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Infrared detection and nitric oxide treatment of bovine respiratory disease

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ABSTRACT

Schaefer AL, Perry BJ, Cook NJ, Miller C, Church J, Tong AKW, Stenzler A *Infrared detection and nitric oxide treatment of bovine respiratory disease, Online Journal of Veterinary Research, 10 (1) : 7-16, 2006.* In a spontaneous induction model, 11 healthy calves were exposed for 3 days to 15 multiple sourced commercial calves carrying bovine respiratory disease (BRD) viruses (2 were uninfected controls). The calves received respiratory nitric oxide gas (NO) either immediately upon exposure to the BRD carriers or upon early identification of BRD from infrared orbital scans (IRT). A third group were treated with NO upon the appearance of clinical signs. All animals treated early with NO (preventative and early detection group) displayed IRT and clinical scores (36.2 C±0.2; 2.7±0.4) similar to controls (36.3 C ±0.2; 1.4±0.5) but lower than the clinical treatment group (37.0 C±0.2; 3.9±0.4, P <0.05). The data suggest infrared thermography is able to detect signs of bovine respiratory disease earlier than conventional clinical scoring systems: nitric oxide may be an effective adjuvant in treating bovine respiratory disease.

Key words: beef cattle, early disease detection, bovine respiratory disease, infrared thermography, nitric oxide

Short title: Detection and Treatment of Bovine Respiratory Disease

INTRODUCTION

Infectious diseases such as bovine respiratory disease (BRD) are known to have a significant economic impact on the cattle industry both with respect to treatment costs as well as the negative impact on animal performance and welfare ([Basarab et](#)

[al. 1997; Wittum et al. 1996](#)). Although the term BRD can refer to a host of complex diseases this term is generally used to refer to an animal displaying an undifferentiated fever as well as some number of clinical signs. As discussed by [Jericho and Kozub \(2004\)](#) the presence of BRD is common in intensively raised calves and the industry dependence on antibiotic treatment is high. Such management practices are becomingly increasingly scrutinized in many countries due to concern regarding antibiotic resistant microbes ([Flemming 1998; Jericho and Kozub 2004](#)). Even with cattle, the ability to treat (BRD) especially in multiple sourced and co-mingled animals is becoming more difficult. [Shahriar et al. \(2002\)](#) for example report the presence of antibiotic resistant pneumonia in feedlot cattle. As a result, a more targeted and selective use of antimicrobials in the animal industries is sought.

As discussed by [Cusack et al. \(2003\)](#) the effectiveness of treating BRD depends primarily on the early recognition and treatment. Unfortunately, traditional clinical signs of BRD are known to often occur late into the course of the disease. Recent research has demonstrated that under controlled laboratory conditions using a single source animal induction model under level 3 biocontainment conditions that the earlier identification of bovine respiratory disease onset has been possible using infrared thermography ([Schaefer et al. 2004a](#)). One of the objectives of the present study was to extend this work and investigate the use of infrared thermography in the early identification of spontaneously occurring BRD.

In terms of treating animals displaying symptoms of BRD, the industry standard practice has been to use one or more antibiotics for this purpose. In fact it is considered by some researchers to be difficult to even place and raise calves into feedlots without using antibiotics ([Jericho and Kozub 2004](#)). Again, this is a practice that is considered undesirable in some societies. The European Community's Scientific Steering Committee for example has recommended against the abundant use of antibiotics in the livestock industries ([Jericho and Kozub 2004](#)).

As an alternative to conventional antibiotics, the natural product nitric oxide may have utility in the treatment of BRD. Nitric oxide (NO) is a primary signalling molecule in biological systems and among other functions, demonstrates cytotoxic activity towards microorganisms ([De-Groote and Fang 1995](#)). In eukariotes, nitric oxide is known to be produced by macrophages as a primary defence mechanism against microbes and is empirically demonstrated to be toxic to a host of microbial organisms ([De-Groote and Fang 1995](#)). It is possible to deliver NO safely as a respiratory gas ([Miller 2003](#)) or topical antimicrobial agent ([Ghaffari et al. 2005](#)) as has been done in humans. A second objective of the present study thus was to test the efficacy of NO respiratory gas to provide targeted prevention or treatment of BRD.

MATERIALS AND METHODS

Animals and Management: A total of 28 crossbred weaned beef calves were used and 15 were sourced and co-mingled from an auction 8h distance from the Lacombe Research Centre, Agriculture and Agri-Food Canada. The animals had been exposed to viral and bacterial infections carrying high titers for respiratory viruses including BVD, PI3, IBR, Corona and BRSV (one titer was 1:1458 on a serum neutralization test for bovine viral diarrhoea). The calves served as a source of infective viruses within a spontaneous induction model. These 15 calves were weighed, monitored for

core and orbital thermal properties, blood sampled and placed onto conventional cereal grain silage. with access to shelter and clean water.

In addition to the aforementioned animals, 13 single source weaned calves were obtained from the BVD and IBR virus free herd at the Animals Diseases Research Institute at Lethbridge, Alberta. These calves were a British crossbred (Hereford X Angus) genetics and had been weaned approximately 1 week before transportation to Lacombe. The calves weighed on average 200 kg, were raised on native grass pasture and had been given a de-worming medication two weeks prior to weaning. The characteristics of these calves and the verification of their BVD/IBR free status has been referred to previously (Schaefer et al. 2004a). Prior to transport these 13 calves were monitored for infrared thermal properties as well as blood collections for verification of serology status.

The calves were transported on a conventional horse trailer which had been disinfected. On arrival at Lacombe Research Centre, a transport time of approximately 5 h, the calves were separated into pre determined random treatment groups as Control (n = 2), Preventative (n = 4), Early Detection (n = 4) and Clinical Detection (n = 3) designations. Specifically, a random number generation table was used to determine treatment designation for calves as they came off the cattle transport. The control calves were placed into their designated pen within a fully enclosed animal housing building. The building measured approximately 60 X 50 meters with a high ceiling (approximately 5 meters) and the calf pens approximately 3 X 3 meters. The building was open to natural ventilation as the large animal doors were closed but not sealed, however, barn temperatures were maintained at between 10-15 C using overhead infrared heaters.

The calves within the other three treatment groups were co-mingled with the 15 “infectious calves” for a period of approximately 52 h. All calves were observed to have periodic contact with each other by touching noses as well as sharing the same water bowl, salt lick, feed bunks and bedding. The treatment calves were then brought into designated pens within the contained housing building. All calves had access to a balanced maintenance ration consisting of smooth hay, a mineral salt block and clean water. The 13 BVD/IBR free calves were monitored daily for orbital infrared and clinical scores and every third day for blood samples for serology and haematology assessment.

Isolation and Containment Procedures: Although not designed or intended to be conducted as a bio-containment facility and trial it was nonetheless important to maintain isolation among the treatment groups of calves. Therefore, four pen systems for the four treatment groups were established within the larger housing facility. These pen systems were approximately 3-4 meters from each other. All pens had been steam cleaned and disinfected. For the control animals, separate feeding tools, clothing, gloves and boots were used by personnel to attend these calves and the animals had their own containment facility used for that pen only. Pens were cleaned daily with fresh straw used for bedding. All feeding, management and research procedures used for the calves met or exceeded code of practice standards for the Canadian Council of Animal Care (1993).

Technical Measurements: Infrared thermography scans for all animals were carried out with a FLIR S60 broadband camera using standard operating procedures for that camera (FLIR Systems Ltd., Burlington, ON). Orbital scans were hand

collected by trained and qualified infrared thermography technicians from a distance of approximately one meter. An orbital maximum value was collected on each animal at each scanning time and a mean of three values were used in subsequent statistical assessment. Camera data storage and retrieval systems were again done by conventional standard operating procedures outlined by FLIR Systems Ltd.

Serology samples were assessed for presence of common bovine respiratory virus including BVD(Bovine Viral Diarrhoea) , IBR (Infectious Bovine Rhinotracheitis), PI3 (Bovine influenza), bovine respiratory syncytial virus (BRSV) and corona by either serum neutralization and/or elisa methods (Prairie Diagnostics, Saskatoon, SK).

Clinical scores were assessed by a qualified veterinarian and respiratory technologist using the following procedures:

Clinical Scores:

Respiratory Insult: (0-5): 0 = no insult, normal breath sounds (NBS); 1 = Very Fine Crackle (rale) (VFCR) on auscultation and/or a moderate cough; 2 = Fine Crackle (subcrepitant) (FCR) on auscultation and/or a moderate nasal discharge and moderate cough; 3 = Medium Crackle (crepitant) (MCR) on auscultation and/or a moderate to severe viscous nasal discharge with cough; 4 = Course Crackles (CCR), tachypnea (> 15% of the norm) and/or a severe discharge with respiratory distress and obtunded lung sounds; 5 = CCR with dyspnea, tachypnea, marked respiratory distress and/or lung consolidation.

Digestive Insult: (0-5): 0 = no insult, normal, eating and drinking; 2 = mild or slight diarrhoea with slight dehydration (< 5%) and reduced eating; 3 = moderate diarrhoea with 10% dehydration and reduced feed intake (< 50%); 4 = moderate to severe diarrhoea with 10% or less of feed intake and more than 10% dehydration; 5 = severe diarrhoea and/or not eating, not drinking and dehydrated.

Temperature Score: Core Temperature (rectal) insult (0-5): 0 = normal temperature of 99-101; 1 = 101.1-102; 3 = 102.1 – 103; 4 = 103.1 – 103.9; 5 = $\geq 104^{\circ}$ F.

Provide Centigrade as well please

Disposition or Lethargy Score: (0-5): 0 = no lethargy, normal posture; 1 = mild anorexia or listlessness, depressed appearance; 2 = moderate lethargy and depression, slow to rise, anorectic; 3 = recumbent or abnormal posture, largely depressed; 4 = prostrate, recumbent or abnormal posture; 5 = death.

Nitric Oxide Treatment: Calves received a respiratory treatment of nitric oxide gas. The gas was administered from a 100,000 ppm source tank (Pulmonox Medical Comp. Edmonton, Alberta) using a breathing activated delivery system with an 8.27 mL pulse dose per breath. Each treatment consisted of 600 breaths (actually counted via the breath activation system) of 10,000 ppm NO via a nasal PVC “J” tube inserted 2-3 cm alternatively into each nostril (300 breaths per nostril). Treatment occurred on four consecutive days. The estimated daily dose was 160 ppm of NO.

The treatment required approximately 20 minutes to administer. In the case of the animals designated to the preventative group the NO was administered on days 1, 2 and 3 of exposure to the co-mingled induction calves plus one additional day post exposure to the calves. In the case of the early detection animals, NO was administered immediately upon the day of discovery of illness plus an additional three days. Illness criteria included any animals displaying an infrared temperature

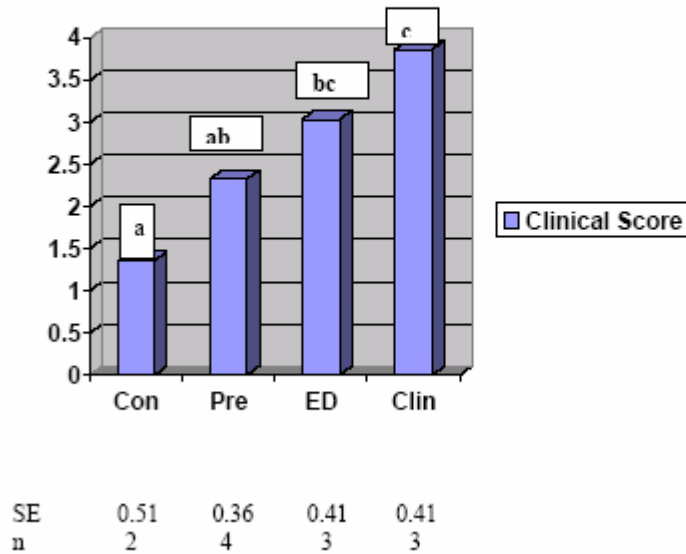
above a threshold level or had changed their temperature by a minimum of 10% per day. For the clinical detection calves, nitric oxide treatment was given the day of discovery plus an additional three days when the animals were deemed to be clinically ill displaying a clinical score above 2. The control animals received a sham oxygen administration via an identical nasal tube on four consecutive days.

Statistics: Group least squares means were statistically tested using a SAS mixed model (SAS Institute Inc. 1989). In the mixed model, treatments and day/treatment were fixed effects and animal identification nested within treatment was a random effect.

RESULTS AND DISCUSSION

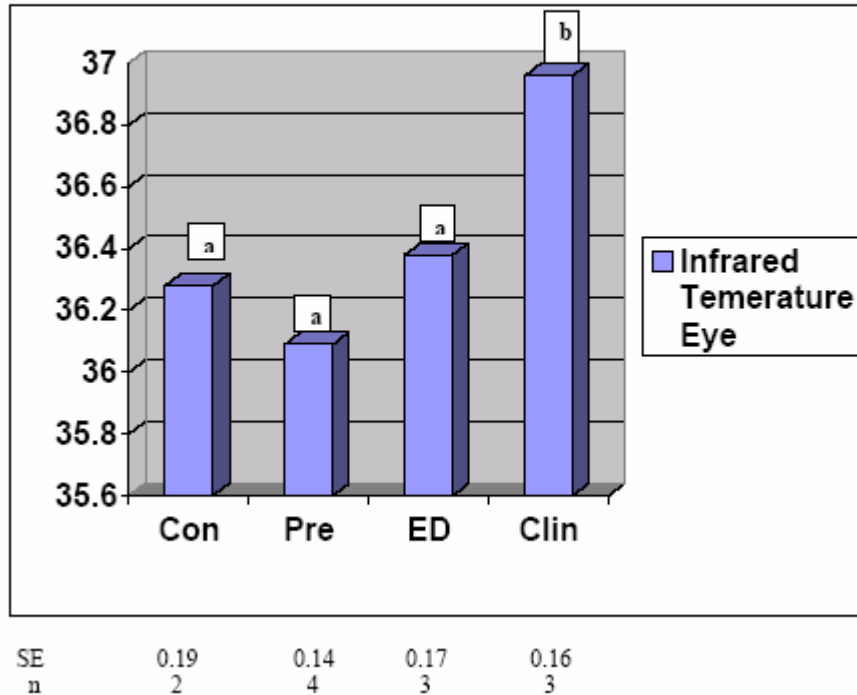
The group least squares mean data for clinical scores and orbital (eye) infrared temperatures are shown in **Figures 1** and **2** respectively.

Figure 1: Group means for clinical scores over ten days. a,b,c = means with different superscripts are statistically different at P<0.05. SE = Standard error. . Con = Control animals, Pre = Preventative treatment animals, ED = Early detection animals, Clin = Clinical detection animals.



One animal initially designated as an early detection treatment animal was removed from the data set as this animal displayed severe respiratory, clinical and thermal distress on initial testing post induction. In other words, although this animal was verifiably healthy when initially transported to Lacombe, the calf became ill during the three day induction period and was clearly ill by the start of the study or day one post induction. Hence, the animal had no opportunity to truly be early detected and would not meet the criteria for this treatment group.

Figure 2. Mean orbital (eye) infrared temperatures for treatment groups collected over ten days. a, b means with different letters are statistically different $P < 0.05$. SE = Standard Error. Con = Control animals, Pre = Preventative treatment animals, ED = Early detection animals, Clin = Clinical detection animals.



Please put in meaning of Con, Pre, ED, Clin here, also IRT Eye?

The lowest scores for health aberrations were seen in the control calves and the highest scores in the clinical group. On a given day the clinical scores demonstrated a lowest value of 0, typically seen in a control calf and a highest value of 10 seen in one of the clinical group animals. Of interest also was the observation that the 15 induction calves obtained from multiple sources and under commingled auction conditions displayed a high rate of clinical illness. Fifty-three percent (53%) of the 15 calves contracted BRD and required medical treatment with 15% of the animals requiring more than one medical treatment. These induction calves showed some of the highest levels of titter for BRD viruses tested (again as an example, a titter as high as 1:1458 for BVD based on a serum neutralization test) and also high clinical and orbital infrared scores. The control animals appeared to remain BRD free throughout the trial demonstrating low clinical and infrared scores throughout the period and also low titres for the BRD viruses tested for including BVD, IBR, Corona, PI3 and BRSV.

By contrast, all of calves within the preventative, early detection and clinical groups displayed higher titters for at least one of the four BRD viruses with BRSV being the most common virus detected. These calves also displayed higher values for clinical scores and infrared values with infrared temperatures in general showing a response in infected calves several days ahead of rectal temperature increases. Again, this is consistent with data reported from our lab that infrared is able to detect illness earlier even in animals displaying spontaneously occurring disease ([Schaefer et al. 2004b](#)). However, of interest was the observation that the calves receiving an earlier respiratory treatment of nitric oxide (Preventative and Early Detection groups) all demonstrated a reduced severity of illness.

Nitric oxide has been demonstrated to be cytotoxic for numerous viral and bacterial organisms ([De-Groote and Fang, 1995](#)). The current study is, to the author's knowledge, the first to demonstrate the ability of a respiratory application of nitric oxide gas to effectively treat bovine respiratory disease in cattle. All calves known to be exposed to BRD vectors and shown to display titers to several common BRD viruses displayed attenuation and earlier resolution of the symptoms following exposure to respired nitric oxide. The data further suggest that such treatment is most effective if applied preferentially as a prophylactic or preventative measure or at least upon the early discovery of BRD. The use of infrared thermography was shown to be effective as an early indicator of BRD in a spontaneously occurring model. Again, this observation is consistent with earlier findings using an induction model ([Schaefer et al. 2004a](#)) and with a further earlier report from our lab using a spontaneously occurring model ([Schaefer et al. 2004b](#)). The use of nitric oxide gas to treat BRD may be a viable adjuvant to the current industry practice of antibiotic application. An exploration of treatment efficacy by combined use of nitric oxide with vaccines and/or anti-biotics would be seemingly merited. Furthermore the efficacy of such treatments may be improved if applied either upon the early discovery of BRD or used as a preventative treatment.

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