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Randomized, non-inferiority trial comparing a nitric oxide releasing solution with a macrolide antibiotic for control of bovine respiratory disease in beef feedlot calves at high-risk of developing respiratory tract disease

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ABSTRACT

Nitric oxide, a molecule produced in most mammalian cells, has bactericidal and virucidal properties. Nasal instillation of a nitric oxide releasing solution (NORS) on arrival at the feedlot was recently reported as non-inferior to a parenteral injection of a macrolide antibiotic, tilmicosin, for control of bovine respiratory disease (BRD) in cattle at low-to-moderate risk of developing BRD. The objective of this study was to evaluate whether NORS was non-inferior to tilmicosin for control of BRD in cattle at high-risk of developing BRD (the target population for many BRD control programs). High-risk Angus-cross heifers (n = 840) were randomly allocated to 2 treatment groups on arrival at a feedlot and received either NORS or tilmicosin for BRD control. Non-inferiority was assessed by calculating the difference in prevalence of heifers diagnosed with BRD during the first 40 d after arrival between NORS and tilmicosin treatment groups. The non-inferiority margin (δ) was set at 8.5%. Thirty-six and 19% of heifers were diagnosed with BRD in the NORS and tilmicosin groups, respectively. Because the lower bound of the 2-sided 95% confidence interval (CI) of the difference in BRD prevalence between the 2 treatment groups (17%; 95% CI = 11–23%) was higher than δ, an inferiority of NORS was concluded. Although on-arrival nasal administration of NORS can be viewed as a more rational control strategy than parental injection of antibiotics, further research is needed to improve NORS efficacy before it can be recommended to prevent BRD in high-risk cattle.

1. Introduction

Bovine respiratory disease (BRD) is one of the most important health problems in the beef feedlot industry (USDA, 2013). Despite substantial advances in antimicrobials and vaccines against bovine respiratory pathogens, BRD remains an important cause of morbidity (16.2%) and mortality (0.8%) in feedlot cattle, causing considerable economic losses and decreasing animal welfare (Miles, 2009; USDA, 2013).

Cattle are most likely to be affected by BRD during the first 40 d after entrance into the feedlot, as they are exposed to a wide range of pathogenic viruses and bacteria (as a result of co-mingling) at a time when various stressors suppress their immune system (Babcock et al., 2010; Taylor et al., 2010). Indeed, although BRD in feedlot cattle is ultimately a bacterial disease, it is a multifaceted problem, with host, management and environmental factors combining to predispose cattle to respiratory disease (Griffin et al., 2010; Taylor et al., 2010).

Presently, control of BRD in feedlots mainly relies on mass-medication (metaphylaxis) with injectable antibiotics given at or soon after arrival (Nickell and White, 2010). This control strategy aims at reducing the number of pathogenic bacteria in the cattle's nasopharynx and thus, at preventing bacterial proliferation (due to stressors) and potential inhalation into the lungs, which could lead to bacterial pneumonia (Duff and Galyean, 2007). Although highly effective to decrease BRD morbidity and mortality in feedlots (Nickell and White, 2010), metaphylaxis is increasingly regarded as an irrational use of antibiotics, as it is suspected to favor emergence of resistant bacteria, especially by exposure of gut microbiota to antibiotics (Lipsitch et al., 2002).

A promising alternative to injection of antibiotics for control of BRD in feedlot cattle is nasal instillation of a nitric oxide releasing solution (NORS; Bovinex, Bovicor Pharmatech Inc., BC, Canada) on-arrival at the feedlot (Regev-Shoshani et al., 2013). Nitric oxide, a molecule

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produced in most mammalian cells, has bactericidal and virucidal properties (Regev-Shoshani et al., 2014). Therefore, nasal installation of NORS could prevent BRD by reducing the number of pathogenic bacteria and viruses in the cattle's nasopharynx without affecting the gut microbiota (i.e., local action). In a recent study, NORS was non-inferior to an injectable macrolide antibiotic (Tilmicosin, Micotil, Elanco, Guelph, ON, Canada) for control of BRD in cattle at low-to-moderate risk of developing BRD (e.g. body weight >350 kg at feedlot entry, weaned at farm of origin and/or ranch-derived; Regev-Shoshani et al., 2015). In that study, 3.2 and 5.2% of cattle that received on-arrival tilmicosin or NORS, respectively, were subsequently treated for BRD during the first 50 d after arrival. These results were promising; however, further research is needed to investigate the efficacy of NORS to control BRD in cattle at high-risk of developing BRD (e.g. recently weaned, body weight <350 kg at feedlot entry, commingled and auction market-derived), the target population for many metaphylaxis programs (Nickell and White, 2010).

The objective of the current study was to evaluate whether NORS was non-inferior to a reference macrolide antibiotic (tilmicosin) for control of BRD in beef feedlot cattle at high-risk of developing BRD. Our hypothesis was that NORS is non-inferior to tilmicosin for control of BRD in high-risk cattle.

2. Materials and methods

This study was conducted at a commercial feedlot with a one-time capacity of 5000 cattle located near Beiseker, AB, Canada. This study was conducted in strict accordance with recommendations of the Canadian Council on Animal Care (Olfert et al., 1993). Furthermore, the research protocol was reviewed and approved by the University of Calgary Animal Care Committee (AC13-0104).

2.1. Study design: randomized, non-inferiority trial

This study was designed to demonstrate that NORS is at least as effective, within a predefined margin of non-inferiority (δ), as tilmicosin for prevention of BRD in feedlot cattle at high-risk of developing BRD. The null hypothesis (H_0) was that NORS is inferior to tilmicosin for prevention of BRD, whereas the alternative hypothesis (H_a) was that NORS is not inferior by more the predefined non-inferiority margin. The hypotheses were:

H_0: [P_{BRD} (NORS) – P_{BRD} (tilmicosin)] ≥ δ.

H_a: [P_{BRD} (NORS) – P_{BRD} (tilmicosin)] < δ.

where P_{BRD} is the prevalence of cattle diagnosed at least once for BRD during the first 40 d after arrival at the feedlot and δ is the non-inferiority margin.

2.2. Determination of the non-inferiority margin

The non-inferiority margin (δ) was determined using a 2-step approach (Freise et al., 2013). In a first step, the smallest reliable effect size (δ_0) of tilmicosin was determined based on previous superior studies having compared tilmicosin with no treatment (i.e., placebo) for prevention of BRD. A systematic review was performed in Pubmed (tilmicosin [MeSH] AND BRD [MeSH] AND prevention AND metaphylaxis) and 10 studies were included in a meta-analysis based on the following criteria: randomized trial, presence of a negative control, North American feedlots, cattle population at high-risk of developing BRD (recently weaned, body weight <350 kg at feedlot entry, commingled, auction market-derived), similar period of observation, and similar definition of a BRD case (i.e., visual sign of BRD and rectal temperature ≥40 °C) (Corbin et al., 2009; Guthrie et al., 2004; Kilgore et al., 2005; McClary et al., 2008; Morck et al., 1993; Schumann et al., 1990; Schumann et al., 1991; Vogel et al., 1998). Meta-analysis was performed using the package "meta" of R (R Foundation for Statistical Computing, Vienna, Austria) and δ_0 was defined as 17% (Fig. 1). This value corresponded to the lower bound of the 2-sided 95% confidence interval (CI) of the mean absolute difference (risk-difference) between tilmicosin and no treatment: 21%, 95% CI = 17–25% (Fig. 1). In a second step, δ was calculated to maintain at least half the smallest reliable effect of tilmicosin (δ_0), as suggested (Freise et al., 2013), i.e., $\delta = ½^*\delta_0$. Therefore, δ was defined as 8.5%.

2.3. Sample size calculation

The primary outcome used for *a priori* sample size calculation for this study was the prevalence of cattle diagnosed at least once with BRD during the first 40 d after arrival at the feedlot. Sample size calculation was based on a non-inferiority study design using StudySize 3.0 (Creostat HB, Frolunda, Sweden). A total of 385 heifers per group were estimated to be required to demonstrate non-inferiority, assuming $\alpha = 0.05$ and a power at 80%, δ set at 8.5% and a prevalence of heifers diagnosed at least once with BRD in the active control group (tilmicosin group) of 23% (95% CI: 17–32%). This prevalence of BRD in the active control group was calculated based on the meta-analysis conducted to determine δ_0 (data not shown).

2.4. Cattle, randomization and health data collection

Two successive groups (i.e., replicates) of Angus-cross heifers at high-risk of developing BRD (recently weaned, commingled and auction-market derived) were studied during the first 40 d after arrival at the feedlot. The first group of heifers was studied from October to December 2014, whereas the second group was studied from December 2014 to January 2015.

Upon arrival at the feedlot, heifers were allowed to rest for at least 12 h with *ad libitum* access to hay and water before processing. At processing, heifers were weighed, vaccinated against infectious bovine herpes virus-1, bovine viral diarrhea virus (Types I and II), bovine parainfluenza-3, bovine respiratory syncytial virus, *Mannheimia haemolytica* (Pyramid FP 5 + Presponse SQ, Boehringer Ingelheim, Burlington, ON, Canada), *Histophilus somni* and clostridial pathogens (Ultrabac 7/Somnubac, Zoetis, Kirkland, QC, Canada), dewormed with pour-on ivermectin solution (Bimectine, Bimeda-MTC, Cambridge, ON, Canada) and implanted with zeranol (Ralgro, Merck Animal Heath, Kirkland, QC, Canada). The rectal temperature of each heifer was also measured at processing and heifers with a rectal temperature ≥40 °C were excluded from the study. Heifers with a rectal temperature < 40 °C °C were enrolled in the study and randomly allocated to 2 treatment groups i.e., tilmicosin or NORS, using a randomization table. Heifers in the tilmicosin group received a subcutaneous injection of tilmicosin (Micotil, Elanco, Guelph, ON, Canada) at the labelled dose (10 mg per kg of body weight). Heifers in the NORS group received intranasally 32 mL of a NORS solution (Regev-Shoshani et al., 2015; Bovicor Pharmatec Inc., Vancouver, Canada) using a delivery device specifically designed by the manufacturer for the present study. This solution consisted of a nitrite strength of 60 mM that was previously tested to release 160 ppm NO in a 3 L/min flow of gas (Regev-Shoshani et al., 2014). After processing, heifers were allocated in large pens (200–220 cattle per pen) by treatment. They were fed twice daily, at 0630 and 1430 h, a 55–63% concentrate receiving/backgrounding diet formulated to meet or exceed (NRC, 2000) nutrient requirements. Each morning before feeding, bunks were visually evaluated and feed deliveries were adjusted to ensure that feed was offered for *ad libitum* consumption. On d 0 and d 40, heifers were individually weighed.

During the study period, heifers were observed daily by experienced pen checkers for detection of clinical illness; the pen checkers were not blinded to treatment. Heifers with visual BRD signs (e.g. depression, decreased rumen fill compared to pen mates, nasal or ocular discharge, cough, increased respiratory rate, or labored breathing) were removed from the pen and clinically examined by a veterinarian. Clinical examination included rectal temperature measurement, complete lung auscultation using a conventional stethoscope to detect abnormal lung