.

During the 45 day trial (35 days from the time the last animal entered the study), all animals were provided with a total mixed ration and permitted ad libitum feed consumption according to the standard operating procedure of the feedlot. The animals also had free access to water.

2.2. Screening

Animals were screened for enrolment criteria into the study. One thousand one hundred and nine animals were screened for enrolment and within 10 days, 1080 were enrolled in the study.

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