

Nitric Oxide Gas is an Effective Anti-Viral Agent in a Bovine Inoculation Model

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BACKGROUND

We have previously reported that *in vitro* exposure to 160 ppm nitric oxide gas (NO), was 100% cytotoxic to a broad range of microbes including gram positive, gram negative and multi drug resistant strains of bacteria, yeast and mycobacteria. Safety of this dose was demonstrated with a wide variety of host cell lines. Bovine Respiratory Disease (BRD) has a negative economic impact on the cattle industry. Further, there is dependence on prophylactic antibiotic use with resistant microbes emerging. We hypothesized that inhaled NO would be effective against BRD related viruses.

METHOD

A natural inoculation pilot study using a Bovine Respiratory Virus (BRV) model was undertaken. Thirteen uninfected naïve calves were exposed for 52 hrs to a commercial BRV positive induction herd. Calves were then pre-randomized into one of four treatment groups as follows; 1) Prophylactic (n=4); with NO treatment started immediately after herd exposure, 2) Early Detection (n=4); with treatment starting upon thermal infrared (IRT) detected signs of infection (Fig.2), 3) Clinical detection (n=3); treatment initiated only upon evidence of industry standard clinical signs for BRD and 4) Controls (n=2); naïve calves were isolated and served as uninfected controls receiving a daily air placebo treatment. Once initiated, a single NO treatment was given each day for 4 consecutive days. Inhaled NO (160 ppm) was administered during the inspiratory phase via a nasal J-tube into each nares (Fig.1) for 600 breaths (approx. duration 20 minutes). The presence of BRD related viruses were verified by relevant clinical signs. All calves had similar temperatures and clinical scores upon entry.



Fig. 1: NO delivery via J-tube during inspiration

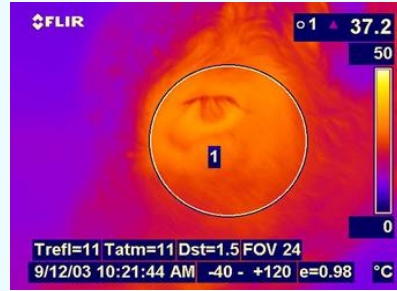


Fig. 2: Orbital infrared thermography (IRT) indicating Early Detection of viral infection in BRD

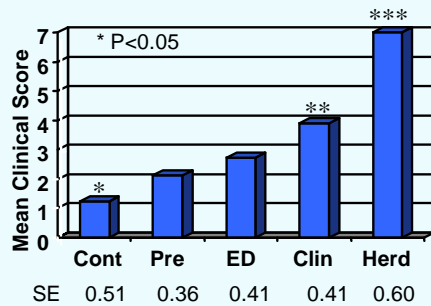


Fig. 3: NO effect on 10 day mean clinical infection score (industry standard signs)

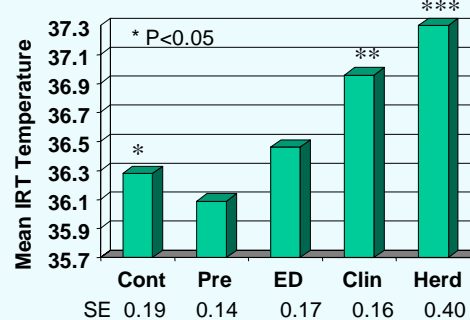


Fig. 4: NO effect on 10 day mean IRT Temperatures

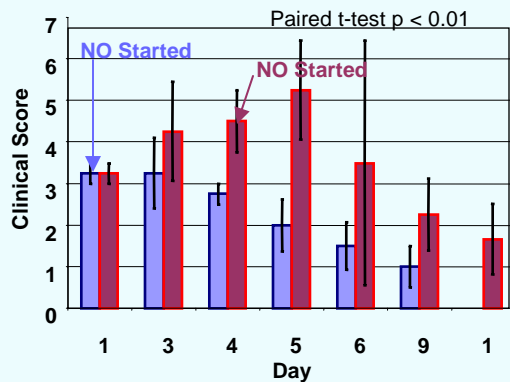


Fig. 5: Effect of NO given Prophylactically (blue) or at the time of IRT Early Detection (red) on clinical Infection score (industry standard signs)

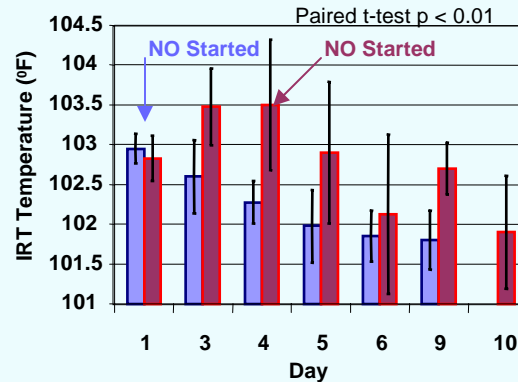


Fig. 6: Effect of NO given Prophylactically (blue) or at the time of IRT Early Detection (red) on IRT Temperatures

RESULTS

NO treated groups and the induction herd had positive serology for Para-influenza virus, Bovine Coronavirus, Respiratory Syncytial Virus, Bovine Virus Diarrhea and Infectious Bovine Rhinotracheitis. Prophylactic NO treatment was effective in preventing infection (Fig.3-6). After 6 months, none of the NO treated animals demonstrated infection regardless of NO treatment timing. Whereas, the induction herd became ill and 13/15 required antibiotic treatments. Of these, 8/15 were treated directly for severe respiratory infections. When NO was given at the time of Early Detection (IRT) or at the point of Clinical Detection, the calves' temperatures and clinical scores rapidly resolved. The induction herd had a clinical score of 7.1(0.6) and also exhibited aberrant lab values. Paired t-test differences were highly significant (p<0.01) with similar scoring patterns. NO of 160 ppm was delivered safely without incident. An acute rise in NO metabolites resulted during NO delivery as compared to naïve calves confirming NO kinetics. However, this was not statistically nor clinically significant when compared to the induction herd.

CONCLUSION

- Inhaled NO is an effective treatment for Bovine Influenza, SARS, Respiratory Syncytial and other viruses in this BRD population.
- Early use of inhaled NO reduces clinical symptoms.
- Inhaled NO of 160 ppm is safe and well tolerated.
- Studies in humans are warranted to determine the effectiveness of NO for antiviral use.

REFERENCES

1. BRD accounts for 65-77% morbidity 44-72% mortality with high economic impact in beef industry (Quimby et al. Can. Anim. Sci.2001; 81: 315-320)
2. Industry dependence on antibiotics (Jerico and Kozub. Can. J. Vet Research 2004; 68:118)
3. Gaseous nitric oxide acts as a non-specific antimicrobial (McMullin et al. Respiratory Care 2005; November. Ghaffari et al. Nitric Oxide Biol Chem 2005; in press)
4. Nitric oxide is an anti-viral agent (Rimmelzwaan et al. J Virol 1999; 73:8880)
5. Early detection of BRD with Infrared orbital thermography (Schaefer et al. Can. J. An. Sci. 2004; 84:73)