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THE RELATIONSHIP BETWEEN 3-METHYLINDOLE, BOVINE RESPIRATORY SYNCPYTIAL VIRUS, AND BOVINE RESPIRATORY DISEASE IN FEEDLOT CATTLE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the
Graduate School of The Ohio State University

By

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ABSTRACT

This research investigated the relationship between 3-methylindole (3MI, skatole), bovine respiratory disease (BRD, shipping fever, pneumonia), and bovine respiratory syncytial virus (BRSV) in feedlot cattle. The abrogation of the pneumotoxic effects of 3MI by acetylsalicylic acid (aspirin), d-α-tocopherol (vitamin E), and humoral immunity (both naturally acquired and induced by both inactivated and modified-live BRSV vaccines) in feedlot cattle was also investigated. Four separate studies were performed.

The first study was designed to evaluate the potential synergy between BRSV and 3-MI in inducing respiratory disease in cattle. Twenty mixed-breed beef calves were randomly assigned in a 2 X 2 factorial study design to the following 4 challenge exposure groups: unchallenged control, BRSV challenge exposure, 3-MI challenge exposure, and combined BRSV-3MI challenge exposure. Clinical respiratory disease was more acute and severe in the BRSV-3MI challenge exposure group. All 5 cattle in this group and 3 of 5 cattle treated with 3MI alone died or were euthanatized prior to termination of the experiment. Mean lung displacement volume was greatest in the BRSV-3MI challenge exposure group. Gross and histologic examination showed that pulmonary pathology was also most severe in this group. Feedlot cattle are commonly infected with BRSV, and 3MI is produced by microflora in the rumen of all cattle. Our results suggest that there is
synergy between BRSV and 3MI. Thus, controlling combined exposure may be important in preventing respiratory disease in feedlot cattle.

The purpose of the second experiment was to determine if d-alpha-tocopherol (vitamin E) and acetylsalicylic acid (aspirin), either alone or in combination, can mitigate the pneumotoxic effects of 3-methylindole in cattle. Twenty mixed-breed beef calves were randomly assigned within gender in a 2 X 2 factorial study design to the following 4 treatment groups: untreated control, vitamin E treatment, aspirin treatment, or combined aspirin-vitamin E treatment. Calves were pre-treated with vitamin E and aspirin. All calves were challenged with 3MI. Aspirin and vitamin E did not abrogate the pneumotoxic effects of 3MI in these calves. However, the results suggest potential toward protection in the group treated with both aspirin and vitamin E. This treatment group had lower mortality, delayed onset of depression, lower lung weight and volume, and lower gross and histologic pulmonary lesion scores compared to all other treatment groups, while comparisons of these outcomes were similar among the control, aspirin treatment, and vitamin E treatment groups. Subclinical lung damage caused by 3MI metabolism may contribute to the susceptibility of the bovine lung to common respiratory pathogens. Inhibition of 3MI metabolism by aspirin and vitamin E at feedlot entry may be possible because serum 3MI concentrations under natural conditions are much lower than those observed in this experiment.

The purpose of the third study was to evaluate the ability of oral aspirin administered to cattle upon feedlot arrival to mitigate 3MI induced respiratory disease and reduced rate of gain. Two hundred forty-four beef cattle were systematically randomized
to either receive a single oral dose of aspirin (31.2 grams) upon entry into a feedlot or to serve as an untreated control. Serum 3MI concentrations were measured on day 0, 3, and 6 after feedlot entry. Rumen 3MI concentration was also measured on day 3. Mean daily gain (MDG) in cattle treated with aspirin compared to control cattle was 0.06 kg higher in the backgrounding unit and 0.03 kg higher for the overall feeding period. However, serum or rumen 3MI concentrations did not appear to modify this effect. This may have been influenced by relatively low peak 3MI production in these cattle and by relatively slow rates of gain. Cattle in the aspirin treatment group were more likely to be treated for respiratory disease compared to cattle in the control group. Mortality rates, chronic gross pulmonary lesions, and serum and rumen 3MI concentrations were similar between groups. Increased rumen 3MI concentration was associated with a small difference in the risk of post mortem lung fibrosis.

The purpose of the fourth study was to evaluate the ability of immunity against BRSV to mitigate the effects of 3MI on respiratory disease and rate of gain in feedlot cattle. Two hundred fifty-four mixed breed beef cattle entering a feedlot were systematically randomized to one of three groups: unvaccinated control, inactivated BRSV vaccination, or modified-live BRSV vaccination. Increasing serum 3MI concentrations early in the feeding period were associated with lower mean daily gain for the feeding period and increased risk for clinical respiratory disease after 3 days on feed. Cattle with lower BRSV antibody titers on arrival had higher risk of treatment for respiratory disease. Cattle in the control group were more likely to be treated for respiratory disease after 3 days on feed compared to cattle in the modified-live BRSV
vaccine group. Humoral immunity against BRSV did not appear to modify the effect of 3-methylindole on clinical respiratory disease or mean daily gain outcomes. Our results suggest that abrogating the effects of 3-methylindole and BRSV infection may improve the health and growth performance in cattle entering feedlots. However, immunity against BRSV as measured in this study, did not appear to protect against potential synergism between 3MI and BRSV in these cattle. This may have been affected by the relatively slow rates of gain of cattle included in this study or timing of sampling.
DEDICATION

Dedicated to my mother, Joyce N. Bingham, who constantly told me "you can do anything you set your mind to" while I was growing up and my father, Carl W. Bingham, who's lifetime of hard work in the agricultural industry was an inspiration and example which taught me to set goals and work hard.
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CHAPTER 1

INTRODUCTION

Bovine respiratory disease (BRD) is an economically important disease to cattle producers.\(^1\) Death losses attributed to BRD were valued at an estimated $485 million dollars in 1996.\(^2\) Bovine respiratory disease has been intensively researched over the last 30 years. Information from this research has lead scientists and veterinarians to conclude that BRD is a multi-factorial disease complex.\(^3\) Initially, BRD research was largely focused on individual infectious pathogens and as a result, many infectious pathogens have been associated with outbreaks of BRD in feedlot cattle.\(^3\) Recently, interactions between the known infectious causes of BRD have been explored.\(^4-7\) However, interactions between known infectious and non-infectious causes of respiratory disease in cattle are largely uninvestigated.

This research investigated the relationship between 3-methylindole (3MI), bovine respiratory syncytial virus (BRSV), and BRD in feedlot cattle. The abrogation of the pneumotoxic effects of 3MI by acetylsalicylic acid (aspirin), \(d-\alpha\)-tocopherol (vitamin E), and humoral immunity (both naturally acquired and induced by both inactivated and modified-live BRSV vaccines) in feedlot cattle was also investigated.

3-Methylindole is recognized as a pneumotoxin in cattle, sheep, goats, mice, and man.\(^8-11\) 3-Methylindole pneumotoxicosis in cattle has been associated with abrupt
dietary changes. Abruptly changing the diet from poor quality forages to higher quality forages increases intraruminal 3MI production by rumen Lactobacilli, which may cause clinical acute bovine emphysema and edema (ABPE, fog fever). Clinical signs attributable to the respiratory tract predominate in cases of ABPE. During post mortem examination, lung lobes do not collapse due to severe interstitial edema and emphysema. Cranioventral lung consolidation and congestion are also commonly observed. Histologic lesions found in the lung include type II pneumocyte hyperplasia, interstitial edema and emphysema, and hyaline membrane formation.

Bovine respiratory syncytial virus has been demonstrated to be an important cause of BRD in cattle. Exposure to BRSV is common based on seroepidemiologic studies. However, outbreaks of clinical disease attributable to BRSV are less common. Clinical respiratory disease caused by BRSV infection may occur in both immature and adult cattle. Clinical signs attributable to the respiratory tract predominate. Morbidity and mortality in outbreaks of BRSV pneumonia can range from 60 - 80% and 0 - 20% respectively. Post mortem lung lesions may include uncollapsed and grossly distended lungs, cranioventral consolidation, caudodorsal interstitial emphysema and edema, and emphysematous bullae. Interstitial emphysema, alveolar edema, lymphocytic bronchiolitis, type II pneumocyte hyperplasia, and hyaline membrane formation may be observed histologically. Despite many years of basic research, the pathogenesis of severe pulmonary emphysema and edema in some cattle with BRSV induced respiratory disease remains unclear.
Feedlot cattle are commonly moved from poor quality, late summer and fall pastures to public cattle auctions and are eventually transported to feedlots. During this transition, they are co-mingled with cattle from many different sources. Co-mingled cattle in public cattle auctions and feedlots are typically exposed to a wide variety of respiratory pathogens including BRSV. Cattle undergo an abrupt dietary change during this time period as well, changing from relatively poor quality forages of late summer pasture to higher quality forages available in feedlot rations. The incidence of respiratory disease in feedlot cattle is highest during the first 14-21 days following entry. Clinical signs and post mortem lung lesions reported in cattle with respiratory disease attributable to BRSV and 3MI are similar. It is possible that synergism between BRSV and 3MI may account for a proportion of respiratory disease observed in cattle entering feedlots and that prevention of pulmonary damage by one or both agents may improve the health and productivity of feedlot cattle.

The following chapters contain a review of the pertinent literature necessary to understand the justification of the following research and to aid in the interpretation of the findings. Chapter 3 will present the results and interpretation of an experimental challenge exposure of cattle to both 3MI and BRSV and examine the data for evidence of synergism between these two agents. Subsequent chapters expand on this initial experiment by examining the association between 3MI and decreased respiratory health and growth performance in feedlot cattle. The effectiveness of aspirin, vitamin E, or humoral immunity against BRSV to abrogate the potential negative effects of 3MI in feedlot cattle are also investigated.
1.1 References


2.1 Introduction

This chapter will review the pertinent literature for 3-methylindole (3MI) and bovine respiratory syncytial virus (BRSV) which is necessary to understand the justification of the following research and conclusions. The pertinent literature of 3MI and BRSV will be separately reviewed. The science surrounding the discovery of 3MI production in cattle will be reviewed to establish the scientific basis that 3MI is an important pneumotoxin which is related to certain dietary and management conditions which occur in cattle production. The epidemiology, clinical signs, and lung lesions reported in 3MI and BRSV induced respiratory disease will also be included in this review. This will help to identify some of the similarities between 3MI and BRSV. It will also provide some vital background information which is necessary to understand our motive for exploring a potential synergistic relationship between them in feedlot cattle.
2.2 3-Methylindole

2.2.1 History

Acute bovine pulmonary edema and emphysema (ABPE) or fog fever was first reported in 1819 by Knowlson in Great Britain.¹ In North America, it was first reported by Schofield in 1924 and in Montana by W.J. Butler in 1940.² Although ABPE has been recognized for at least 200 years in Europe,³ the experiment that eventually lead to the discovery that 3MI was the causative pneumotoxin of this syndrome came about by accident combined with an astute observation by Dr. G.R. Spencer, a pathologist at Washington State University. In the mid 1960s, animal scientists at Washington State University were performing research on the metabolism of tryptophan in cattle and goats. After dosing cattle with oral tryptophan (0.57 g/kg (0.26 g/lb) body weight (BW)), they reported that 5 of the 8 adult cattle in the study died from pulmonary emphysema one to seven days after the challenge.⁴ Not fully recognizing the significance of their discovery, they thanked Dr. Spencer for the diagnosis of what they referred to as "an anomaly."⁵ The following year Dickinson, Spencer, and Gorham published the first purposeful experimental induction of ABPE in cattle. They challenged adult cattle with oral d,l-tryptophan (0.5 - 0.6 g/kg (0.23 - 0.27 g/lb) BW) and produced clinical signs and lung lesions consistent with a diagnosis of ABPE.⁶ In 1968, Carlson, Dyer, and Johnson extended these findings by challenging mature cattle with d,l-tryptophan orally, intravenously, and by intraperitoneal injection. They reported that cattle in all groups had similar serum concentrations of tryptophan but only cattle given d,l-tryptophan orally developed clinical signs of respiratory disease.⁷ They concluded that ABPE must be
caused by product of D,L-tryptophan metabolism in the rumen. In 1972, Carlson and co-workers reported a series of experiments demonstrating that 3MI was the pneumotoxin causing ABPE in cattle. First, they experimentally challenged steers with oral D-tryptophan or L-tryptophan and observed that only cattle challenged with the L isomer developed clinical signs of respiratory disease. Second, they incubated L-tryptophan in strained rumen fluid under anaerobic conditions and identified 3MI as one of the metabolic end products. Finally, they challenged cattle and goats orally and intravenously with 3MI. Cattle challenged with 0.2 g/kg (0.09 g/lb) 3MI orally developed clinical signs of disease and all died within 73 hours after dosing. Cattle challenged with 0.1 g/kg (0.05 g/lb) 3MI orally developed signs of respiratory disease but did not die from respiratory disease. Cattle given a 2 hour intravenous infusion of 3MI receiving a total dose of 0.06 g/kg (0.03 g/lb) developed signs of respiratory disease and 1 of 3 cattle died 56 hours after challenge. This series of experiments demonstrated that it was only L-tryptophan that was metabolized to 3MI and that 3MI, administered either orally or intravenously, could cause ABPE in cattle and goats. They also demonstrated that the effect of 3MI in cattle was dose dependent. An oral dose of 0.2 g/kg (0.09 g/lb) resulted in 100% mortality whereas an oral dose of 0.1 g/kg (0.05 g/lb) only caused acute respiratory distress.

The metabolic pathway of L-tryptophan conversion to 3MI was further defined in 1974 when Yokoyama and Carlson reported that it was a 2 step process. Their research demonstrated that L-tryptophan was converted to indoleacetate and that the major pathway for 3MI formation was decarboxylation of indoleacetate to 3MI. They also
reported that the antimicrobials kanamycin and neomycin could effectively reduce
decarboxylation of indoleacetate to 3MI. This finding helped further research which
lead to the identification of specific rumen microbes that were capable of decarboxylating
indoleacetate to 3MI and the inhibition of l-tryptophan metabolism by rumen microbes
through the use of polyether antimicrobial drugs.

After experimental intraruminal administration of l-tryptophan (0.35 g/kg (0.16
g/lb) BW), 3MI has been detected in the ruminal fluid and plasma of cattle within 6 hours
of administration. 3-Methylindole concentrations peaked at 3.0-9.0 μg/ml in the plasma
within 12 to 24 hours after administration. Three of 5 cows challenged with l-
tryptophan in this experiment developed clinical signs of ABPE. The authors concluded
that 3MI, from rumen fermentation of l-tryptophan, causes ABPE in cattle, and that
maximal concentration and duration of plasma 3MI may predict the severity of
pulmonary damage. Another observation from this study was that the rise in rumen 3MI
concentrations actually lagged behind the rise in serum 3MI concentrations. The
significance of this observation is unknown.

In 1975, Carlson and coworkers experimentally challenged cattle with
intraruminal and intravenous 3MI. Mean plasma 3MI concentrations (18.5 μg/ml)
peaked 3 hours after challenge and then gradually decreased to baseline levels
approximately 48 hours after cattle were challenged with 0.2 g/kg (0.09 g/lb) 3MI
orally. Mean plasma 3MI concentrations (16.8 μg/ml) peaked 3 hours after challenge
and then gradually decreased to baseline levels 36 hours after cattle were challenged with
0.1 g/kg (0.05 g/lb) 3MI orally. Mean plasma 3MI concentrations (10.7 μg/ml) peaked
9 hours after challenge and then gradually decreased to baseline levels 18 hours after cattle were challenged with 0.06 g/kg (0.03 g/kg) 3MI intravenously.\textsuperscript{10} Goats challenged with similar doses of 3MI exhibited similar plasma 3MI concentrations, clinical signs and pathologic lung lesions at necropsy compared to those reported in cattle.\textsuperscript{11} Sheep, horses, rabbits, rats, and mice have also been reported to be susceptible in varying degrees to the pneumotoxic effects of 3MI.\textsuperscript{12-14}

*In vitro* and experimental challenge studies had implicated 3MI as the cause of ABPE, but the presence of 3MI had not been demonstrated in naturally occurring outbreaks of ABPE. Plasma and rumen fluid 3MI concentrations were therefore measured in 19 mature beef cattle abruptly moved from poor quality pasture to lush green pastures.\textsuperscript{15} One cow developed severe clinical signs and 6 more developed moderate clinical signs of ABPE 102 hours after introduction onto the better quality pasture.\textsuperscript{15} Mean rumen 3MI concentrations (1.8 $\mu$g/ml) peaked at 78 hours and plasma 3MI concentrations ranged from 0.07 to 0.12 $\mu$g/ml over the 174 hour collection period.\textsuperscript{15} Other researchers have reported rumen 3MI concentrations of 3.5 - 9.5 $\mu$g/ml following a natural challenge where cattle were abruptly moved onto lush grass pasture.\textsuperscript{16} Hammond and co-workers reported that plasma 3MI concentrations do not correlate well with serum 3MI concentrations under conditions naturally conducive to developing ABPE and that the induction of clinical ABPE may be related to the duration of rumen 3MI concentration as well as the peak concentration.\textsuperscript{15} Cattle that developed clinical ABPE had mean rumen 3MI concentrations $\geq$ 2.0 $\mu$g/ml for an average of 42 hours, and cattle that did not show clinical signs of ABPE had mean rumen 3MI concentrations of $\geq$ 2.0
\( \mu g/ml \) for an average of 14 hours.\(^{15}\) The same author concluded that plasma 3MI concentrations were correlated to rumen 3MI concentrations (but at lower levels) after cattle were challenged with \( L \)-tryptophan orally.\(^{17}\)

Most experimental challenge studies with 3MI were performed using mature cattle, goats, or sheep until 1979. In 1979, researchers experimentally challenged young calves (3 month old Holsteins) with 0.25 g/kg (0.11 g/lb) BW 3MI orally which caused mild clinical signs and lung lesions in these calves.\(^{18}\) They concluded that there may be age related differences in the metabolism of 3MI in cattle and that young cattle may not be as susceptible to the pneumotoxic effects of 3MI when compared to mature cattle.\(^{18}\) Several studies which followed reported that after repeated oral dosing of young calves with 0.1 g/kg (0.05 g/lb) 3MI once weekly that a proportion of these calves developed clinical signs of ABPE following every challenge, but all other calves become increasingly resistant to the pneumotoxic effects of 3MI.\(^{19,20}\) This suggests that immature cattle have differing susceptibilities to 3MI toxicity. Lekeux et al suggested that some of the calf susceptibility differences may be attributable to diet (milk versus hay and concentrate fed calves).\(^{20}\)

2.2.2 Chemistry

3-Methylindole is a nonpolar, lipophilic compound,\(^{14,21}\) produced by rumen fermentation of \( L \)-tryptophan.\(^{7,8,22,23}\) \( L \)-Tryptophan is deaminated to indoleacetic acid (IAA) by several species of rumen microorganisms.\(^{9,22}\) Rumen \( Lactobacillus \) spp. decarboxylate IAA to form 3MI.\(^{22}\)
3-Methylindole is a volatile compound that is rapidly absorbed from the rumen into the systemic circulation. Type I pneumocytes and clara cells in the lung are most susceptible to damage by 3MI metabolism. Within these cells, 3MI is metabolized in the smooth endoplasmic reticulum (SER) by enzymes in the cytochrome P450 family of mixed function oxidases (MFO) and by prostaglandin H synthase (PHS). Mixed function oxidase enzyme inhibition studies have demonstrated that MFO's are involved in 3MI metabolism in goats. In one study which demonstrated the involvement of the MFO system in the metabolism of 3MI, goats were pretreated with either a MFO inhibitor (piperonyl butoxide) or a MFO inducer (phenobarbital) and subsequently challenged with intravenous [14C] labeled 3MI. Clinical signs of respiratory disease were not observed and lung lesions were minimal in goats that were pretreated with piperonyl butoxide compared to goats in the control group. Clinical signs and gross and histologic lung lesions were severe in goats pretreated with phenobarbital compared to control goats. In vitro studies have reported that cytochrome P450 enzymes are the MFO enzymes involved in the metabolism of 3MI.

The pneumotoxic effects of 3MI in the lungs cannot be completely explained by the MFO metabolic pathway. Prostaglandin H Synthetase (PHS) is also present in the SER and may enhance the development of pulmonary cytotoxicity by MFO induced toxins. High concentrations of PHS are found in type II pneumocytes and clara cells. Prostaglandin H synthetase is an enzyme involved in arachidonic acid metabolism, and is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs). Ram seminal vesicles, which contain high concentrations of PHS and low concentrations of
MFO, were used to examine the potential role of PHS in 3MI metabolism. Formosa and Bray reported that 3MI was co-oxidized by PHS and that this effect could be 98% inhibited by the addition of indomethacin (an NSAID). Acton and co-workers reported successful mitigation of 3MI induced pneumotoxicity in goats experimentally challenged with 0.1g/kg oral 3MI after pre-treatment with aspirin or indomethacin (both NSAIDs). Pulmonary lesions and clinical signs were less severe in goats pretreated with aspirin or indomethacin versus controls or goats given aspirin after 3MI administration. They concluded that "the protective effect of inhibitors when administered before, but not after, 3MI dosing suggests it is the inhibition of PHS activity in activation of 3MI, not in production of prostanoids which prevented the disease process". Therefore, metabolism of 3MI by PHS was mitigated by NSAIDs similar to the way piperonyl butoxide inhibited 3MI metabolism by cytochrome P-450 MFO enzymes. Breeze and co-workers reported that 3MI mediated toxicity was not the initial effect of 3MI on cell membranes. The initial step in 3MI toxicity is the enzymatic activation of 3MI to reactive, electrophilic intermediate molecules that bind to cell macromolecules and initiate cellular toxicity. The formation of N-centered free radicles followed by the formation of C-centered free radicles have been suggested as possible intermediate products that initiate the toxic oxidative cascade. In vitro studies have demonstrated that free radicles can be formed through metabolism of 3MI in MFO and PHS systems. N-centered free radicles may undergo molecular internal rearrangement to form a methylene imine electrophilic species that has been demonstrated as the next molecule formed in the toxic cascade of 3MI metabolites. 3-Methyleneindolenine, 2,3-epoxy-3-methylindoline, and 3-
hydroxy-3-methylindolenine have all been identified in *in vitro* MFO systems with goat lung microsomes as reactive intermediates in the metabolism of 3MI.\textsuperscript{28,40,41} These reactive imines may damage the cell by binding to cellular proteins or DNA forming adducts.\textsuperscript{14} It is unlikely that cellular damage occurs by lipid cell membrane peroxidation because the addition of 3MI to goat lung microsomes *in vitro* inhibited lipid peroxidation and appeared to act as an antioxidant.\textsuperscript{42}

3-Methylindole induced toxicity in goats was reduced when tissue levels of glutathione were increased, suggesting that tissue glutathione levels are important in the chemical pathway for detoxification of reactive 3MI intermediate molecules.\textsuperscript{43,44} Goats pretreated with diethyl maleate (glutathione inhibitor) and subsequently challenged with 3MI had more severe respiratory disease when compared to control goats.\textsuperscript{43,44}

Toxicity that results from 3MI metabolism is cell and organ specific in ruminants. Kaster and Yost reported that the organ specific toxicity in goats in order of most toxic to least toxic was lung > kidney > liver.\textsuperscript{45}

After detoxification, the majority of 3MI metabolites are eliminated in the urine. Eighty-seven to 92% of \[^{14}C\]3MI was excreted in the urine of goats infused intravenously.\textsuperscript{46} A small amount of the radioactivity (0.4 - 0.9%) was in expired air and goats had a negligible amount of radioactivity in the feces.\textsuperscript{46} Derivatives of 3-methyloxindole (68%) and indole-3-carboxylic acid (13%) accounted for the majority of radioactive 3MI metabolites excreted in the urine.\textsuperscript{46} Recently, 3-hydroxy-3-methyloxindole has also been identified as a urinary metabolite in mice.\textsuperscript{47}
2.2.3 Epidemiology

Outbreaks of ABPE typically occur in cattle that have been abruptly moved from poor quality, non-succulent feed to lush green pastures in the spring or fall of the year.\textsuperscript{1,3,48} Cattle have been reported to develop ABPE after grazing succulent pastures containing alfalfa, rape, kale, turnip tops, or silage aftermath.\textsuperscript{2,48,49} However, the plant species composition does not seem to matter as long as the forage is lush.\textsuperscript{3,50} Acute bovine pulmonary emphysema typically develops within two weeks after cattle are moved to the new pasture.\textsuperscript{51} Morbidity rate has been reported to vary between 4.2% to 60% under natural conditions.\textsuperscript{1,48,51} The mortality rate has been reported to be 2.9%, and approximately 30% of severely affected animals will typically die within the first 48 hours after the onset of clinical signs.\textsuperscript{1} Ninety-two percent of clinically affected cattle in one epidemiological study were two years of age or older (OR = 7.75 compared to cattle < 2 years old), and females of this age category were slightly more likely (OR = 1.32) to be observed with clinical signs compared to males of the same age.\textsuperscript{48} It has been suggested that ABPE occurs more frequently in the Hereford breed because it is the most prevalent breed used in North American cow calf operations.\textsuperscript{51} However, ABPE can occur in any breed under the right management circumstances.\textsuperscript{51}

2.2.4 Clinical signs

Clinical signs of ABPE are predominately referable to the respiratory tract but affected animals will be more depressed and tranquil as well.\textsuperscript{50} Tachypnea is commonly noted with respiratory rates ranging from 35-75 breaths per minute or higher.\textsuperscript{50} Dyspnea,
open mouth breathing, loud expiratory grunting, and frothing at the mouth have been reported in severe cases. Both severely and mildly affected cattle improve dramatically after 3 days, but may still exhibit tachypnea and hyperpnea. Coughing is not a dominate clinical sign in affected cattle. Subcutaneous emphysema may also be observed. Lung sounds are soft on initial auscultation despite increased respiratory rate, and crackles are occasionally detected. As clinical signs abate, auscultated lungs sound more harsh and crackles and wheezes may be heard in the caudal lung lobes.

2.2.5 Pathology

As was previously mentioned, 3MI metabolism and toxicity in ruminants occur primarily in the lungs following both experimental and naturally induced episodes of 3MI pneumotoxicosis.

Predominant gross lung lesions found during post mortem examination of the respiratory tract in cattle dying from ABPE include interstitial emphysema and edema in all parts of the lungs. Lung lobes are often distended. Pulmonary edema is prominent in ventral lung segments and gelatinous yellow edematous fluid has been reported in the interlobular septa and around vascular connective tissue. Large emphysematous bullae may also be observed and are typically found in the caudal lung lobes. Cranial lung lobes are purple or deep red and have a smooth, glistening appearance on the cut surface after transection. This appearance is reportedly the result of severe congestion, edema, and hyaline membranes. Upper airways may be filled with a frothy, edema fluid.
Ecchymotic and petechial hemorrhages may be present in the larynx, trachea, and bronchi.  

Bradley and co-workers characterized ultrastructural changes in goat lungs during continuous intravenous infusion with 3MI. Swelling of the mitochondria and intracellular vesicles in the capillary endothelial, alveolar, and nonciliated bronchiolar epithelial cells occurred within 30 minutes after starting the infusion. They reported that the capillary endothelial cells returned to normal two hours after commencing the 3MI infusion. However, morphologic changes in type I pneumocytes and non-ciliated bronchiolar epithelial cells became progressively worse during the experiment leading them to conclude that 3MI produces a rapid cytotoxic effect in these cells.

Histologic lung lesions reported in adult cattle with severe ABPE include congestion, edema, interstitial emphysema, and focal proliferation of alveolar epithelial cells. With the increased passage of time, alveolar epithelialization becomes more extensive and involves all segments in all lobes of both right and left lungs. Histologic lung lesions in cattle that are less severely affected include focal areas of hyaline membrane formation in alveoli and alveolar ducts, alveolar epithelial hyperplasia (type II pneumocyte hyperplasia), and interstitial emphysema and edema. Prominent histologic findings in the interalveolar septa are edema, interstitial cells and eosinophils. Alveolar spaces may contain condensed hyaline membrane deposits, multinucleated giant cells, large mononuclear cells, and a mixture of type II pneumocytes and alveolar macrophages.
2.2.6 Prevention

Several species of rumen bacteria will deaminate L-tryptophan to form indoleacetate, but only *Lactobacillus* spp. has been reported to decarboxylate indoleacetate to form 3MI.\(^{23}\) *In vitro* and *in vivo* inhibition of rumen 3MI production by antimicrobial drugs has been investigated.\(^{8,17,23,53-56}\) Monensin and lasalocid are polyether antibiotics used as feed additives in cattle.\(^ {23}\) Clinical ABPE was successfully prevented in cattle challenged with oral L-tryptophan by pre-feeding cattle monensin or lasalocid 1-3 days prior to the challenge.\(^{17,54,55}\) Clinical signs of ABPE were also prevented in cattle pre-fed monensin and moved from poor quality pastures to lush green pasture.\(^ {56}\)

The pneumotoxic effects of 3MI have also been mitigated in experimental models using goats. Goats treated with acetylsalicylic acid (aspirin) or indomethacin (non-steroidal anti-inflammatory drugs) prior to challenge with oral 3MI had clinical signs and lung lesions that were less severe when compared to goats in the control group or goats given aspirin after 3MI challenge.\(^ {32}\) Aspirin is approved for use in cattle and at the current writing, has no meat or milk withholding time. The serum t\(_s\) of aspirin is 32 minutes, and the t\(_a\) of absorption in the rumen is 2.91 hours.\(^ {57}\) Oral dosing of aspirin at 100 mg/kg (45 mg/lb) every 12 hours has been reported to maintain serum salicylate concentrations > 30 \(\mu\)g/ml in cattle which is considered to be therapeutically effective as an anti-inflammatory based on studies in man.\(^ {57}\) However, Bray and Preston have reported that a single dose of aspirin (32 mg/kg (15 mg/lb), PO) inhibited prostaglandin H synthase for more than 48 hours in cattle.\(^ {58}\) Aspirin may potentially be used to mitigate the toxic effects of 3MI in feedlot cattle by inhibiting the PHS pathway of 3MI.
metabolism. This may potentially prevent a proportion of BRD in feedlot cattle that may be attributed to lung exposure to the toxic metabolites of 3MI. Aspirin is inexpensive, approved for use in food animals, readily available in oral form, palatable to cattle, and could easily be fed to cattle entering a feedlot.

Vitamin E (d or dl-alpha-tocopherol) is a fat soluble vitamin with antioxidant properties. It exerts its primary antioxidant effect by inhibiting the oxidation of membrane lipids. Vitamin E has been studied as a potential preventative for 3MI induced pneumotoxicity in goats, but not in cattle. In one study, goats were pre-treated with vitamin E, and either cysteine (increases tissue concentration of glutathione) or diethyl maleate (decreases tissue concentration of glutathione) to vary the tissue levels of glutathione because glutathione plays a major role in the detoxification of reactive intermediate molecules produced by 3MI metabolism in the lung. Two additional groups of goats were pre-treated with cysteine or diethyl maleate alone. After experimental challenge with intravenous 3MI, they reported that goats with high tissue concentrations of glutathione (cysteine treated) had mild lung lesions and goats with low tissue concentrations of glutathione (diethyl maleate treated) had severe lung lesions regardless of vitamin E status. They reported that the initial toxicological event in 3MI pneumotoxicosis is probably the result of the 3MI free radical covalently binding to cellular protein rather than lipid peroxidation. Vitamin E(d-alpha-tocopherol acetate) and aspirin have also been reported to potentially have a synergistic antioxidant effect, although via different pathways, in cultured endothelial cells incubated with hydrogen peroxide.
2.3 Bovine Respiratory Syncytial Virus

2.3.1 Molecular biology

Bovine respiratory syncytial virus (BRSV) is classified in the family *Paramyxoviridae* and genus *Pneumovirus*. Other viruses within the genus *Pneumovirus* are human respiratory syncytial virus (HRSV), ovine respiratory syncytial virus, caprine respiratory syncytial virus, pneumovirus of mice, and turkey rhinotracheitis virus. Bovine respiratory syncytial virus may be closely related to both the HRSV (distinct viruses but antigenically related) and caprine respiratory syncytial virus.

Bovine respiratory syncytial virus is a single stranded, negative sense, enveloped RNA virus. It replicates in the cytoplasm of infected cells and matures by budding from the apical cell membrane. In general, respiratory syncytial viruses are 80 to 500 nanometers in diameter and round or pleomorphic in form with occasional filamentous forms observed. It is thermolabile and susceptible to inactivation by freeze-thawing, ether, chloroform, trypsin, sodium deoxycholate, and acidic environments (labile at pH ≤ 3; stable at pH ≥ 4). Respiratory syncytial viruses were named for the characteristic cytopathic effect they produce in cell culture. Infected cells form a multinucleated mass of protoplasm (syncytial cell) by fusing or merging two or more cells. Syncytium formation does not occur under all circumstances. It is dependent on the isolate, culture conditions, and cell type.

The genome for BRSV encodes for 10 messenger RNAs which is similar to that of HRSV. The protein structure of BRSV and HRSV are also similar; each is composed of 10 viral proteins. Except for proteins 1 B and 1 C, all viral proteins are structural.
The proteins and their functions are as follows. The F and G proteins are glycosylated, transmembrane surface proteins and are important proteins for immunity.\textsuperscript{64,65} The function of the G protein is viral attachment to the target cell.\textsuperscript{64,65} Neuraminidase and hemagglutinin are not associated with the G protein which is unique to viruses in the genus \textit{Pneumovirus} compared to other \textit{Paramyxoviruses}\.\textsuperscript{65} Disulfide bonds link the G protein to the F protein.\textsuperscript{65} The function of the F protein is fusion of the virus or host cell membrane with the membrane of uninfected cells.\textsuperscript{65} The F protein is first synthesized as a precursor molecule ($F_0$).\textsuperscript{65} The $F_0$ precursor molecule is proteolytically cleaved into disulfide-linked peptide fragments (F1, F2) which can then initiate virus or infected cell membrane fusion with the uninfected cell membrane.\textsuperscript{65} The N, P, and L proteins are associated with the nucleocapsid of the virus.\textsuperscript{64} The N protein is a structural protein and the L and P proteins are associate with polymerase functions.\textsuperscript{64} The other 5 proteins are M, M2, SH (1A), NS1 (1C), and NS2 (1B).\textsuperscript{64} The M protein is an internal membrane or matrix protein.\textsuperscript{64} The functions of the M2, SH, NS1, and NS2 proteins are unknown.\textsuperscript{64}

Two antigenic subgroups of HRSV have been identified (A, B) based differences between the G, F, N, and P proteins.\textsuperscript{63} However, differences in the attachment protein (G) constitute the main difference.\textsuperscript{63} Subgroup B has also been subdivided into B1 and B2 groups.\textsuperscript{63,64} Bovine respiratory syncytial virus was initially considered to be a monotypic virus but recent research suggests that there may be two antigenic subgroups.\textsuperscript{66} This could be important in the development of vaccines effective against both BRSV subgroups.\textsuperscript{63}
2.3.2 Epidemiology

Bovine respiratory syncytial virus was first isolated in Switzerland in 1967.\textsuperscript{67} In the United States, it was first reported in Iowa and Missouri in 1974.\textsuperscript{68,69} Since that time, it has been demonstrated that BRSV infection in cattle is a common event and its distribution is world wide.\textsuperscript{62} Seroepidemiologic studies completed before vaccination against BRSV became widespread reported that 65-81\% of cattle in North America had antibodies specific for BRSV.\textsuperscript{70-74} Seroepidemiological studies in France and England reported 50\% and 94\% of cattle respectively had antibodies against BRSV.\textsuperscript{75,76} Cattle are the principle reservoir for BRSV.\textsuperscript{77} Cattle infected with BRSV transmit the virus to uninfected cattle by aerosolizing secretions containing the virus.\textsuperscript{77} The virus gains entry into uninfected cattle via the respiratory tract.\textsuperscript{63,77} This mode of transmission enables the virus to spread rapidly from infected to susceptible cattle.\textsuperscript{63} Overcrowding in holding facilities and during transportation may also facilitate rapid transmission of the virus.

Bovine respiratory syncytial virus is susceptible to environmental destruction which makes close contact even more likely as a prerequisite for transmission.\textsuperscript{77,78} Although respiratory syncytial virus infections have been reported in humans, sheep, and goats among other species, interspecies transmission is unlikely.\textsuperscript{77,79} A chronic carrier state has not been proven but bovine cell lines can be persistently infected with BRSV.\textsuperscript{80} Bovine respiratory syncytial virus has also been isolated in asymptomatic cattle and cattle 7 months after initial infection with BRSV.\textsuperscript{77,78}

In outbreaks of respiratory disease attributable to BRSV, morbidity may be as high as 60 - 80\%\textsuperscript{63} and mortality can range from 0 - 20\%.\textsuperscript{81} Outbreaks of BRSV
pneumonia can occur at any time of the year in both immature and mature cattle of beef or dairy breeds. However, BRSV pneumonia is most commonly reported in the fall and winter. Transportation, overcrowding, and extreme temperature fluctuations are stress factors which may play a role in outbreaks of BRSV pneumonia. The occurrence of BRSV initiated pneumonia is highest in beef cattle from 6 weeks to 13 months of age and highest in dairy cattle from 2 weeks to 9 months of age. However, the most severe form of the disease (acute respiratory distress) occurs most commonly from 2 to 4½ months of age. Red beef breeds in North America and the Belgian blue-white breed in Europe have been reported to be more susceptible to infection with BRSV compared to other breeds. Active immunity which develops as a result of primary BRSV infection is not completely protective against re-infection. Cattle re-infected with BRSV typically develop mild or subclinical disease. Active immunity which develops as a result of primary BRSV infection is not completely protective against re-infection. The presence of maternal antibody against BRSV does not prevent infection, but does ameliorate the severity of the disease. Bovine respiratory syncytial virus has also been shown to act synergistically with Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus, and bovine viral diarrhea virus to cause respiratory disease.

2.3.3 Pathogenesis

The complete pathogenesis of BRSV infection is unclear primarily because attempts to reproduce BRSV pneumonia have only been occasionally successful. Under both experimental and natural conditions, BRSV infects and causes cellular
damage to several ciliated and non-ciliated cell types in the airways of the lung and may also infect respiratory epithelial cells within the alveoli. Cellular damage from necrotizing bronchiolitis and debris from infected cells are characteristic findings in BRSV infected lungs. Cellular debris from extensive viral replication may also cause bronchiolitis obliterans as a sequella resulting in partial to complete blockage of the airways. This may contribute to dyspnea and emphysema seen in cattle severely affected with BRSV infection. Cellular damage is found mainly in the cranioventral lobes of the lung. However, in some cases the most dramatic gross lesion in lungs from animals dying from BRSV infection is severe edema and emphysema in the caudal lung lobes. The exact mechanism which produces severe emphysema and edema reported in cattle with severe BRSV pneumonia has not been determined, although there are several hypotheses. Baker et al suggested that dyspnea and increased forced respiration as a result of bronchiolitis obliterans may change airflow patterns and contribute to pulmonary emphysema. Others have proposed that the immune system plays a role in the pathogenicity of the virus. Infants and children immunized with a formalin inactivated HRSV vaccine in the late 1960's had increased severity of respiratory disease when they were subsequently infected naturally with respiratory syncytial virus. Because clinical disease observed in HRSV vaccinated children who were subsequently infected with HRSV and children with severe natural HRSV infection were similar, this led some researchers to hypothesize that the pathogenesis of naturally produced respiratory syncytial virus disease involved a type III hypersensitivity (arthus) immune reaction. Later studies reported that formalin inactivation of HRSV may have changed functional
antigenic epitopes on the F protein and consequently, formalin inactivated HRSV vaccines primarily produced non-neutralizing and non-fusion inhibiting antibodies to the F glycoprotein.\textsuperscript{101,102} Human respiratory syncytial viral antigen binding by non-neutralizing antibody produced by the vaccine may have induced a type III hypersensitivity reaction in the vaccinates.\textsuperscript{101,102} This phenomenon has been recently reproduced in cattle experimentally immunized with a formalin inactivated BRSV vaccine.\textsuperscript{92} However, Kimmen has suggested that type III hypersensitivity may not play a big role in the pathogenesis of natural infections because high levels of maternal BRSV or HRSV antibody in cattle and children respectively, decrease the severity of clinical disease.\textsuperscript{97} In addition, although cell free antigen and BRSV specific antibodies have been found in the lungs of cattle infected with BRSV, the presence of immune complexes have not been reported.\textsuperscript{103} He has suggested that the pathogenesis of severe naturally occurring BRSV disease may involve complement.\textsuperscript{97} Evidence which he cites in support of complement mediated pathogenesis include the simultaneous presence of BRSV antigen and antibodies in the lungs of infected animals, deposition of complement factor C3 and neutrophil influx in the cranioventral lung lobes, a lowered number of mast cells and mast cell granules and a lower level of histamine (which is indicative of mast cell degranulation), and severe edema and emphysema throughout the lungs.\textsuperscript{97} However, definitive evidence that a type III hypersensitivity is involved in the pathogenesis of BRSV infection has not been reported.\textsuperscript{104}

Other potential mechanisms of BRSV immunopathogenesis in severe naturally occurring infections have been reviewed.\textsuperscript{97,104} Briefly, type I and type 4 hypersensitivity
reactions have been examined as potential immunopathogenic mechanisms in BRSV infections.\textsuperscript{97,104} The involvement of a type I hypersensitivity was hypothesized based on reports that two stages of clinical disease (mild clinical signs initially followed by severe respiratory distress in 2-14 days) were observed in some cases of BRSV induced respiratory disease and that the second stage of severe respiratory distress responded positively to treatment with corticosteroids and antihistamines.\textsuperscript{104} However, epizootic and experimental evidence does not support the hypothesis that prior sensitization to BRSV plays a role in the pathogenesis of the disease,\textsuperscript{91,105} and the clinical response to corticosteroids and antihistamines in cattle with severe respiratory distress associated with BRSV infection has never been established in controlled field trials.\textsuperscript{104} The hypothesis that a type IV hypersensitivity is involved in the pathogenesis of BRSV is based on observations in human infants and young children with severe HRSV induced bronchiolitis.\textsuperscript{104} Infants and young children who developed severe HRSV induced bronchiolitis had an increased systemic cell mediated immune response (as measured by lymphocyte transformation) when compared to infants who developed less severe forms of the HRSV induced disease (pneumonia or upper respiratory tract infection).\textsuperscript{104} Other research has failed to demonstrated this association.\textsuperscript{104} To date, type I and type IV hypersensitivities have not been definitively shown to play a role in the pathogenesis of BRSV infection.\textsuperscript{104}
2.3.4 Clinical signs

Infection with BRSV can result in a wide range of clinical signs. While many BRSV infections result in subclinical or mild infections, some BRSV infections cause severe clinical disease. The incubation period for BRSV is 3-5 days. Cattle in the early stages of clinical disease may have a mucopurulent nasal and ocular discharge, slight anorexia, pyrexia (40° - 42.5° C [104° - 108° F]), tachypnea, and ptyalism. Clinically affected cattle may appear slightly depressed but become much brighter if disturbed. Sudden death may also be the only clinical sign observed in cattle that are infrequently observed. Signs of respiratory disease are progressive in cattle with the acute form of the disease. Cattle may be observed with a dry, non-productive cough, severe dyspnea with heads lowered and neck extended, and expiratory grunting. Increased lung sounds and crackles may be heard on lung auscultation in severely affected cattle. Subcutaneous emphysema over the dorsal shoulders and in the cervical and submandibular areas may also be present due to alveolar rupture and migration of air through the mediastinum in cattle with severe pulmonary emphysema. Cattle may be observed standing around the watering trough, although, due to severe dyspnea, they may not consume enough water to prevent dehydration. Diarrhea followed by constipation (due to dehydration) and severe dyspnea has been reported when severely affected cattle are required to ambulate. The duration of clinical disease may be from 1-2 weeks. Abortion has not been reported as a common sequella to infection with BRSV.
A biphasic disease pattern has been reported in naturally occurring outbreaks of BRSV pneumonia.\textsuperscript{63,84,107-109} Cattle which exhibit this pattern initially have clinical signs of mild respiratory disease.\textsuperscript{63,106} They appear to recover from this initial event.\textsuperscript{63,106} The second phase of the disease may occur several days to 2 weeks following the primary event and is typically characterized by severe respiratory distress due to the presence of pulmonary edema and emphysema.\textsuperscript{63,106} However, this disease pattern has not been reported in dairy calves and experimental BRSV challenge models attempting to reproduce this pattern have been unsuccessful.\textsuperscript{91,110}

2.3.5 Pathology

Gross lung pathology in naturally occurring outbreaks of BRSV pneumonia initially involves the cranioventral lobes.\textsuperscript{93,111} The cranioventral lung lobes may have multifocal areas of dark red or purple colored lobular consolidation. Consolidated lobules may coalesce which can result in consolidation of much of the individual lobe.\textsuperscript{93,96,112,113} In severe cases, cattle have severe interstitial emphysema and edema primarily in the caudodorsal lobes although the cranial lobes may also be affected as well.\textsuperscript{63,93} Lungs from severely affected cattle are large, heavy, wet, and fail to collapse.\textsuperscript{63,93,114} Subpleural emphysema may be present in both cranial and caudal lobes.\textsuperscript{93} Caudal lung lobes are commonly distended as a result of severe interstitial emphysema and interlobular septa may be swollen due to edema.\textsuperscript{93} Emphysematous bullae of varying size may also be present. Subcutaneous emphysema may be present in the tissues of the back, neck and shoulders, around the kidneys and around the pericardial sac.\textsuperscript{63,93}
Mucopurulent exudate may be found in the small bronchi. Infections with BRSV are often complicated by secondary bacterial pneumonia. In these cases, the primary pathologic finding may be severe, exudative fibrinous, or suppurative bronchopneumonia.

Lung lesions in cases of severe BRSV pneumonia are similar to lesions reported for atypical interstitial pneumonia in cattle. Atypical interstitial pneumonia (AIP) has multiple potential etiologies that have previously been described. Many studies have attempted to associate BRSV infection with the occurrence of atypical interstitial pneumonia in cattle. In one study, feedlot cattle with fatal respiratory tract disease diagnosed as AIP post mortem were more likely (OR = 7.15, P = 0.01) to have BRSV identified in the lungs when compared to cattle diagnosed with other causes of respiratory disease.

Histologic lung lesions in BRSV pneumonia vary depending on the stage of viral infection and the presence of secondary bacterial pneumonia. One consistent finding is bronchointerstitial pneumonia with severe bronchiolitis in a cranioventral distribution. The cranioventral lung lobes may be consolidated with lymphocyte and plasma cell infiltration in the walls of the bronchi and bronchioles. Multinucleated syncytial cells (with or without eosinophilic intracytoplasmic inclusion bodies) have been reported to project from bronchiolar walls and may also be present in the bronchiolar or alveolar lumen as free cells. Although bovine respiratory syncytial virus was named for its characteristic syncytia formation, it is important to note that parainfluenza
type 3 viruses can also cause syncytia formation. Bronchiolar epithelial hyperplasia or necrosis may also be observed in the craniaoventral lung lobes depending on the stage of viral infection. The bronchiolar lumen may be partially or totally occluded by exudate depending on the size of the bronchiole.

Alveolar lesions in the craniaoventral lung lobes from natural BRSV infections include alveolar epithelialization (type II pneumocyte hyperplasia), thickening of the walls by cellular infiltrates, edema and exudate in alveolar lumen space, emphysema, and syncytial cell formation.

In severe natural infections, histologic lesions may also be observed in the caudal lung lobes. They include interstitial emphysema, alveolar edema, alveolar epithelialization (type II pneumocyte hyperplasia), hyaline membrane formation, and occasional rupture of alveolar walls. Characteristic lesions of BRSV infection found in the craniaoventral lung lobes (bronchiolitis and syncytial cell formation) are not typically present in the caudal lung lobes. It has also been reported that viral antigen is commonly absent in the caudodorsal lung lobes, but a recent investigation of a naturally occurring outbreak of severe BRSV pneumonia in adult dairy cattle reported identifying viral antigen in the caudal lung lobes.

It is difficult to experimentally induce BRSV pneumonia and many attempts have resulted in no clinical disease or pathologic findings, or at most very mild lung lesions. However, a small number of experiments have been published that describe successful production of macroscopic pulmonary consolidation in the cranioventral lung lobes and mild emphysema in the most severely affected calves after experimental challenge with
The most severe lesions have been induced by using a virulent, low passage level strain of BRSV and then administering the viral challenge via combined intranasal and intratracheal routes. Histologic lung lesions in cattle successfully challenged with BRSV were similar to those found in natural outbreaks of BRSV pneumonia. Consolidated regions in the cranioventral lungs had bronchitis, proliferative and necrotizing bronchiolitis, interstitial pneumonia, alveolar edema, epithelial syncytial formation on the bronchiolar and alveolar walls, and type II pneumocyte hyperplasia. Bovine respiratory syncytial virus antigen has also been detected by immunoperoxidase staining or immunofluorescent antibody staining of the bronchiolar and alveolar epithelium in the cranioventral lung lobes. Important cellular targets of BRSV include ciliated and non-ciliated bronchiolar epithelial cells and type II alveolar pneumocytes. Bovine respiratory syncytial virus has also been reported to replicate in ciliated and non-ciliated tracheobronchial epithelial cells and mucus cells. Damage to the ciliated tracheobronchial epithelial cells and mucus cells may damage the mucociliary defense system. This may be one factor which predisposes cattle with BRSV infections to secondary bacterial infections.

2.3.6 Diagnosis

Diagnosis of BRSV can be made by virus isolation, antigen detection, or antibody measurement. Isolating BRSV is difficult and results are therefore unpredictable. Tissues that have high concentrations of BRSV antigen rarely yield virus in cell
This may be due to free virus neutralization by the immune response if tissues are collected after several days of clinical disease or at post mortem. Neutralization of free virus may also occur during tissue preparation. The virus is thermolabile and sensitive to freeze thawing so it may lose viability during transportation to the laboratory. The best sample for viral isolation of BRSV is tracheal wash or lung lavage fluid obtained early in the disease process when highest quantities of free virus are maximized. The samples should be transported to the laboratory within 24 hours after collection. Nasopharyngeal swabs and lung samples from lesion margins in the cranioventral lung lobes may also be useful for viral isolation.

Bovine respiratory syncytial virus antigen may be detected using an antigen detection enzyme immunoassay, immunofluorescent antibody (IFA) staining, immunohistochemistry, and polymerase chain reaction (PCR).

Antigen detection enzyme immunoassay for human respiratory syncytial virus has been developed and is cross reactive for several BRSV proteins. Sampling and use of this assay for BRSV has been previously reviewed. An antigen detection enzyme immunoassay specifically for BRSV has also been developed.

Immunofluorescent antibody staining is a rapid, reliable, and sensitive test for the detection of BRSV antigen. The reliability and accuracy of the test depend on the specificity of the antibody reagent used. Monoclonal antibodies to human respiratory syncytial virus that are cross reactive with BRSV, and BRSV specific monoclonal antibodies have been reported to vastly improve the sensitivity of the IFA. Some of the proteins detected with human respiratory syncytial virus monoclonal antibodies are
represented as distinct intracytoplasmic inclusions.\textsuperscript{124} Monoclonal antibodies combined with the antigen distribution have been reported to allow the detection of BRSV infection in a single cell.\textsuperscript{124} Optimum specimens to select for IFA are similar to that for viral isolation. The advantages of using IFA for detection of BRSV are that it can be performed on frozen lung sections and it exhibits viral specificity in lungs complicated with severe secondary bacterial pneumonia.\textsuperscript{124}

Detection of BRSV using immunohistochemistry is also possible. Immunohistochemistry is the process of antigen detection in tissues using monoclonal or polyclonal antibodies and avidin-biotin complex.\textsuperscript{63} Using immunohistochemistry, BRSV antigen can be identified in formalin fixed, paraffin embedded lung tissue.\textsuperscript{124,130} Immunohistochemistry for BRSV antigen is just as sensitive as IFA and diminishes the need for immediate transportation to the lab. Histological examination of the lung tissue can also be performed at the same time.\textsuperscript{63} One disadvantage of immunohistochemistry is that preparation of tissues requires more time when compared to IFA.\textsuperscript{63}

Detection of BRSV antigen using polymerase chain reaction techniques to detect specific nucleic acid sequences of BRSV has also been reported.\textsuperscript{63,131} The polymerase chain reaction technique is reported to be more sensitive compared to IFA,\textsuperscript{132} but is not used routinely for the diagnosis of BRSV.\textsuperscript{63}

Serum antibodies specific for BRSV can be detected using virus neutralization techniques, enzyme immunoassays, or fusion inhibition assays. These techniques and their application for the diagnoses of BRSV infection have been previously reviewed.\textsuperscript{63,124,133}
2.3.7 Prevention

Vaccines against BRSV have been commercially available since the mid 1980's. In North America, a modified-live (MLV) BRSV vaccine was introduced in 1984 and an inactivated BRSV vaccine became available in 1988.\textsuperscript{63}

Modified-live BRSV vaccines are generally attenuated by multiple passage of strains in cell culture.\textsuperscript{63,80} It is commonly injected intramuscularly and has been reported to undergo an abortive cycle of replication at the injection site, but it still induces an immune response.\textsuperscript{63} Adverse reactions to BRSV MLV vaccines have been uncommon during the last fifteen years since becoming commercially available. However, one report in the literature suggests that concurrent vaccination with a BRSV MLV vaccine during natural infection with BRSV may have enhanced the severity of clinical disease.\textsuperscript{135} In this clinical report, approximately 50% of vaccinated cattle developed lower respiratory tract disease within several days after vaccination with a BRSV MLV vaccine.\textsuperscript{135}

Commercially available inactivated (KV) BRSV vaccines are generally whole cell vaccines manufactured by using a fixative agent to preserve cells infected with BRSV. Cells infected with BRSV often have viral proteins on the lipid cellular membrane. Vaccination with inactivated BRSV infected cells stimulates an immune response to these proteins when injected into the target animal. Most commercially available BRSV KV vaccines also contain an adjuvant to help increase the immune response to the vaccine. Immunopotentiation by a BRSV KV vaccine in cattle has not been reported. However, children immunized with a formalin inactivated human respiratory syncytial virus (HRSV) vaccine were reported to develop severe clinical respiratory disease which was
similar in severity to disease observed in unvaccinated children who subsequently underwent natural infection. Later, studies in humans and cotton rats reported that the formalin inactivated HRSV vaccine produced high concentrations of antibody specific for the HRSV F protein, however, these antibodies did not exhibit neutralizing or fusion inhibiting activity. This suggests that formalin inactivation of respiratory syncytial virus may have altered important viral epitopes. Disproportionate concentrations of non-neutralizing antibody produced in response to the formalin inactivated HRSV vaccine is thought to have contributed to the pathogenesis of the disease in these children. A recent study reported vaccinating 7-8 week old calves with a formalin inactivated BRSV vaccine and subsequently challenged them 44 days later with virulent BRSV by nebulization. They reported producing severe clinical disease and histologic lung lesions in these calves which were similar to those previously reported in children immunized with a formalin inactivated HRSV vaccine and then naturally infected with HRSV. The experimental BRSV vaccine produced non-neutralizing BRSV specific IgG antibodies.

Most currently available BRSV KV vaccines in North America do not use formalin as an inactivating agent. Because information about vaccine manufacturing processes by North American biological companies is proprietary, a review of common inactivating agents and adjuvants used in manufacturing BRSV vaccines in North America will not be reported. However, the results of a field trial in which cattle were inoculated with a killed BRSV vaccine composed of glutaraldehyde-fixed bovine nasal mucosa cells persistently infected with BRSV in an oil adjuvant reported increased
protection when compared to cattle administered a BRSV MLV vaccine or controls when naturally challenged with BRSV. A field trial conducted by Howard et al reported decreased morbidity in cross-bred beef calves inoculated with a vaccine containing glutaraldehyde-fixed cells infected with BRSV using Quil A as an adjuvant compared to unvaccinated controls.

Recently, there have been several studies published comparing the antibody response of commercially available BRSV vaccines in cattle. It has been demonstrated that several commercially available BRSV KV vaccines produce higher serum concentrations of non-neutralizing antibodies when compared to a BRSV MLV vaccine. The clinical significance of this finding in commercial feedlot cattle operations has not been reported. However, studies involving mice that were given respiratory syncytial virus specific monoclonal antibodies and then experimentally challenged with respiratory syncytial virus demonstrated that protection was best correlated with antibody that inhibited fusion between cells and was less correlated with neutralizing antibody or complement-dependent lysis of virally infected cells. The authors also report that only half of the neutralizing epitopes on the F protein inhibited cell-to-cell fusions but all fusion inhibiting antibody neutralized the virus. Another recent study by Taylor and co-workers reported differences in the bovine immune response to the F, G, N, and M2 proteins of BRSV. They immunized calves with recombinant vaccinia virus (rVV) vaccines encoding the F, G, N, or M2 proteins of BRSV, and then challenged them with BRSV 6-7 weeks later. They concluded that the F, G, and N protein of BRSV induced resistance to the subsequent challenge with BRSV.
and protected against the development of lung lesions. However, only the rVV encoding for the F protein induced high concentrations of neutralizing antibody. The rVV vaccines encoding for the G protein and N proteins induced low or no neutralizing antibody, respectively, yet were still protective against experimental challenge with BRSV. The mechanism of resistance to BRSV by antibody induced via immunization is still unclear.

Efficacy trials of BRSV vaccines have been reviewed elsewhere. Briefly, both MLV and KV BRSV vaccines appear to be generally safe except for one incident where BRSV MLV vaccine was given to cattle concomitantly undergoing natural infection with BRSV. The results from most field trials involving BRSV vaccination have been marginally positive or equivocal regarding the vaccines' ability to prevent undifferentiated bovine respiratory disease. Baker et al suggested that these studies, taken as a whole, suggest there is some degree of efficacy associated with vaccination. Whether this protection is reliably induced in all management situations or is cost effective is debatable.
2.4 Experimental challenge with 3-methylindole and bovine respiratory syncytial virus

3-Methylindole and BRSV are both agents which have been associated with spontaneous diffuse interstitial pneumonia in cattle (i.e. atypical interstitial pneumonia).\(^\text{116,143}\) Castleman and co-workers reported experimentally challenging 30–45 day old calves with 3MI and BRSV to determine if 3MI would increase the susceptibility of calves to severe interstitial pneumonia induced by BRSV.\(^\text{143}\) Calves were dosed with oral 3MI (0.25 g/kg [0.11 g/lb], PO) 3 days prior to intratracheal inoculation with BRSV.\(^\text{143}\) They concluded that young calves were susceptible to the pneumotoxic effects of 3MI, however, their results did not indicate that 3MI induced lung damage makes the bovine lung more susceptible to replication or injury by BRSV.\(^\text{143}\) They also conceded that the BRSV isolate used in this challenge underwent minimal replication in the alveolar tissue in these calves and suggested that additional studies using a more virulent strain of BRSV may be necessary to ultimately answer the question of synergism between 3MI and BRSV.\(^\text{143}\)
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CHAPTER 3

SYNERGISTIC EFFECTS OF CONCURRENT CHALLENGE WITH BOVINE RESPIRATORY SYNCYTIAL VIRUS AND 3-METHYLINDOLE IN CALVES

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3.1 Abstract

Objective  To evaluate the potential synergy between bovine respiratory syncytial virus (BRSV) and 3-methylindole (3MI) in inducing respiratory disease in cattle.

Animals  20 mixed breed beef calves

Procedure  A 2 x 2 factorial study design was used with random assignment to the following 4 challenge exposure groups: unchallenged control, BRSV challenge exposure (5 X 10^4 TCID_{50} by aerosolization and 5.5 X 10^4 TCID_{50} by intratracheal inoculation), 3-MI challenge exposure (0.1 g/kg of body weight, PO), and combined BRSV-3MI challenge exposure. Clinical examinations were performed daily. Serum 3MI concentrations, total white blood cell counts, packed cell volume, total plasma protein, and fibrinogen concentrations were determined serially throughout the experiment. Surviving cattle were euthanatized 7 days after challenge exposure. Gross and histologic pulmonary lesions were evaluated post-mortem.

Results:  Clinical respiratory disease was more acute and severe in the BRSV-3MI challenge exposure group. All 5 cattle in this group and 3 of 5 cattle treated with 3MI alone died or were euthanatized prior to termination of
the experiment. Mean lung displacement volume was greatest in the BRSV-3MI challenge exposure group. Gross and histologic examination showed that pulmonary pathology was also most severe in this group.

Conclusions: Feedlot cattle are commonly infected with BRSV, and 3MI is produced by microflora in the rumen of all cattle. Our results suggest that there is synergy between BRSV and 3MI. Thus, controlling combined exposure may be important in preventing respiratory disease in feedlot cattle.
3.2 Introduction

Bovine respiratory disease complex (BRD) is an economically important disease syndrome of beef cattle.\textsuperscript{1,2} Many infectious agents have been linked to this multifactorial disease complex.\textsuperscript{3} However, little attention has been paid to potentially important non-infectious causes, or to possible interactions between infectious and non-infectious causes of BRD.

Three-methylindole (3MI) is a documented cause of respiratory disease in cattle. Acute bovine pulmonary emphysema and edema (ABPE) can be induced by 3MI toxicosis and is typically observed in mature cattle that have been moved from poor quality pastures to lush, green pastures that are rich in tryptophan.\textsuperscript{4,5} Lactobacilli in the rumen convert dietary tryptophan to 3MI, which is rapidly absorbed into the systemic circulation. Three-methylindole in cattle acts specifically in pulmonary tissues through cytochrome-dependant mixed function oxidase and prostaglandin H synthase found in the smooth endoplasmic reticulum of type I pneumocytes and Clara cells.\textsuperscript{6} Conjugation produces free radicals and causes oxidative damage to pulmonary tissues, causing interstitial emphysema, edema, type II pneumocyte hyperplasia, and hyaline membrane formation.\textsuperscript{6} Dyspnea, tachypnea, respiratory distress, and acute death can be observed in affected animals within 14 days after dietary changes.\textsuperscript{7} Similar syndromes have been documented in association with exposure to other toxic compounds such as 4-ipomeanol and Perilla mint ketone.\textsuperscript{8,9}

Young cattle entering feedlots also undergo similar dietary changes that may influence 3MI production. These cattle are typically moved from pasture in late summer.
and offered feedlot rations with high concentrations of tryptophan. Production of 3MI may increase in rumen Lactobacilli, causing subclinical pulmonary damage in these calves similar to that seen in cattle with ABPE. Oxidative damage to airways may allow opportunistic viral and bacterial pathogens to invade and cause clinical BRD.

Clinical and post-mortem findings in cattle affected by ABPE are similar to those in feedlot cattle with pneumonia attributed to bovine respiratory syncytial virus (BRSV). Feedlot cattle with pneumonia caused by BRSV often have an acute onset of dyspnea and tachypnea. Post-mortem findings in severely affected cattle include interstitial emphysema, edema, hyaline membrane formation, and type II pneumocyte hyperplasia. These similarities suggest that the pathogenic mechanisms of 3MI toxicosis and BRSV infection also may be similar.

Bovine respiratory syncytial virus has been studied extensively and is thought to be capable of producing clinically important pneumonia in cattle of all ages. Estimates of seroprevalence in herds in the United States and Western Canada range from 65-81%. However, clinical disease appears to be far less common than serologic evidence of BRSV infection. Possibly, the development of pathologic changes in the respiratory tract attributed to BRSV requires additional factors that act as initiators or promoters of clinical disease.

Synergistic pathogenic effects have been shown when mice received a combined challenge of 4-ipomeanol and viral respiratory pathogens. These effects appeared to be partially attributable to increased protease activity, which can cleave viral attachment glycoproteins, and increased recruitment of alveolar macrophages, which can support
greater viral replication.\textsuperscript{15-17} A previous investigation\textsuperscript{18} did not identify a similar synergistic effect between BRSV and 3MI in cattle; however, the BRSV isolate that was used had low virulence because of repeated passage in tissue culture, which may have affected results of that investigation. This purpose of the study reported here was to evaluate potential synergistic effects of simultaneous exposure to 3MI and low-passage virulent BRSV in cattle.
3.3 Materials and methods

3.3.1 Study design

Animal care and treatment protocols were reviewed and approved by the animal care and use committee at The Ohio State University prior to initiation of this study. Twenty unvaccinated mixed-breed beef cattle were purchased from a single ranch and transported approximately 100 miles to research facilities at the Ohio State University. A balanced $2 \times 2$ factorial study design was used. Calves were randomly assigned into four challenge exposure groups: an unchallenged control group, a group challenge exposed with BRSV, a group challenge exposed with 3MI, and a group receiving combined BRSV-3MI challenge exposure. Each calf was individually housed in a stall ($1.2 \times 3.0$ m), and calves were separated by an empty stall. The BRSV and BRSV-3MI challenge exposure groups were housed separately from the control group and the 3MI challenge exposure group. To prevent viral transmission between calves and between groups, animal caretakers followed a strict biosecurity protocol that included the following features: BRSV challenge exposure animals were cared for last, boots were washed in a virucidal solution before entering stalls, boots and coveralls were changed between groups, and disposable gloves were worn when caretakers handled calves and changed between calves.

Physical examinations and postmortem evaluations were performed by investigators who were unaware of treatment group assignments. Data collected during physical examinations included rectal temperature, heart rate, respiratory rate, thoracic
auscultation findings, and a subjective assessment of each animal's mentation and physical condition.

3.3.2 Experimental challenge exposure

Shortly after arrival, each calf was weighed. Bovine respiratory syncytial virus used in the challenge was a low passage field isolate obtained during an outbreak of severe respiratory disease in dairy cattle. Calves were challenge exposed by means of aerosolization and by intratracheal inoculation. Three milliliters of culture supernatant containing $5 \times 10^4$ TCID$_{50}$ BRSV was aerosolized during a 20 to 30 minute period. After aerosolization, a 14-gauge needle was used to introduce a 10-F polyethylene catheter into the trachea, where it was advanced approximately 25 cm to the area of the tracheal bifurcation, and 3 milliliters of culture supernatant containing $5.5 \times 10^4$ TCID$_{50}$ BRSV was injected into the tracheal lumen. All noninfected calves were sham inoculated intratracheally with sterile tissue culture media but did not undergo sham aerosolization because of time limitations.

Calves in the 3MI and BRSV-3MI challenge exposure groups were given crystalline 3MI$^b$ (0.1 g/kg, PO) in gelatin capsules. 3-methylindole was given to the BRSV-3MI challenge exposure group immediately after completion of the BRSV inoculation.
3.3.3 Euthanasia

During the course of the study, calves that were not considered likely to survive 24 hours on the basis of evidence of dyspnea, respiratory distress, lethargy, and dehydration were euthanatized using sodium pentobarbitol (43mg/kg, IV) and sodium phenytoin (5.5mg/kg, IV). All calves that survived were euthanatized on day 7.

3.3.4 Postmortem examination

Pulmonary tissues of all calves were examined within 6 hours of death. Gross examination findings were recorded, lung weight was measured to the nearest 0.1 kg, and lung displacement volume was determined by submerging the lungs in water and measuring the volume of water displaced to the nearest 0.01 L. Specimens from a centrally located transverse section of each lung lobe were collected from each calf and processed for histopathologic examination, bacterial culture, and viral identification. A pathologist who was unaware of treatment group assignments scored histopathologic evidence of parenchymal and airway changes, using an interval scoring system. Changes in airways and the pulmonary parenchyma were assessed separately in each of the 7 sections and assigned a score of 0 to 4 (0 = no pathologic changes; 4 = severe pathologic changes). Airways were examined for evidence of epithelial necrosis or hyperplasia and evidence of inflammatory cell infiltrates in lumina and lamina propria. Parenchyma was examined for evidence of edema, hyaline membrane formation, thickening of interalveolar septae, proliferation of type-II pneumocytes, and intra-alveolar or interstitial infiltration by inflammatory cells.
3.3.5 Laboratory analyses

Whole blood samples were collected, and serum was obtained at the time of challenge exposure and after 0.5, 1, 2, 6, 12, 24, 48, 72, 96, 120, and 144 hours; packed cell volume was determined by use of centrifugation, total plasma protein concentration was determined using refractometry, fibrinogen concentration was determined using heat precipitation, and total white blood cell count was measured. Serum 3MI concentrations were determined by use of a microplate method adapted from procedures as described. Serum BRSV neutralizing antibody titers was determined for each calf, using samples collected at the time of challenge exposure. Immunohistochemical techniques were used to detect BRSV in specimens obtained from each lung lobe: direct immunofluorescence was used to detect BRSV in pooled lung tissue specimens from each calf. Pooled lung specimens from each calf were plated onto blood agar, MacConkey’s agar, and chocolate agar to detect Pasteurella spp. or Haemophilus spp. Lung tissue from each calf was tested for bovine viral diarrhea virus (BVDV) by use of a direct fluorescent antibody technique.

3.3.6 Statistical analyses

Descriptive statistics were calculated and data were examined graphically. Differences among challenge exposure groups were assessed using ANOVA. Although clinical signs were recorded throughout the experiment, repeated measures ANOVA were not performed because several animals died prior to completion of the experiment and
complete time-series information was unavailable. These data instead were summarized graphically.
3.4 Results

3.4.1 Descriptive

Mean ± SEM body weight for all calves at arrival was 142 ± 10 kg and did not differ among groups. Screening prior to purchase suggested that calves in the source herd were seronegative to BRSV. However, low serum neutralizing antibody titers to BRSV were identified in serum samples obtained prior to challenge exposure in 8 calves. The remaining calves were seronegative. Two seropositive animals were assigned to each group through randomization. Of the 8 seropositive animals, 4 had a titer of 1:4, 2 had a titer of 1:8, and 2 had a titer of 1:16.

One calf in the BRSV challenge exposure group unexpectedly regurgitated and aspirated rumen contents at the time of inoculation, developed severe aspiration pneumonia, and was removed from the study.

3.4.2 Clinical evaluation

Calves in the BRSV-3MI challenge exposure group began to show signs of lethargy, anorexia, and mild dyspnea 2-4 days after inoculation. The condition of affected calves deteriorated rapidly following the onset of clinical signs as dyspnea and respiratory distress became progressively worse. Tachypnea and expiratory grunting were commonly noted as disease became more severe (Figure 3.1). All calves in the BRSV-3MI challenge exposure group died or were euthanatized prior to termination of the study: 1 calf died on day 2, 2 on day 4, and 2 on day 6.
Three of 5 calves in the 3MI challenge exposure group began to develop signs of anorexia, lethargy, and dyspnea within 4-5 days after challenge exposure. Dyspnea and respiratory distress became progressively more severe, and these calves were euthanatized on day 6. The remaining calves in the 3MI challenge exposure group were lethargic but did not develop severe respiratory distress or dyspnea.

Two calves in the BRSV challenge exposure group and 2 calves in the control group had mild mucopurulent nasal discharge on days 3 and 4. All other clinical observations of calves in the BRSV challenge exposure group and the control group were unremarkable.

Mean respiratory rates of the BRSV-3MI and 3MI challenge exposure groups increased during the study (Figure 3.1). Mean heart rates and rectal temperatures did not differ among challenge exposure groups (Figures 3.2 & 3.3).

3.4.3 Laboratory analyses

Serum 3MI concentrations gradually increased and peaked 6 hours after administration in the BRSV-3MI and 3MI challenge exposure groups (Figure 3.4). Serum 3MI concentrations gradually decreased and returned to baseline concentrations after approximately 48 hours. Peak serum 3MI concentrations were similar between the BRSV-3MI and the 3MI challenge exposure groups. Serum fibrinogen concentrations, total plasma protein concentrations, packed cell volumes, and white blood cell counts were similar for all 4 groups (Figures 3.5 - 3.8).
3.4.4 Gross post mortem findings

At necropsy, lungs from all BRSV-3MI challenge exposed calves were severely distended, had extensive intralobular and interstitial edema and emphysema, and had various degrees of subpleural emphysema (Figure 3.9). Large emphysematous bullae (4 to 8 cm diameter) were in the caudal lobes. Lungs from calves in this group also collapsed less after removal compared which lung from other groups. Lungs from calves in the 3MI challenge exposed group were moderately distended, and interlobular edema and emphysema were grossly visible (Figure 3.9), but only one small bulla (2 to 3 cm diameter) was seen in 1 calf. Pathologic changes were observed grossly in 3 calves in the BRSV challenge exposure group. One calf had mild fibrinous pleural adhesions, and 2 calves had multifocal mild, cranioventral lung consolidation (Figure 3.9). Pulmonary lesions were not observed grossly in lungs from control group calves (Figure 3.9).

Least-squares (LS) mean lung weights and lung displacement volumes were calculated, controlling for differences in body weight. Lungs of the BRSV-3MI challenge exposure group were larger than those of other groups. Although not statistically significant ($P = 0.07$), the LS mean lung weight of the BRSV-3MI challenge exposure group was 1.04 kg heavier than the 3MI challenge exposure group. The LS mean lung weight of the BRSV-3MI challenge exposure group was 2.05 kg heavier ($P < 0.01$; Figure 3.10) that the BRSV challenge exposure group. The LS mean lung displacement volume of the BRSV-3MI challenge exposure group was 1.60 L greater ($P < 0.05$) than the 3MI challenge exposure group and 3.36 L greater ($P < 0.05$; Figure 3.11) than that of the BRSV challenge exposure group.
3.4.5 Histologic post mortem findings

Histologic lesions in calves that received 3MI alone or in combination with BRSV were similar in nature but were more severe than lesions in calves receiving the combined challenge. With few exceptions, the parenchyma of all lobes was affected in these calves. Alveolar septae variably were thickened because of proliferation of type II pneumocytes (epithelialization). Many alveoli contained protein-rich fluid, and hyaline membrane formation had developed in some alveoli. In particular, calves that received BRSV and 3MI had severe interlobular emphysema (Figure 3.12).

Most calves challenge exposed with BRSV alone had mild inflammatory lesions scattered in 1 or 2 lung lobes. In 1 calf, however, there were moderate to severe lesions that were typical of BRSV infection. In larger bronchioles, there was necrosis or hyperplasia of epithelium with mixed inflammatory cell infiltrates in the lumina and lamina propria. In small bronchioles, there was necrosis of lining cells and syncytia formation. The lumina of bronchioles contained aggregates of necrotic cells and inflammatory cells. In the surrounding parenchyma, there was congestion, accumulation of protein-rich fluid in the alveolar spaces with multifocal incipient hyaline membrane formation, and mild to moderate mixed inflammatory cell infiltration in the interstitium and alveolar spaces. Two of the 5 control calves did not have histologic pulmonary lesions. In 3 of the 5 control calves, there were minimal inflammatory changes scattered in 1 or 2 lung lobes consisting of mild infiltration of small airways and alveoli by neutrophils.
Parenchymal histologic score for calves in the BRSV-3MI challenge exposure group (median, 17; range, 6 to 22) was not different ($P = 0.06$) than that for the 3MI challenge exposure group (median, 17; range 4 to 28). Parenchymal histologic scores for the BRSV challenge exposure group (median, 1; range, 0 to 12) and the control group (median, 3; range, 0 to 5) were similar. Airway histologic score was 0 (range, 0 to 9) for the BRSV-3MI challenge exposure group, 0 (range, 0 to 6) for the 3MI challenge exposure group, 1 (range, 0 to 14) for the BRSV challenge exposure group, and 0 (range, 0 to 6) for the control group.

3.4.6 Microbiologic findings

The BRSV was identified with immunohistochemical techniques (Figure 3.13) and immunofluorescence in lung tissue from 1 calf challenge exposed with BRSV and 3MI and 1 calf challenge exposed with BRSV alone. The BRSV was cultured from lung tissues of the calf challenge exposed with BRSV alone. Lung tissue specimens from all calves did not yield Pasteurella spp or Haemophilus spp.
3.5 Discussion

Results of this study suggest that BRSV and 3MI can act synergistically to induce more severe respiratory disease than would be expected from simple addition of effects of exposure to each agent individually. The clinical and postmortem findings of the control and BRSV challenge exposure groups were similar, implying that BRSV challenge exposure alone did not result in measurable disease. Thus, if the effect of combined exposure to BRSV and 3MI had been additive, clinical and postmortem findings for the BRSV-3MI and 3MI challenge exposure groups would have been similar. However, lesions in the BRSV-3MI challenge exposure group were more severe than in the 3MI challenge exposure group, supporting our hypothesis that the effects of the agents were synergistic. Respiratory disease developed earlier, clinical disease was most severe, and mortality rate was highest among cattle in the BRSV-3MI challenge exposure group. In addition, interstitial edema, interstitial emphysema, and emphysematous bullae were most severe in the group receiving the combined exposure, as indicated by greater lung displacement volume. Synergy between BRSV and 3MI may contribute to the variability in the incidence of BRD in feedlots where exposure to infectious agents may be ubiquitous.

3-Methylindole is produced by microflora in the rumen of all cattle. It has been established that management changes can affect the amount of 3MI produced by cattle. Severe 3MI toxicosis, as typified by ABPE in pastured cattle, has not been recognized in feedlot cattle, and although the quantity of 3MI produced by feedlot cattle has not been documented, it is unlikely that such quantities would be as large as those used in this
experiment. However, small increases in 3MI production possibly could cause subclinical pulmonary damage, which might predispose cattle to BRD typically seen in feedlots.

Pathologic findings attributed to ABPE, BRSV, and atypical interstitial pneumonia (AIP) are very similar; however, conclusive evidence of a direct link between these conditions has not been identified. 3-Methylindole might function as an initiator or promoter that increases the likelihood that clinical respiratory disease will develop when cattle become infected with BRSV or other respiratory pathogens.

The pathogenesis of 3MI toxicosis has been reviewed. Synergism between 3MI and BRSV may be the result of pulmonary damage that compromises pulmonary defense mechanisms and predisposes to infection. Free radicals are formed as products of 3MI metabolism and cause oxidative damage in pulmonary tissues. Further inflammation and damage in pulmonary tissues may interfere with effective viral clearance by the immune system.

In typical livestock production programs, young, immunologically naive cattle are stressed by weaning, exposure to auction markets, and transport to feedlots. During this process, cattle typically have inadequate feed intake because of stress, competition, socialization with strange cattle, and limited access to feed. After arriving at feedlots, cattle are offered optimal quantities of a balanced rations that include high quality protein. These changes alter the population balance of microbes in the rumen environment, and favor proliferation of Lactobacillus spp. The availability of adequate substrate together with changes in rumen microflora may predispose cattle to produce
larger quantities of 3MI than normal. At the same time, these young cattle are exposed to multiple infectious agents through co-mingling of animals from diverse sources. Infection with BRSV is common in feedlot cattle as has been revealed in serologic studies, but clinical disease does not always result from these infections.\textsuperscript{12,14,30,31} It is possible that an initiator or promoter such as 3MI exposure is needed to induce disease, and that the synergy between BRSV and 3MI identified in this experiment is clinically relevant in feedlot cattle.

Clinical disease that would be considered significant in a feedlot setting was not identified in the BRSV challenge exposure group in this study. Other researchers have reported similar difficulties in inducing clinical disease after experimental inoculation with BRSV.\textsuperscript{13,32-35} However, 8 of the 20 calves used in this study had evidence of previous exposure to BRSV, as is typical of young calves entering feedlots.\textsuperscript{30} Although titers were low in these calves, evidence of previous exposure suggests that anamnestic responses mediated by memory B or T cells may have enhanced immune-mediated clearance of the virus. However, the paucity of histologic changes in the BRSV infected calves, together with the more severe gross and histopathologic changes in the cattle that received BRSV and 3MI, suggests that subclinical BRSV infection could be an important cofactor in the pathogenesis of ABPE or AIP in feedlot cattle.

Results of the study reported here were more dramatic than was seen in a previous study examining combined exposure to BRSV and 3MI.\textsuperscript{18} Despite the fact that cattle in this previous study were given a 2.5 times greater dose of 3MI in combination with intratracheal BRSV challenge, calves did not develop disease as severe as reported
herein, and disease was not enhanced by combined exposure. There is evidence that repeated passage of BRSV isolates affects the virulence of that virus. Castleman et al. used a high passage BRSV isolate that had minimal replication in alveolar tissue of calves, and suggested that this likely affected results of their investigation. The BRSV isolate used in our study was a low passage isolate that was obtained from an adult cow during an outbreak of severe respiratory disease. Aerosol challenge used in this study also may have influenced the success of viral challenge and the observed synergy. In addition, calves used by Castleman et al. were much younger than those used here, and age may affect expression of this synergy. It is possible that the metabolic pathways for 3MI change as cattle mature. Differences in immunity also could have affected these results as the young calves used by Castleman et al. could have had higher concentrations of maternally derived serum antibody.

Evidence of synergy between BRSV and 3MI suggests that control combined exposure may be important in feedlot cattle. Lasolocid and monensin have been used to control ABPE in pastured cattle and may aid in prevention of respiratory disease in feedlot cattle through inhibition of rumen Lactobacilli that convert tryptophan to 3MI. Pulmonary damage caused by 3MI can be prevented or attenuated by influencing production of oxidative metabolites. Improving immunity to BRSV may be more important than has been previously realized.
3.6 Footnotes

\(^a\) Stroke Environ\(^{\textregistered}\), Merck & Co. Inc., St. Louis, MO 63110

\(^b\) 3-Methylindoie (Skatole), Sigma Chemical Company, St. Louis, MO 63178

\(^c\) Beuthanasia\(^{\textregistered}\)-D Special, Schering-Plough Animal Health Corp., Kenilworth, NJ 07033

\(^d\) Unopette\(^{\textregistered}\) White Cell Test, Becton-Dickinson, Franklin Lakes, NJ 07417
3.7 Acknowledgments

The authors thank Drs. Beverly Byrum, Paul French Sally Goclan, Sheila Grimes, Kevin Lahmers, Sarah Lathrop, Bimbo Welker, Diane Gross, and Ram Mohan for technical assistance.
Figure 3.1—Respiratory rate (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.2—Heart rate (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.3—Rectal temperature (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.4—Serum 3-methylindole concentrations (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.5—Serum fibrinogen concentrations (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.6—Serum total plasma protein concentrations (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.7—Packed cell volume (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.8—Total white blood cell count (mean ± SEM) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI).
Figure 3.9—Photographs of lungs (dorsal view) from calves challenge exposed with BRSV-3MI (a), 3MI (b), BRSV alone (c), or neither (control group, d). Notice the subpleural emphysema and edema in the caudodorsal regions and the failure of lungs to collapse in calves challenge exposed with BRSV-3MI or 3MI. Distention caused by bullae formation in caudal lobes was prominent in lungs from cattle challenge exposed with BRSV-3MI. Also notice the lobular consolidation in the cranioventral regions of lungs from the calf challenge exposed with BRSV alone.
Figure 3.10—Lung weight (least squares mean ± SEM, controlling for differences in body weight) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI). Different superscript letters indicate significantly (P < 0.05) different mean values.
Figure 3.11—Lung displacement volume (least squares mean ± SEM, controlling for differences in body weight) in cattle (n = 5/group on day 0) after challenge exposure with bovine respiratory syncytial virus (BRSV), 3-methylindole (3MI), or a combination of BRSV and 3MI (BRSV-3MI). Different superscript letters indicate significantly (P < 0.05) different mean values.
Figure 3.12—Photomicrographs of representative histologic sections of lungs from calves challenge exposed with BRSV and 3MI (a, severe interstitial pneumonia; 3MI alone (b, moderate interstitial pneumonia); BRSV alone (c, mild bronchointerstitial pneumonia); or neither agent (d, control group). Notice the prominent hyperplasia of type-II pneumocytes, as well as inflammatory cells and fibrin, in lungs of calves that received 3MI alone or in combination with BRSV. H&E stain; bars = 100 μm.
Figure 3.13—Photomicrograph of BRSV (dark stain) in numerous bronchial epithelial cells in severe necrotizing lesion in the lung of a calf challenge exposed with BRSV. Immunoperoxidase stain; bar = 50 μm.
3.8 References


CHAPTER 4

THE POTENTIAL PROPHYLACTIC EFFECTS OF TREATMENT WITH ACETYLSALICYLIC ACID (ASPIRIN) AND D-α-TOCOPHEROL (VITAMIN E) TO PREVENT 3-METHYLINDOLE PNEUMOTOXICOSIS IN CALVES

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4.1 Abstract

**Objective** To determine if d-alpha-tocopherol (vitamin E) and acetylsalicylic acid (aspirin), either alone or in combination, can mitigate the pneumotoxic effects of 3-methylindole (3MI) in calves.

**Design** Two by two factorial, masked experiment, with random assignment within gender to the four treatment groups: control, vitamin E, aspirin, or aspirin and vitamin E.

**Animals** Twenty mixed-breed beef calves.

**Procedure** Calves were pre-treated with vitamin E (1500 IU IM q 24 hours x 4 days) and oral aspirin (100 mg/kg [45 mg/lb] PO). All calves were challenged with 0.1 g/kg intraruminal 3MI. Clinical examinations were performed daily before and after 3MI challenge. Serial serum 3MI and vitamin E concentrations were determined throughout the experiment. Surviving calves were euthanatized 7 days after challenge. Pulmonary tissues were examined for gross and histologic lesions post-mortem.

**Results** Aspirin and vitamin E alone did not abrogate the pneumotoxic effects of 3MI in these calves. However, these results suggest potential protection in calves treated with both vitamin E and aspirin. The combined treatment
group had lower mortality, delayed onset of depression, lower lung weight and volume, and lower gross and histologic pulmonary lesion scores when compared to all other treatment groups. Comparisons of these outcomes were similar among the control, aspirin, and vitamin E treatment groups.

**Conclusions** Subclinical lung damage caused by 3MI metabolism may contribute to the susceptibility of feedlot calves to common respiratory pathogens. Inhibition of 3MI metabolism by oral aspirin and injectable vitamin E at feedlot entry may be possible because serum 3MI concentrations under natural conditions are much lower than those induced in this experiment.
3-Methylindole (3MI) is recognized as the pneumotoxic agent associated with acute bovine pulmonary edema and emphysema (ABPE). Under natural conditions, mature cattle abruptly changed from poor quality feedstuffs to high quality forages may develop ABPE. The ingestion of poor quality forages favors the proliferation of \textit{Lactobacillus} spp. which predominate under low energy conditions in the rumen. An abrupt dietary change to better quality forages provide rumen \textit{Lactobacillus} spp. with higher concentrations of dietary l-tryptophan which is converted to 3MI. In cattle, 3-methylindole is absorbed into the systemic circulation and is metabolized by cytochrome P-450 mixed function oxidase (MFO) and prostaglandin H synthase (PHS) enzymes located in the smooth endoplasmic reticulum of Clara cells and type I pneumocytes. This metabolic step produces unstable oxidative intermediates which have been characterized as being peroxides, superoxides, and N-centered free radicals. These reactive oxygen species may bind to cell membranes, intracellular proteins, and nucleic DNA causing cellular damage and death.

The metabolism of 3MI by prostaglandin H synthase in goat lung microsomes has been shown to be inhibited \textit{in vitro} by indomethacin, a cyclooxygenase inhibitor. Also, Acton et al has reported that goats treated with aspirin, another cyclooxygenase inhibitor, prior to experimental challenge with 3MI were reported to have less severe clinical signs and gross and histologic pulmonary lesions when compared to goats treated with aspirin post-challenge or control goats. Vitamin E is a fat soluble vitamin that inhibits peroxidation of lipid cell membranes, and partially inhibits production of prostaglandin
by blocking *in vitro* metabolism of arachidonic acid.\textsuperscript{13} A possible synergistic relationship between aspirin and vitamin E has also been reported.\textsuperscript{14} Pre-incubation of cultured endothelial cells with both aspirin and vitamin E increased their viability after hydrogen peroxide was added to the cultures when compared to the additive effects of each individual drug.\textsuperscript{14}

The purpose of this study is to evaluate whether treatment with aspirin and vitamin E, either alone or in combination, prior to experimental challenge with oral 3MI may mitigate the pneumotoxic effects of 3MI in cattle.
4.3 Materials and methods

4.3.1 Study design

Twenty mixed-breed beef calves were purchased from a single farm and transported approximately 80 miles to The Ohio State University on day -7. Calves were co-mingled in a small pasture (approximately 30,000 sq. ft.). They grazed this pasture and were also fed 0.45 kg (1 lb) per head per day of a commercial concentrate mix along with grass/legume hay.

Calves were randomized within gender and assigned to four equal sized groups in a 2x2 factorial study design. The treatment groups were as follows: cattle treated with aspirin prior to 3MI challenge, cattle pre-treated with vitamin E, cattle pre-treated with both aspirin and vitamin E, and an untreated control group that was not treated with either drug prior to challenge.

All calves were examined and treated with tilmicosin\(^a\) (9.9 mg/kg [4.5 mg/lb], SQ) on day -4 and day -1. Rectal temperature was recorded once daily, and respiratory rate was recorded 1 to 2 times per day throughout the experiment (day 0 through day 7). All calves were also evaluated for the presence of clinical signs of depression, anorexia, dehydration, nasal discharge, dyspnea, open mouth breathing, and expiratory grunting throughout the experiment. The same investigator performed all assessments and treatment group was masked throughout the experiment. Handling of calves was minimized after oral challenge with 3MI to reduce exacerbation of disease due to stress.

Calves in the vitamin E and aspirin-vitamin E groups were treated with vitamin E (d-alpha-tocopherol,\(^b\) (1500 IU IM q 24 hours) on day -4 through day 3 post-challenge.
Calves in the aspirin and control groups were treated with a similar volume of propylene glycol (5ccs IM q 24 hours). Syringes were prepared for each calf prior to the study and were painted with opaque paint to mask contents. Calves in the aspirin and aspirin-vitamin E groups were treated with a single bolus of aspirin (15.4 g PO) 2 hours prior to challenge with 3MI. Calves in the vitamin E and control groups were given an oral gelatin capsule containing sucrose as a placebo.

3-Methylindole used in the challenge was dissolved in 99% ethanol to form a solution containing 350mg 3MI per milliliter. All calves received a single intraruminal dose of 3MI (0.1 g/kg [0.045 g/lb] PO) delivered via an orogastric tube at time = 0.

Animal care and treatment protocols were reviewed and approved by the Animal Care and Use Committee at OSU prior to initiation of this study. Study personnel strictly adhered to all guidelines for the care and use of animals.

4.3.2 Euthanasia

During the course of the study, calves that were not considered likely to survive another 24 hours after examination were euthanized using sodium pentobarbital (43mg/kg [20 mg/lb] IV) and sodium phenytoin (5.5 mg/kg [2.5 mg/lb] IV). This assessment was made by investigators who were unaware of treatment group assignments and was based upon evidence of dyspnea, respiratory distress, depression, and dehydration. All calves that survived through the observation period were euthanized on day 7 post-challenge.
4.3.3 Post mortem examination

Lung tissues of all calves were examined within 6-8 hours of death. Lung weight was measured to the nearest 0.1 kg [0.05 lb], and lung displacement volume was determined by submersing the lungs in water and measuring the volume of water displaced to the nearest 0.01 L for each calf. Gross pulmonary parenchymal and pleural lesions were scored for each lung lobe using an interval scoring system (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The presence of edema, congestion, hemorrhage, emphysematous bullae, interstitial emphysema, fibrin, and fibrosis was scored in each lung. Pulmonary consolidation in each lung lobe was estimated as a percentage of the tissue mass in that lobe. Lung tissue samples were collected from each lung lobe at similar anatomic locations and processed for histologic examination and bacterial and viral culture. Histologic pulmonary parenchymal lesions were assessed for each sample and assigned a numerical score (0 = no pathological changes through 4 = severe pathological changes) by a pathologist who was unaware of treatment group assignments. Parameters assessed histologically included evidence of pulmonary edema, hyaline membrane formation, thickening of interalveolar septa, proliferation of type II pneumocytes, and intra-alveolar or interstitial infiltration by inflammatory cells.

4.3.4 Laboratory analyses

A whole blood sample was collected from each calf on day -4 and at 0, 3, 6, 12, 24, 48, 72, 96, 120, and 144 hours post 3MI challenge. Blood samples were placed on ice
and serum was harvested within two hours after collection. Serum samples were stored at
-20 °C (±4 °F) until processed to determine serum 3MI and vitamin E concentrations.

Serum samples were analyzed for 3MI concentration using a technique modified
from Mortensen and Sorensen. Briefly, samples were extracted using 99.9% ethanol and
then centrifuged. The supernatant was mixed with a solution containing
4-dimethylaminobenzaldehyde which reacted with 3MI to form a purple reaction product
that was used to determine the concentration of 3MI spectrophotometrically by
comparison to a standard curve.

Serum vitamin E concentrations were measured using high pressure liquid
chromatography (HPLC). Briefly, ascorbic acid in ethanol and saturated potassium
chloride was added to samples and mixed. Petroleum ether was added and layered with
nitrogen. This was mixed for 5 minutes and then centrifuged. The petroleum ether layer
was pipetted into a glass tube and evaporated with no heat and nitrogen gas. Petroleum
ether was added to the evaporated sample and layered with nitrogen, mixed and
evaporated again. Evaporated samples were reconstituted with methanol and analyzed for
vitamin E concentration using HPLC.

Pooled lung lobe samples from each calf were cultured for Pastuerella spp.,
Actinomyces spp., Haemophilus spp, and Mycoplasma spp., and inoculated in tissue
culture for viral isolation.
4.3.5 Statistical analyses

Descriptive statistics were calculated and data were examined graphically. Physical examination data were recorded as dichotomous variables (yes/no) and reported graphically. Serum vitamin E and 3MI concentrations were also summarized graphically. While clinical information was collected over time during this experiment, repeated measures analyses of variance were not performed because several calves died prior to completion of the experiment and complete time-series information were therefore unavailable. These data were instead summarized graphically. Differences between treatment group morbidity and selected physical examination parameters were assessed using the chi-square test. Multivariable ANOVA (MIXED Procedure) was used to assess differences in lung weight and lung displacement volume among treatment groups. Calf body weight was forced into all models to control its potential confounding effect on both lung weight and lung displacement volume. Multiple comparisons among treatment groups were evaluated using the Tukey-Kramer method of correction. Gross and histologic lung lesion data was converted to a weighted overall lung score by multiplying the gross or histologic lung lesion score of the individual lobe by the percent the lobe contributes to the overall lung volume. Comparisons of weighted gross and histologic lung lesion scores among groups were performed using the Kruskal-Wallis test.
4.4 Results

4.4.1 Descriptive

Mean ± SD weight of calves upon arrival was 198 ± 22.6 kg (436 ± 50 lbs) and was not different among treatment groups. Five of the 20 calves were females, and at least 1 of the 5 heifers was randomly assigned to each group.

4.4.2 Laboratory analyses

Overall mean ± SD serum 3MI concentration on day 0 (immediately prior to challenge with 3MI) was 2.27 ± 1.65 μg/ml. Mean serum 3MI concentrations for each treatment group over time are summarized (Figure 4.1). The highest serum 3MI concentrations were measured at 3 hours in all treatment groups and concentrations returned to baseline values 24 to 48 hours after challenge. Overall mean serum 3MI concentration was 16.62 ± 2.04 μg/ml at 3 hours post-challenge.

Overall mean ± SD serum vitamin E concentration upon arrival was 5.63 ± 1.60 μg/ml and was not different among treatment groups. Mean serum vitamin E concentrations for each treatment group over time are summarized (Figure 4.2). As expected, mean serum vitamin E concentration on day 0 was higher in calves in the vitamin E and aspirin-vitamin E treatment when compared to calves in the control and aspirin treatment groups ($P < 0.001$) and remained higher in those two treatment groups through 96 hours post-challenge ($P < 0.005$).
4.4.3 Clinical evaluation

Two of 5 calves in the control, aspirin, and vitamin E treatment groups died during the study period compared to 0 of 5 in the group that received both aspirin and vitamin E. However, statistical differences in mortality among treatment groups could not be detected. Calves died on days 2 and 6 in the control group, on days 2 and 3 in the vitamin E treated group, and on days 3 and 6 in the aspirin treated group.

Physical examination parameters varied greatly within treatment groups after challenge with 3MI, but rectal temperature, anorexia, and coughing were not different among treatment groups. Mean respiratory rates for all treatment groups over time are summarized (Figure 4.3). Respiratory rates appeared similar in all treatment groups, and were within normal limits (30-40 breaths per minute) before 3MI challenge. They increased over time and peaked 4-6 days post-challenge at greater than 80 breaths per minute.

The proportion of surviving calves exhibiting signs of depression over time are summarized (Figure 4.4). Depression was recorded in all treatment groups, but the onset of depression occurred 36 hours later and the proportion of surviving calves exhibiting depression was lower in the aspirin-vitamin E treatment group compared to the other treatment groups.

The proportion of surviving calves in each group exhibiting dyspnea over time are summarized (Figure 4.5). Surviving calves in the control, aspirin, and vitamin E treatment groups began exhibiting dyspnea 2 days after challenge with 3MI and high proportions of surviving calves in these three treatment groups exhibited dyspnea from
day 3 to 5 after 3MI challenge. Calves in the aspirin-vitamin E treatment group began to exhibit dyspnea 36 hours after it was first observed in the other 3 treatment groups. A lower proportion of calves in the aspirin-vitamin E treatment group exhibited dyspnea on days 3 to 5 post 3MI challenge when compared to the other three treatment groups.

4.4.4 Gross and histologic post mortem findings

Least square mean ± SEM lung weight for each treatment group is summarized in Figure 4.6. Least square mean lung weight was 3.77 ± 0.54 kg (8.29 ± 1.19 lbs) for the control group, 3.70 ± 0.53 kg (8.14 ± 1.17 lbs) for the aspirin treatment group, 4.50 ± 0.53 kg (9.90 ± 1.17 lbs) for the vitamin E treatment group, and 2.74 ± 0.53 kg (6.03 ± 1.17 lbs) for the aspirin-vitamin E treatment group. Least square mean lung weight was not statistically different among treatment groups.

Least square mean ± SEM lung displacement volume for each treatment group is summarized in Figure 4.7. The least square mean lung displacement volume was 4.82 ± 0.64 L for the control group, 5.21 ± 0.63 L for the aspirin treatment group, 5.18 ± 0.63 L for the vitamin E treatment group, and 3.74 ± 0.63 L for the aspirin-vitamin E treatment group. Least square mean lung displacement volume was not statistically different among treatment groups.

Weighted gross and histologic lung lesion scores for each treatment group are summarized in Table 4.1. Differences in weighted gross and histologic lung lesion scores compared among treatment groups were not detected, but suggested a protective effect in the aspirin-vitamin E treatment group compared to other groups.
4.4.5 Microbiologic findings

*Pasteurella hemolytica, Pasteurella multocida, Actinomyces pyogenes, Haemophilus somnus,* and *Mycoplasma* spp. were not cultured from pooled lung samples of any calf. Viral cultures for all calves were negative for bovine herpes virus type 1, bovine viral diarrhea virus, parainfluenza virus type 3, and bovine respiratory syncytial virus.
4.5 Discussion

While we did not have adequate statistical power to detect differences, these data suggest that concurrent treatment with aspirin and vitamin E may partially mitigate the pneumotoxic effects of 3MI in calves. The aspirin-vitamin E treatment group had lower mortality, delayed onset of depression and dyspnea, and lower gross and histologic lung lesion scores when compared to all other treatment groups. In contrast, these outcomes were similar among the control, aspirin, and vitamin E treatment groups.

Others have reported that goats treated with aspirin prior to experimental challenge with oral 3MI had less severe signs of clinical respiratory disease and post mortem lung lesions compared to untreated goats or those administered aspirin after challenge with 3MI. The antioxidant effects of aspirin and vitamin E have also been shown to protect endothelial cells in vitro from the oxidizing effect of hydrogen peroxide. Although combined treatment with aspirin and vitamin E did not result in complete protection of calves against 3MI induced pneumotoxicity and could not be detected statistically, the clinical signs and post mortem lung findings observed in the aspirin-vitamin E treatment group were less severe compared to those observed in the other treatment groups. Thus, these data suggest that concurrent treatment with aspirin and vitamin E prior to challenge with 3MI may partially protect against 3MI induced pulmonary toxicity in calves. However, the dose of 3MI administered in the experiment reported here may have overwhelmed individual protective effects of aspirin and vitamin E.
In general, clinical signs (tachypnea, depression, dyspnea) observed in calves in the aspirin-vitamin E treatment group occurred later and were not as severe as those observed in calves in the other 3 treatment groups. However, clinical examination outcomes were difficult to interpret due to deaths among calves in the control, aspirin, and vitamin E treatment groups. Mortality within the first 3 days after oral challenge with 3MI eliminated calves within the respective treatment groups which were exhibiting the most severe clinical signs of respiratory disease. For this reason, clinical outcome data was summarized graphically.

Calves simultaneously challenged with 3MI and BRSV have been reported to develop more severe respiratory disease when compared to calves challenged with either agent alone. It is possible that synergy between 3MI and common respiratory pathogens may occur under natural conditions. Cattle moving from farm to feedlot are typically changed from relatively poor quality pastures to better quality rations in the feedlot. High quality rations contain optimum quantities of L-tryptophan which may increase rumen 3MI concentrations. At the same time, cattle are potentially exposed to many common respiratory pathogens, including BRSV, due to co-mingling cattle from many different sources in cattle auctions and in feedlots. It is possible that a combination of aspirin and vitamin E administered to feedlot cattle upon entry may prevent a proportion of bovine respiratory disease attributable to this synergistic relationship. Data generated by our group and several studies reporting plasma and rumen 3MI concentrations after natural induction of ABPE suggest that serum 3MI concentrations in field cases of ABPE are not as high as serum 3MI concentrations in cattle experimentally dosed with oral 3MI. It
is possible that combined treatment with aspirin and vitamin E may abrogate 3MI pneumotoxicity at lower levels of exposure to 3MI. Bray\textsuperscript{19} has reported that the duration of PHS inhibition by aspirin, as measured by thromboxane B\textsubscript{2} production in bovine platelets, is greater than 48 hours. Thus, a single dose of oral aspirin and injectable vitamin E administered to cattle upon feedlot entry may be effective for a sufficient time period to mitigate the pneumotoxic effects of 3MI after an abrupt dietary change similar to those experienced by cattle entering feedlots.
4.6 Footnotes

a Micotil® 300, Elanco Animal Health, Indianapolis, IN 46285

b Vital E™-300, Schering-Plough Animal Health Corp., Union, NJ 07083

c 1,2-Propanediol (propylene glycol), Sigma-Aldrich Chemical Company,
   P.O. Box 14508, St. Louis, MO 63178

d Aspirin Boluses, The Butler Company, 5000 Bradenton Ave., Dublin, OH 43017-0753

e 3-Methylindole (Skatole), Sigma-Aldrich Chemical Company,
   P.O. Box 14508, St. Louis, MO 63178

f Beuthanasia®-D Special, Schering-Plough Animal Health Corp., Union, NJ 07083

g SAS version 6.12, SAS Institute Inc, Cary, NC 27513-2414
4.7 Acknowledgments

The authors thank Drs. Beverly Byrum, Sheila Grimes, Ram Mohan, Diane Gross, and Bimbo Welker for technical assistance.
Figure 4.1—Serum 3-methylindole concentrations (mean ± SEM) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole.
Figure 4.2—Serum vitamin E concentrations (mean ± SEM) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole.
Figure 4.3—Respiratory rates (mean ± SEM) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole.
Figure 4.4—Depression (% of live remaining cattle affected) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole.
Figure 4.5—Dyspnea (% of live remaining cattle affected) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole.
Figure 4.6—Lung weight (least squares mean ± SEM, controlling for differences in body weight) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole. Different superscript letters indicate significantly ($P < 0.05$) different mean values.
Figure 4.7—Lung displacement volume (least squares mean ± SEM, controlling for differences in body weight) in cattle (n = 5/group on day 0) that were treated with aspirin, vitamin E, or a combination of aspirin and vitamin E (aspirin & vitamin E) prior to challenge exposure to 3-methylindole. Different superscript letters indicate significantly ($P < 0.05$) different mean values.
<table>
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<th>Score (± SE)</th>
<th>Control</th>
<th>Aspirin</th>
<th>Vitamin E</th>
<th>Aspirin + Vitamin E</th>
</tr>
</thead>
<tbody>
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<td>0.67 ± 0.61</td>
<td>0.39 ± 0.61</td>
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Table 4.1—Weighted gross and histologic mean (± SEM) lung lesion scores for each treatment group. Gross lung lesions were graded from 0-3 (0 = absent, 3 = severe) and histologic lung lesions were graded from 0-4 (0 = absent, 4 = severe). Statistical differences in gross or histologic lung lesion scores were not detected among treatment groups.
4.8 References


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CHAPTER 5

THE ABILITY OF ORAL ASPIRIN TO MITIGATE THE EFFECTS OF 3-METHYLINDOLE PRODUCTION BY FEEDLOT CATTLE

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Supported by grants from The Ohio State University and The Ohio State University College of Veterinary Medicine. Facilities and cattle used were provided courtesy of the Ohio Department of Rehabilitation and Correction.
5.1 Abstract

Objective: To evaluate the ability of oral aspirin administered to cattle upon feedlot arrival to mitigate 3-methylindole (3MI) induced respiratory disease and reduced rate of gain.

Design: Masked, randomized, controlled field trial.

Animals: Two hundred forty-four beef cattle.

Procedure: Calves were systematically randomized to either receive a single oral dose of aspirin (31.2 grams) upon entry into a feedlot or to serve as an untreated control. Serum 3MI concentrations were measured on day 0, 3, and 6 after feedlot entry. Rumen 3MI concentration was also measured on day 3. Cattle were observed daily for clinical signs of respiratory disease by feedlot personnel. Lungs were evaluated at slaughter for the presence of chronic gross pulmonary lesions.

Results: Mean daily gain (MDG) in cattle treated with aspirin compared to control cattle was 0.06 kg higher in the backgrounding unit and 0.03 kg higher for the overall feeding period. However, serum or rumen 3MI concentrations did not appear to modify this effect. Cattle in the aspirin treatment group were more likely to be treated for respiratory disease compared to cattle in
the control group. Mortality rates, chronic gross pulmonary lesions, and serum and rumen 3MI concentrations were similar between groups. Increased rumen 3MI concentration was associated with a small difference in the risk of post mortem lung fibrosis.

**Conclusions:** Cattle given a single oral dose of aspirin upon feedlot entry had higher MDG in the backgrounding unit and for the overall feeding period. However, we could not demonstrate that the observed effect of aspirin treatment on mean daily gain was due to the mitigation of a negative 3MI effect on respiratory disease or mean daily gain in these cattle. This may have been influenced by relatively low peak 3MI production in these cattle and by relatively slow rates of gain.
5.2 Introduction

Historically, strategies to prevent bovine respiratory disease (BRD) in feedlot cattle have focused primarily on vaccination against viral and bacterial pathogens that are commonly associated with BRD. Prophylactic or metaphylactic injections of long acting antimicrobials on arrival have also been shown to improve health and dramatically increase productivity.\textsuperscript{1-5} The association between a single dose of an antimicrobial drug with improved health and performance underscores the likelihood that subclinical respiratory disease is common near the time that cattle enter the feedlot. Intervention during this critical window of opportunity may be particularly effective for improving the overall health and productivity of feedlot cattle.

Feedlot cattle are commonly required to make a dietary transition from poor quality, late summer pastures to high quality rations offered in feedlots. This abrupt dietary change may increase rumen production of 3-methylindole (3MI) which could result in pulmonary damage. Under conditions similar to this, ruminal \textit{Lactobacillus} spp. in adult cattle abruptly moved from poor quality pasture to lush green pastures have been shown to convert L-tryptophan contained in the forage to 3MI.\textsuperscript{6,7} This increased production of 3MI is known to result in clinical cases of acute bovine pulmonary emphysema and edema in some cattle.\textsuperscript{6,7} 3-Methylindole is naturally produced in the colon and rumen of several species and is further metabolized to pneumotoxic compounds in the bovine lung by the cytochrome P-450 mixed function oxidase system and prostaglandin H synthase found in the smooth endoplasmic reticulum of type I pneumocytes and clara cells.\textsuperscript{8} It has been previously suggested that the small amounts of
3MI that are constantly produced in the rumen of cattle, although insufficient to cause clinical respiratory disease, may be sufficient to cause subclinical lung damage. It is also possible that low levels of 3MI act synergistically with common feedlot pathogens to cause a proportion of the morbidity attributed to undifferentiated BRD in feedlot cattle.

Previous work has shown that cattle experimentally challenged with oral 3MI and simultaneously infected with bovine respiratory syncytial virus developed more severe clinical disease and lung lesions compared to cattle challenged with either agent alone.

Aspirin is a non-steroidal anti-inflammatory drug which has been shown to inhibit prostaglandin H synthase in human endothelial cells in vitro. Goats given a single dose of aspirin prior to experimental challenge with oral 3MI were reported to have less severe clinical disease and lung lesions compared to goats that did not receive aspirin or goats that were dosed with aspirin after oral administration of 3MI.

The purpose of this study was to evaluate the effectiveness of administering a single oral dose of aspirin to cattle upon feedlot entry to mitigate effects of 3MI on respiratory disease and rates of gain.
5.3 Materials and methods

5.3.1 Study design

Two hundred forty-four mixed breed beef cattle were purchased at a public cattle auction over a four week period and transported approximately 60 miles to a backgrounding facility. Routine processing occurred within 24 hours after arrival (day 0) and all cattle were individually weighed and identified with an ear tag. Cattle were vaccinated with bovine herpesvirus type 1, bovine viral diarrhea virus, parainfluenza virus type 3, bovine respiratory syncytial virus, Pasteurella haemolytica, P. multocida, Haemophilus somnus, a multivalent Clostridium spp. bacterin, and a multivalent Leptospira spp. bacterin. Cattle received ivermectin (0.2 mg/kg [0.09 mg/lb], SQ) and were dehorned and castrated as necessary. After 45-70 days in the backgrounding operation, cattle were transported to 1 of 4 feedlots and fed for the remainder of the feeding period. All cattle were weighed prior to transportation to calculate mean daily gain during the backgrounding period.

Cattle were randomly assigned to 2 treatment groups for this study. One group (n = 123) received 31.2 g (approximately 100mg/kg [45 mg/lb], PO) aspirin at the time of processing and the other group (n = 121) served as untreated controls.

Farm personnel who were unaware of treatment group assignments observed the calves at least once daily during the feeding period to identify calves exhibiting clinical signs consistent with respiratory disease. For this field trial, cattle were classified as having undifferentiated respiratory disease when their rectal temperature was ≥ 39.7 C (103.5 F) and they did not have clinical signs referable to disease in another body system.
Calves diagnosed with undifferentiated respiratory disease were treated with tilmicosin phosphate\(^b\) (9.9 mg/kg [4.5 mg/lb], SQ) or long acting oxytetracycline\(^c\) (22 mg/kg [10 mg/lb], IM) and returned to the pen of origin. The date, clinical findings, presumptive diagnosis, antimicrobial drug used, dose, and route of administration were recorded.

5.3.2 Post mortem examination

The identity of individual cattle was maintained through slaughter. Estimated finished weight for overall mean daily gain calculation was calculated by multiplying the hot carcass weight by a factor of 1.667 to represent expected dressing percentage. Lungs were evaluated for the presence of gross lesions within 6 hours of slaughter by a single investigator. Lung weight was recorded for each animal after excess mediastinal tissue, pericardial tissue, and trachea were trimmed away from lungs. Pulmonary consolidation was graded by estimating the percentage of consolidation within each lung lobe. Each lung lobe was also classified for the presence of chronic lesions (consolidation, fibrosis) as dichotomous (yes/no) variables.

5.3.3 Laboratory analyses

Blood was collected on day 0, 3, and 6 for determination of serum 3MI concentration. Blood samples were placed on ice in a dark cooler after collection and transported to the laboratory where they were processed for storage within 8 hours after collection. Rumen fluid was collected from all calves by per cutaneous ruminocentesis on day 3 for determination of rumen 3MI concentration. Rumen fluid was frozen in
liquid nitrogen immediately after collection. Serum and rumen fluid samples were stored at -20 C (-4 F) until analyzed for 3MI concentration.

Concentrations of 3MI in serum and rumen fluid samples were measured using a colorimetric assay adapted from a previously described technique. Briefly, samples were extracted using absolute ethanol and then centrifuged. The supernatant was mixed with a solution containing 4-dimethylaminobenzaldehyde which reacted with 3MI to form a purple reaction product that was used to determine the concentration of 3MI spectrophotometrically by comparison to a standard curve.

5.3.4 Statistical analyses

The outcomes of interest were measures of respiratory disease and growth performance during the feeding period. Two measures of respiratory disease were investigated: clinical respiratory disease that was diagnosed after initial processing (yes/no), and the presence of chronic pulmonary lesions at slaughter (yes/no) was used as an indicator of respiratory disease that was not detected or not completely resolved by treatment. Mean daily gain was used as a measure of growth performance during the backgrounding period and for the overall feeding period.

The independent variables of primary interest to the analysis were rumen 3MI concentration, the maximum of serum 3MI concentration measured on day 0, 3, or 6, and aspirin treatment (yes/no). Multivariable logistic regression (GENMOD Procedure) was used to assess the effect of the independent variables on the dichotomous disease outcomes. Multivariable ANOVA (MIXED Procedure) was used to assess the effect of
independent variables on continuous outcomes (MDG during backgrounding and overall feeding periods). For each outcome, an initial model was specified that included the independent variables of primary interest: 3MI concentration and aspirin treatment group. Both maximum serum 3MI concentrations and rumen 3MI concentrations were evaluated in the same manner but in separate models because of collinearity.

Additional variables assessed for potential confounding included purchase group (lot), feedlot where cattle were finished, gender, body weight on day 0, treatment with an antimicrobial drug on day 0, dehorning (yes/no), and castration (yes/no). A design variable representing the purchase group (lot) was forced into all models because of the potential confounding effect on outcomes for both respiratory disease and rate of gain. Other potential confounding variables were evaluated using manual forward selection with a critical alpha for retention of 0.05. Once all potential confounding variables had been evaluated, the interaction between measures of 3MI concentration (rumen or serum) and aspirin treatment were assessed in order to evaluate data for evidence that aspirin mitigated the pneumotoxic effects of 3MI.

Final linear regression model goodness-of-fit was evaluated visually by graphing the predicted versus the residual values of the dependent variable. Effects on disease occurrence were evaluated by estimation of odds ratios (OR) and exact 95% confidence intervals (95% CI) as well as estimation of the likelihood ratio χ². Final logistic regression models were evaluated for goodness-of-fit using the Hosmer-Lemeshow statistic.
5.4 Results

5.4.1 Descriptive

Mean ± SD entry weight for all cattle was 268 ± 30 kg (590 ± 66 lbs). Cattle in the aspirin treatment group were 7.7 ± 3.8 kg (± SE; 17 ± 8.4 lbs) heavier at entry into the backgrounding unit than were cattle in the control group ($P = 0.04$). Fifty-three percent of the cattle were female and 48% were male. The mean number of days in the feedlot was 305 ± 52 (± SD) days and was not different between aspirin treatment groups. Fourteen percent of cattle were dehorned and 6% were castrated upon feedlot entry. Sixty-three percent of all cattle were treated for BRD during the feeding period, but none of them died.

5.4.2 Laboratory analyses

Mean serum 3MI concentration was 1.12 μg/ml (range = 0.30-2.90) on day 0, 0.97 μg/ml (range = 0.39-1.88) on day 3, and 1.01 μg/ml (range = 0.33-3.51) on day 6. Mean serum 3MI concentration was higher on day 0 compared to day 3 or 6 post entry ($P < 0.01$). Mean rumen 3MI concentration was 2.58 μg/ml (range = 1.22-12.47) on day 3. Differences in serum and rumen 3MI concentrations were not detected between groups.

5.4.3 Clinical evaluation

Cattle in the aspirin treatment group had slightly higher odds of treatment for clinical BRD after day 0 (OR = 1.70, 95% CI = 1.01-2.89) compared to cattle in the control group (Table 5.1). Maximum serum 3MI concentrations (OR = 0.68, 95% CI =
0.34-1.35) and rumen 3MI concentrations (OR = 0.95, 95% CI = 0.76-1.21) were not associated with clinical BRD in these cattle. A statistical interaction was not detected between 3MI concentrations and aspirin treatment, suggesting that aspirin did not modify an effect of 3MI on clinical BRD occurrence.

The least square mean ± SEM MDG for the overall feeding period was 0.78 ± 0.014 kg (1.72 ± 0.032 lbs) in aspirin treatment group cattle and 0.75 ± 0.015 kg (1.64 ± 0.032 lbs) in control group cattle (P = 0.09, Table 5.2). Maximum serum 3MI concentrations or rumen 3MI concentrations were not associated with overall MDG in these cattle. A statistical interaction was not detected between 3MI concentrations and aspirin treatment, suggesting that aspirin did not modify an effect of 3MI on overall MDG in these cattle.

The least square mean ± SEM MDG during the 45-70 day backgrounding period was 0.24 ± 0.027 kg (0.54 ± 0.057 lbs) in the aspirin treatment group and 0.18 ± 0.026 kg (0.39 ± 0.057 lbs) in the control group (P = 0.08, Table 5.3). Maximum serum 3MI concentrations or rumen 3MI concentrations were not associated with MDG during the backgrounding period. A statistical interaction was not detected between 3MI concentrations and aspirin treatment, suggesting that aspirin did not modify an effect of 3MI on MDG in the backgrounding unit in these cattle.

5.4.4 Post mortem evaluation

Cattle in the aspirin treatment group had similar risk for post mortem lung consolidation (OR = 0.71, 95% CI = 0.13-3.47) compared to cattle in the control group.
Maximum serum 3MI concentration was not associated with post mortem lung consolidation. Cattle in the aspirin treatment group had similar risk for post mortem lung fibrosis (OR = 0.66, 95% CI = 0.30-1.42) compared to cattle in the control group, and maximum serum 3MI concentration was not associated with post mortem lung fibrosis (Table 5.5). However, rumen 3MI concentration was associated with a higher likelihood of post mortem lung fibrosis (OR = 1.39, 95% CI = 1.02-1.90 per 1 μg/ml increase, Table 5.6). A statistical interaction was not detected between 3MI concentrations and aspirin treatment, suggesting that aspirin did not modify an effect of 3MI on the occurrence of post mortem lung consolidation or fibrosis in these cattle.
5.5 Discussion

We found that oral aspirin administered to cattle at feedlot arrival gained an average of 0.06 kg (0.15 lbs) more per day during the first 45-70 days in the backgrounding unit and 0.03 kg (0.07 lbs) more per day during the entire overall feeding period when compared with the control animals. This relatively small, but clinically significant difference is sufficiently large enough to warrant consideration and further research. Controlling for differences in entry weight, gender, and lot, cattle in the aspirin group were an average of 3.36 kg (7.4 lbs) heavier when exiting the backgrounding unit and 9.15 kg (20.13 lbs) heavier at slaughter compared to cattle in the control group. Higher mean daily gain in the aspirin treated group suggests that this group was healthier overall compared to the control group. However, aspirin treatment was also associated with a slightly higher risk of respiratory disease after day 0. A potential mechanism to explain why cattle administered aspirin were at slightly higher risk of respiratory disease is unknown.

Long term oral aspirin administration has been reported to increase the clinical condition and weight gain in young calves (30-60 days old) with clinical respiratory disease, but no effect was reported in clinically healthy animals. Aspirin has also been recommended as an ancillary treatment for clinical respiratory disease in feedlot cattle. Aspirin may protect against oxidative lung damage by limiting the inflammatory response associated with infectious agents and by trapping hydroxyl radicals produced by the immune system.
Outbreaks of respiratory disease in feedlot cattle are most commonly seen 10-14 days after entering the feedlot and typically last for 2-3 weeks after the first case is observed. This suggests that the timing of preventive measures is likely critical in altering the course of respiratory disease. A single injection of tilmicosin given prophylactically/metaphylactically upon feedlot entry has been reported to decrease morbidity, decrease mortality, improve mean daily gain, and improve feed conversion efficiency in cattle. Although the mechanism of action of this preventive measure has not been definitely established, it is logical that the administration of an antimicrobial drug at feedlot entry may have decreased the bacterial pathogen load in the lungs which had a positive affect on the overall health and productivity in these cattle. This demonstrates that a single, appropriately timed preventive measure can improve the long term health and productivity of feedlot cattle.

It is not possible to determine the mechanism by which cattle receiving aspirin had improved rates of gain in this study, but there are some intriguing possibilities. This study and others have reported that cattle normally produce 3MI in the rumen and colon. Metabolites of 3MI are pneumotoxic and have been shown to induce acute bovine pulmonary emphysema or fog fever in mature cattle abruptly moved from poor quality forages to lush pastures that have high concentrations of L-tryptophan. Severe respiratory disease has also been reported to occur as a result of synergy between 3MI and the bovine respiratory syncytial virus under experimental conditions. Cattle moved from poor quality, late summer or fall pastures commonly have limited feed intake while being transported from farm to a feedlot. Upon feedlot entry, cattle have access to abundant
quantities of high quality feed. This abrupt dietary change may provide rumen microbes with the needed substrate to increase rumen concentrations of 3MI. Thus, common management practices for beef cattle may actually increase rumen 3MI production. 3-Methylindole is metabolized to unstable oxidative intermediates by prostaglandin H synthase enzymes and the cytochrome P-450 mixed function oxidase system found in the smooth endoplasmic reticulum of Clara cells and type I pneumocytes in the lung.⁵,¹¹,²¹ Oxidative free radicals cause cellular damage and may impair pulmonary defenses.⁸ It has been reported that aspirin inhibits prostaglandin H synthase in vitro.¹⁰ Goats given aspirin before (but not after) an oral challenge with 3MI showed a reduction in clinical signs of respiratory disease and had reduced pulmonary damage.¹¹ A similar protective mechanism may be present in feedlot calves given aspirin upon feedlot entry.

Aspirin administration upon feedlot entry may have positive effects on pulmonary defenses. Bactericidal dysfunction caused by parainfluenza virus type 3 infection in alveolar macrophages was partially restored when a cyclooxygenase inhibitor was added to the culture one hour before the start of an alveolar macrophage bactericidal assay.²² If aspirin administration during this critical window improved bactericidal effects of alveolar macrophages, an improvement in mean daily gain in cattle treated with aspirin or other cyclooxygenase inhibitors at entry into the feedlot may be observed similar to that observed with prophylactic antimicrobial drug administration upon entry.⁴,⁵ Aspirin has also been shown to increase bacterial phagocytosis by mammary gland neutrophils in vitro.²³
Similarities in overall morbidity, mortality and pulmonary pathology between aspirin treatment groups may have been partially the result of beef production practices of the Ohio Department of Rehabilitation and Correction. Their main objective is to provide a continuous and economic supply of beef for consumption by the state’s inmate population. Therefore, many cattle participating in this study were not fed to maintain optimal growth rates after leaving the backgrounding unit. The manner in which these cattle were fed may limit the ability to extrapolate these results to commercial feedlots until they can be validated. Additional research into this area may give feedlot managers and their veterinarians an additional tool to help prevent subclinical BRD and improve the health and productivity of feedlot cattle without the risk of antibiotic residues.
5.6 Footnotes

\( ^a \)Ohio Department of Rehabilitation and Correction, 1050 Freeway Dr. North.

Columbus, OH 43229

\( ^b \)Micotil\( ^\text{®} \) 300, Elanco Animal Health, Indianapolis, IN 46285

\( ^c \)Liquamycin\( ^\text{®} \) LA-200\( ^\text{®} \), Pfizer Inc., North American Region, Animal Health Group.

812 Springdale Dr., Exton, PA 19341

\( ^d \)SAS version 6.12, SAS Institute Inc, Cary, NC 27513-2414
5.7 Acknowledgments

The authors thank Larry McChesney, Mike Clark, Garson Spencer, Robert Schleppi, Mark Levy, Dr. Beverly Byrum, and the Ohio Department of Rehabilitation and Correction for technical assistance.
Table 5.1—Final logistic regression model for estimating adjusted effects of aspirin treatment administered to cattle (n = 244) upon feedlot entry on treatment for clinical respiratory disease after day 0.

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*Maximum serum 3-methylindole concentration (µg/ml) on day 0, 3, or 6.
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*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6. F = test statistic.

Table 5.2—Final ANOVA model for estimating adjusted effects of aspirin treatment administered to cattle (n = 204) upon feedlot entry on mean daily weight gain (kg) during the overall feeding period (x̄ = 305 days).
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*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6. F = test statistic.

Table 5.3—Final ANOVA model for estimating adjusted effects of aspirin treatment administered to cattle (n = 244) upon feedlot entry on mean daily weight gain (kg) during the backgrounding period (x = 56 days).
### Table 5.4—Final logistic regression model for estimating adjusted effects of aspirin treatment administered to cattle (n = 199) upon feedlot entry on lung consolidation detected during post mortem examination.

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*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6.
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<td>3MI*</td>
<td>-0.0086</td>
<td>0.5377</td>
<td>0.99</td>
<td>0.32 - 2.69</td>
<td>0.2633</td>
</tr>
<tr>
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<td>....</td>
<td>....</td>
<td>....</td>
<td>0.32 - 2.69</td>
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</tr>
</tbody>
</table>

*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6.

Table 5.5—Final logistic regression model for estimating adjusted effects of aspirin treatment administered to cattle (n = 199) upon feedlot entry on pleural fibrosis detected during post mortem lung examination.
<table>
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<th>Variable</th>
<th>Beta (β)</th>
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<th>Odds Ratio</th>
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<th>P-value</th>
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*Rumen 3-methylindole concentration (µg/ml) on day 3.

Table 5.6—Final logistic regression model for estimating adjusted effects of aspirin treatment administered to cattle (n = 199) upon feedlot entry on pleural fibrosis detected during post mortem lung examination. Rumen 3MI concentrations on day 3 are included in this model. Maximum serum 3MI concentrations were not included in the model due to a potential confounding effect with rumen 3MI concentrations.
5.8 References


CHAPTER 6

THE EFFECTS OF 3-METHYLINDOLE PRODUCTION AND VACCINATION AGAINST BOVINE RESPIRATORY SYNCYTIAL VIRUS ON RESPIRATORY DISEASE AND RATE OF GAIN OF FEEDLOT CATTLE

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Supported by grants from The Ohio State University and the OSU College of Veterinary Medicine. Facilities and cattle used were provided courtesy of the Ohio Department of Rehabilitation and Correction. Serum neutralizing antibody assays were performed courtesy of the Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture.
6.1 Abstract

**Objective:** To evaluate the ability of immunity against bovine respiratory syncytial virus (BRSV) to mitigate the effects of 3-methylindole on respiratory disease and rate of gain in feedlot cattle.

**Design:** Masked, randomized, controlled field trial

**Animals:** Two hundred fifty-four mixed breed beef cattle

**Procedure:** Cattle entering a feedlot were systematically randomized to one of three groups: unvaccinated control, inactivated BRSV vaccination, or modified-live BRSV vaccination. Serum antibodies specific for BRSV were determined at feedlot entry and after 28 days on feed using ELISA and virus neutralization assays. Serum 3-methylindole concentrations were measured at feedlot entry and 3 days post entry. Morbidity, mortality, and mean daily gain were observed throughout the feeding period. At slaughter, lungs were weighed and evaluated for the presence of chronic gross lesions.

**Results:** Increasing serum 3-methylindole concentrations early in the feeding period were associated with lower mean daily gain for the feeding period and increased risk for clinical respiratory disease after 3 days on feed. Cattle
with higher BRSV antibody titers on arrival had lower risk of clinical respiratory disease. Cattle in the control group were more likely to be treated for respiratory disease after 3 days on feed compared to cattle in the modified-live BRSV vaccine group. Humoral immunity against BRSV did not appear to modify the effect of 3-methylindole on clinical respiratory disease or mean daily gain outcomes.

**Conclusions:** Our results suggest that abrogating the effects of 3-methylindole and BRSV infection may improve the health and growth performance in cattle entering feedlots. However, immunity against BRSV as measured in this study, did not appear to protect against potential synergism between 3-methylindole and BRSV in these cattle. This may have been affected by the relatively slow rates of gain of cattle included in this study or timing of sampling.
6.2 Introduction

Bovine respiratory disease (BRD) in feedlot cattle is a complex, multifactorial disease where many sufficient causes contribute to the overall incidence rate of disease. In a recent experiment, our group found that 3-methylindole (3MI) and bovine respiratory syncytial virus (BRSV) acted synergistically to produce more severe respiratory disease in young cattle when compared to cattle challenged with either agent alone. Cattle experimentally challenged with both 3MI and BRSV had greater mortality, greater lung weight, and greater pulmonary displacement volume when compared to cattle challenged with 3MI or BRSV alone. Gross and histologic lung lesions were also more severe. This previous research suggests that a proportion of BRD seen in cattle entering feedlots may be the result of synergism between 3MI and BRSV.

All cattle produce 3MI through metabolism of tryptophan by Lactobacillus spp. in the rumen. However, cattle that have recently entered the feedlot may experience a sudden increase in 3MI exposure at the same time that they are exposed to BRSV and other respiratory pathogens. Feed intake of cattle is generally limited while they move through public cattle auctions and during transportation to feedlots. After entering the feedlot, cattle are fed abundant quantities of high quality feed. Abruptly changing the diet and increasing intake of high quality protein is known to increase serum and rumen 3MI concentrations in adult cattle. Serological data from cattle suggest that exposure of feedlot cattle to BRSV is also common. This concurrent exposure to both 3MI and BRSV may account for a proportion of respiratory disease in feedlot cattle.
The purpose of this study was to evaluate the effect of 3MI production and immunity to BRSV (both at entry and vaccine induced) on respiratory disease and rates of gain of feedlot cattle.
6.3 Materials and methods

6.3.1 Study design

Two hundred fifty-four mixed breed beef cattle with unknown vaccination and disease histories were purchased from a commercial auction market in southern Ohio. Cattle were purchased in 3 groups over a six week period in the fall of 1996. They were transported approximately 60 miles from the auction market to a backgrounding unit operated by the Ohio Department of Rehabilitation and Correction, and given access to grass hay and water and then processed the following morning (day 0). At processing on day 0, all cattle were individually identified, weighed, and systematically randomized into 1 of 3 groups: one group received inactivated BRSV vaccination (KV), one group received modified-live BRSV vaccination (MLV), and one group was not vaccinated against BRSV and served as controls. Cattle in the BRSV MLV and BRSV KV groups were re-vaccinated against BRSV on day 14. Groups of cattle arriving at the backgrounding unit were assigned to treatment groups in approximately equal numbers and co-mingled. Treatment group assignments were masked from all personnel involved in the trial. At processing on day 0, all cattle were vaccinated with bovine herpesvirus type 1, bovine viral diarrhea virus, parainfluenza virus type 3, Pasteurella haemolytica, P. multocida, Haemophilus somnus, a multivalent Clostridium spp. bacterin, and a multivalent Leptospira spp. bacterin. All cattle were re-vaccinated on day 14 against bovine herpesvirus type 1, bovine viral diarrhea virus, and parainfluenza virus type 3. Cattle received ivermectin (0.2 mg/kg [0.09 mg/lb], SQ) and were dehorned and castrated as necessary.
Rectal temperatures of calves were recorded during processing on days 0, 3, 14, and 28. Feedlot personnel also observed the cattle once daily to identify sick cattle for closer examination. Cattle were diagnosed as a case of respiratory disease when rectal temperature was greater than 40.0 C (104 F) in the absence of clinical signs referable to disease in another organ system. Cattle diagnosed with clinical respiratory disease were treated with tilmicosin phosphate (9.9 mg/kg [4.5 mg/lb], SQ) or florfenicol (19.8 mg/kg [9 mg/lb], IM). Throughout the feeding period, feedlot personnel responsible for identifying and treating sick cattle recorded the presumptive diagnosis, antimicrobial drug used, dose, route of administration, and date of treatment. Cattle were returned to the pen of origin after examination and treatment. After 40-60 days in the backgrounding unit, cattle were transported to 1 of 3 feedlots operated by the Ohio Department of Rehabilitation and Correction and fed for the remainder of the finishing period.

6.3.2 Post mortem examination

The identity of individual cattle was maintained through slaughter. Estimated finished weight for overall mean daily gain (MDG) calculation was calculated by multiplying the hot carcass weight by a factor of 1.667 to represent expected dressing percentage. Lungs were evaluated for the presence of gross lesions within 6 hours of slaughter by a single investigator. Lung weight was recorded for each animal after excess mediastinal tissue, pericardial tissue, and trachea were trimmed away from lungs. Pulmonary consolidation was graded by estimating the percentage of consolidation within
each lung lobe. Each lung lobe was also classified for the presence of chronic lesions (consolidation, fibrosis) as dichotomous (yes/no) variables.

6.3.3 Laboratory analyses

Serum was obtained from all cattle on days 0 and 3 for determination of serum 3MI concentrations. Serum was also obtained on days 0 and 28 for determination of BRSV virus neutralizing and ELISA antibody concentration. Blood samples were placed on ice in a dark cooler and transported to the laboratory. Aliquots of serum were frozen within 8 hours of collection and stored at \(-20\) C (\(-4\) F) until analysis.

Concentrations of 3MI in serum samples were measured using a colorimetric assay adapted from a previously described technique.\(^1^3\) Briefly, samples were extracted using absolute ethanol and then centrifuged. The supernatant was mixed with a solution containing 4-dimethylaminobenzaldehyde which reacted with 3MI to form a purple reaction product that was used to determine the concentration of 3MI spectrophotometrically by comparison to a standard curve.

Bovine respiratory syncytial virus serum neutralizing antibody (BRSV-SN) titers were measured using a standard protocol at the Ohio Department of Agriculture’s Animal Disease Diagnostic Laboratory.\(^1^3,1^4\) Briefly, test sera were diluted 1:2 using Eagle’s minimum essential medium (MEM) with gentamicin and inactivated at \(56\) C (\(133\) F) for 30 minutes in a water bath. Aliquots of 50 \(\mu\)l were placed in a 96-well microtiter plate in duplicate, and 2-fold serial dilutions were made using 25 \(\mu\)l of MEM. Bovine respiratory syncytial virus grown on bovine turbinate cells was added to all wells (25 \(\mu\)l containing
100-500 TCID$_{50}$ BRSV). The plates were mixed and then incubated at 37 C (98.6 F) in a moist 5% CO$_2$ environment. The wells were evaluated for cytopathic effect after 7 days of incubation. The highest dilution of serum that inhibited cytopathic effect in both wells was considered the titration endpoint for the serum sample. Seroconversion to BRSV in this assay was defined as a 4-fold increase in the BRSV-SN titer. The serum BRSV-SN antibody titer was converted to a log$_2$ value for data analysis.

Serum BRSV antibody concentrations were also measured using a whole cell ELISA (BRSV-ELISA) which has been described.$^{15}$ This assay is believed to measure total BRSV specific IgG (both neutralizing and non-neutralizing antibody).$^{15}$ Seroconversion based on ELISA antibody concentration was defined as a twenty unit increase in ELISA value.$^{15}$

Seroconversion to BRSV in the control group was considered to be reflective of natural exposure during the first 28 days in the feedlot. The ratio of BRSV-SN titer to BRSV-ELISA antibody concentration was calculated as a relative measure of neutralizing and non-neutralizing antibody production.$^{15}$

6.3.4 Statistical analyses

Descriptive statistics were calculated and data were examined graphically or in tabular form. The outcomes of interest for inferential analysis were measures of respiratory disease and growth performance during the feeding period. Three measures of respiratory disease were investigated. Respiratory disease that occurred during the first 3 days on feed was thought to reflect disease that most likely initiated prior to feedlot entry
or at least would not have been influenced by vaccination at the time of arrival. Respiratory disease that occurred after 3 days on feed was thought to be reflective of disease that initiated after feedlot entry when vaccination may have influenced disease occurrence. The presence of chronic pulmonary lesions at slaughter was used as an indicator of respiratory disease that may not have been detected or was not completely resolved by treatment.¹⁰

The independent variables of primary interest to the analysis were serum BRSV-SN and BRSV-ELISA concentrations at feedlot entry, maximum observed serum 3MI concentration on day 0 or day 3 post-entry, BRSV vaccination group, and interactions between serum 3MI concentrations and the measures of immunity to BRSV (initial antibody concentrations and vaccination group assignments). Multivariable logistic regression (GENMOD Procedure®) was used to assess the effect of the independent variables on the three dichotomous (yes/no) disease outcomes. The effect of vaccination on BRD that occurred during the first 3 days was not evaluated because it was unlikely that vaccination affected the occurrence of respiratory disease during this period. Multivariable ANOVA (MIXED Procedure®) was used to assess the effect of independent variables on mean daily gain for the feeding period.

For each outcome, an initial model was specified that included the independent variables of primary interest: serum BRSV antibody concentration at arrival or BRSV vaccination group, and maximum observed serum 3MI concentration. The two measures of serum BRSV antibody concentration (ELISA and SN) at arrival were evaluated in the same manner but in separate models.
Additional variables assessed for potential confounding included purchase group (lot), feedlot where cattle were finished, gender, body weight on day 0, treatment with antimicrobial drugs between day 0 and 3 (for the analysis of respiratory disease occurring after day 3), dehorning, and castration. The design variable representing the lot for groups of cattle was forced into all models because of the potential confounding effect on outcomes for both respiratory disease and rate of gain. Other potential confounding variables were evaluated using manual forward selection with critical alpha for retention of 0.05. Once all potential confounding variables had been evaluated, the interaction between measures of maximum observed serum 3MI concentration and immunity against BRSV (antibody concentrations at entry or vaccine group assignment) were assessed in order to test the ability of immunity against BRSV, as measured by these variables, to protect against the potential synergy produced by combined exposure to BRSV and 3MI.

Multiple comparisons among vaccine groups for least square mean daily gain were evaluated using the Tukey-Kramer method of correction. Final linear regression model goodness-of-fit was evaluated visually by graphing the predicted values versus the residual for the dependent variable. Effects on disease occurrence were evaluated by estimation of odds ratios (OR) and exact 95% confidence intervals (95% CI) as well as estimation of the likelihood ratio $\chi^2$. Final logistic regression models were evaluated for goodness-of-fit using the Hosmer-Lemeshow statistic. McNemar's test for correlated proportions was used to evaluate differences between seroconversion rates based on BRSV-ELISA versus BRSV-SN assays.
6.4 Results

6.4.1 Descriptive

Mean ± SD entry weight for all cattle was 269 ± 42 kgs (592 ± 92 lbs). Forty-two percent were heifers and 58% were steers. Nine percent of cattle were dehorned and 2% were castrated upon entry into the feedlot. Cattle were fed for an average of 269 ± 105 days prior to slaughter. There were no differences in mean entry weight, gender, dehorning or castration rates, or mean days on feed prior to slaughter among vaccine treatment groups.

Thirty-five percent of cattle were treated for clinical respiratory disease during the first 3 days in the feedlot and 10% were treated after day 3. The crude overall mortality rate was 2.4%. Crude mean ± SEM MDG was 0.95 ± 0.01 kgs (2.08 ± 0.02 lbs). Lung consolidation was observed in 9.1% of cattle, and pleural fibrosis was recorded in 25.6% of lungs at slaughter.

All morbidity attributable to BRD was observed in the first two weeks after cattle entered the feedlot. The mortality rate was 2.4% overall and was not different among vaccine groups. All mortalities attributable to BRD occurred in one lot.

6.4.2 Laboratory analyses

Natural exposure to BRSV was common among these cattle. Using the BRSV-ELISA, 64% of the control group, 100% of the KV vaccine group, and 90% of the MLV vaccine group seroconverted to BRSV. Using the BRSV-SN assay, 64% of the control group, 92% of the KV vaccine group, and 96% of the MLV vaccine group seroconverted
to BRSV. Seroconversion to BRSV based on BRSV-ELISA versus BRSV-SN was not different. Serum BRSV antibody concentrations for day 0 and 28 are summarized in Table 6.1. Seroconversion based on BRSV-SN titer was lower \( (P < 0.01) \) in the control group compared to the other groups and there was no difference between the KV and the MLV vaccine groups. The crude day 28 mean serum BRSV-ELISA concentration for the KV vaccine group was higher \( (P < 0.001) \) than all other groups. The crude day 28 mean serum BRSV-SN titer of the control group was less \( (P < 0.01) \) than the KV or MLV vaccine groups. The crude day 28 mean serum BRSV-SN titer of the KV and MLV vaccination groups were not different. The crude day 28 mean BRSV SN/ELISA ratio was highest in the MLV vaccine group followed by the control and KV vaccine groups respectively \( (P < 0.01) \).

The overall mean ± SEM serum 3MI concentrations on day 0 and day 3 were 2.21 ± 0.08 \( \mu g/ml \) (median = 2.04, range = 0.05-7.05) and 1.85 ± 0.07 \( \mu g/ml \) (median = 1.54, range = 1.06-9.04) respectively. Crude serum 3MI concentrations on day 0 were higher \( (P < 0.001) \) than day 3. Serum 3MI concentrations were not different among vaccine groups on day 0 or day 3.

6.4.3 Clinical evaluation

The final model evaluating associations between morbidity during the first 3 days and the independent variables of interest (maximum serum 3MI concentration and serum BRSV antibody concentrations at entry) also included confounding variables for entry weight and lot (Table 6.2). Maximum serum 3MI concentrations were not associated
with the occurrence of BRD in the first 3 days on feed (OR = 1.05, 95% CI = 0.84-1.33). Each 2-fold decrease in serum BRSV-SN antibody concentration at entry was associated a slight increase in the risk of clinical respiratory disease during the first 3 days in the feedlot (OR = 1.16, 95% CI = 0.98-1.43). A statistical interaction was not detected between 3MI and BRSV-SN antibody concentration, suggesting that this measure of immunity did not modify an effect of 3MI on BRD occurrence.

The final models evaluating associations between morbidity occurring after day 3 and the independent variables of interest (maximum serum 3MI concentration and serum BRSV antibody concentrations at entry or vaccination) included confounding variables for gender and lot (Table 6.3). An increase of 1 µg/ml in maximum serum 3MI concentration was associated with an increased likelihood of BRD after day 3 post entry (OR = 1.41, 95% CI = 0.97-2.06). A 10 unit decrease in serum BRSV-ELISA antibody concentration at arrival was associated with increased risk of BRD after day 3 (OR = 1.31, 95% CI = 1.04-1.82). Cattle in the control group (OR = 3.15, 95% CI = 1.00-12.08) were more likely to be treated for BRD after day 3 compared to cattle in the MLV vaccine group. A statistical interaction was not detected between 3MI and BRSV-SN antibody concentration, suggesting that this measure of immunity did not modify an effect of 3MI on BRD occurrence.

The final model evaluating associations between mean daily gain for the feeding period and the variables of interest (maximum serum 3MI concentration and serum BRSV antibody concentration at entry or vaccine group) included confounding variables gender and feedlot (Table 6.4). An increase of 1 µg/ml in the maximum serum 3MI
concentration on day 0 or 3 was associated with a $0.02 \pm 0.01 \text{ kg (0.05 \pm 0.02 lb)}$ decrease ($P = 0.02$) in the expected MDG for the feeding period. Serum BRSV antibody concentrations (SN or ELISA) at feedlot entry were not associated with MDG for the feeding period. The KV vaccine group gained $0.06 \pm 0.03 \text{ kg (0.12 \pm 0.06 lb)}$ more ($P = 0.08$) per day over the 269 day feeding period compared to the control group. Differences in MDG among other vaccine group comparisons were not detected. Immunity against BRSV, as measured by day 0 antibody concentration or vaccination status, did not modify the effect of 3MI on MDG (i.e. a statistical interaction was not detected).

6.4.4 Post mortem evaluation

The presence of lung consolidation or fibrosis was not associated with maximum serum 3MI concentration, initial antibody concentration to BRSV or vaccination against BRSV (Tables 6.5 and 6.6).
6.5 Discussion

Statistical interactions between maximum serum 3MI concentration and immunity against BRSV, as measured by antibody concentration or vaccination, were not detected in these cattle. This may suggest that there was no detectable synergy between BRSV and 3MI during this study, or that immunity to BRSV as measured in this study did not protect against the potential synergy. However, higher serum 3MI concentrations at entry were associated with lower rates of gain and slightly higher odds for BRD after day 3 in the feedlot. This suggests that 3MI production, as measured in this study, had a negative effect on productivity in these cattle and that mitigation of the effects of 3MI in cattle upon feedlot entry may improve weight gains and slightly diminish the risk of respiratory disease.

Bovine respiratory disease is an economically important disease to cattle producers. The estimate of economic value of cattle deaths attributable to bovine respiratory disease in 1996 was $485 million dollars. Research efforts have identified many factors causally related to the occurrence of BRD in feedlot cattle including several infectious agents. While recent research has explored potential interaction among infectious agents that cause BRD, little research has explored the interaction between infectious and non-infectious causes of BRD in feedlot cattle.

3-Methylindole is a natural product of L-tryptophan metabolism and is the pneumotoxin which causes acute bovine pulmonary edema and emphysema (ABPE). Abruptly moving cattle from poor quality forages to better quality diets has been shown to result in higher rumen concentrations of 3MI. Cattle are commonly moved
from poor quality autumn pastures and through public cattle auctions before transportation to a feedlot. During this transition period, they have decreased access to feed and decreased feed intake. Cattle have access to abundant quantities of high quality feed after entering the feedlot. This abrupt dietary change may lead to increased rumen and serum concentrations of 3MI.

Co-mingling cattle from many different sources occurs at public cattle auctions and in the feedlot exposing naive cattle to many different infectious pathogens. Seroepidemiologic studies have reported that exposure to BRSV is common and that cattle entering feedlots with low serum BRSV antibody titers were at greater risk for respiratory disease. It is possible that a proportion of respiratory disease and production losses eventually attributed to BRSV infection may have been initiated by increased production of 3MI. 3-Methylindole has been shown to typically initiate clinical respiratory disease within two weeks after an abrupt dietary change. Serum concentrations of 3MI decrease to baseline levels within 48 hours in cattle after experimental challenge with oral L-tryptophan or 3MI, suggesting that it would be possible for 3MI concentrations to increase and return to baseline prior to the time clinical respiratory disease is observed. While we showed an association between increased serum 3MI concentrations and decreased mean daily gain and increased likelihood of respiratory disease after day 3 post entry, the limited number of times we measured serum 3MI concentration (day 0 and 3) may have inhibited our ability to detect a greater association between serum 3MI concentrations and outcomes of respiratory disease and rate of gain.
These data suggest that daily gain and respiratory disease were negatively affected by 3MI production in these cattle. Data from experimental inductions of ABPE in cattle have reported that the severity of pulmonary lesions was related to maximal concentration and duration of 3MI in the plasma. Serum 3MI concentrations in these cattle varied from 0.05 to 9.04 μg/ml during the 2 days of measurement. Mature cattle experimentally challenge with L-tryptophan or naturally challenged by abruptly changing their diet have been reported to develop respiratory disease attributed to 3MI. These cattle develop rumen 3MI concentrations ranging from 3.0-9.5 μg/ml.

Data from our laboratory suggest that 3MI and BRSV may act synergistically to produce more severe respiratory disease. Although serum BRSV antibody did not appear to mitigate potential synergy between BRSV and 3MI, the mitigation of the effects of 3MI alone may be important in cattle entering feedlots due to it’s effect as a pulmonary pneumotoxin and potential synergy with BRSV.

The association of serum BRSV antibody concentration upon feedlot entry and protection against respiratory disease independent of an abrogation of the negative 3MI effect during both time periods of interest (before day 3, after day 3) corroborated a previous report and emphasize the importance of BRSV immunity in cattle entering the feedlot.

Both inactivated and modified-live BRSV vaccines were used in this study because there have been suggestions that either the KV or MLV could potentiate respiratory disease. More severe respiratory disease has been reported when modified live BRSV vaccines were administered to cattle concurrently infected with
BRSV. Others have suggested that inactivated BRSV vaccines may not be as efficacious compared to modified-live BRSV vaccines because inactivated vaccines produce disproportionately higher concentrations of non-neutralizing antibody compared to MLV vaccines. Results of this study also indicated that the inactivated vaccine produced more non-neutralizing antibody, but similar amounts of neutralizing antibody. Work in humans and cotton rats with the human respiratory syncytial virus (RSV) suggest that the production of non-neutralizing antibody by formalin inactivated RSV vaccines may be caused by critical changes in the antigenic epitopes of either the F or G glycoproteins, and that higher concentrations of non-neutralizing RSV antibody may potentiate disease when the vaccinates are subsequently naturally exposed to the virus.

Based on the seroconversion of control cattle, natural exposure to BRSV was common during the first 30 days in the feedlot. These data do not support immunopotentiation by either the inactivated or MLV BRSV vaccines used in this study during the first 30 days in the feedlot. Rates of respiratory disease and mortality were similar among vaccine groups. This may be due to the timing of BRSV exposure versus protective immunity stimulated by immunization against BRSV. Wikse has suggested that preventive measures against BRD initiated upon feedlot arrival may be applied too late in the beef production cycle to mitigate proliferation and shedding of some infectious respiratory pathogens. The majority of clinical cases of BRD in these cattle occurred during the first two weeks after entering the feedlot. However, amnestic responses from cattle previously exposed to BRSV may account for the slight protective effect of MLV vaccination detected in this study.
Our inability to find an association between chronic lung lesions and our independent variables of interest (serum 3MI concentration, BRSV antibody titers upon feedlot entry, and BRSV vaccination) may have been partially the result of beef production practices of the Ohio Department of Rehabilitation and Correction.\textsuperscript{a} Their main objective is to provide a continuous and economic supply of beef for consumption by the state's inmate population. Therefore, many cattle participating in this study were not fed to maintain optimal growth rates after leaving the backgrounding unit. The extent that these production practices had on the outcomes of this study is unknown.
6.6 Footnotes

aOhio Department of Rehabilitation and Correction, 1050 Freeway Dr. North.
   Columbus, OH 43229

bAnimal Disease Diagnostic Laboratory, Ohio Department of Agriculture,
   8995 E. Main St., Reynoldsburg, OH 43068

cSynshield™, Grand Laboratories, inc., 1447 140th St., Larchwood, IA 51241

dBovishield® 4 + L5, Pfizer Inc., North American Region, Animal Health Group,
   812 Springdale Dr., Exton, PA 19341

eMicotil® 300, Elanco Animal Health, Indianapolis, IN 46285

fNuflor®, Schering-Plough Animal Health Corporation, 1095 Morris ave.,
   Union, NJ 07083

gSAS version 6.12, SAS Institute Inc, Cary, NC 27513-2414
6.7 Acknowledgments

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<td>70.0 (3.0)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.0638&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0485&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0862&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*M<sup>LV</sup> = BRSV modified-live vaccine. KV = BRSV inactivated vaccine.  *geometric mean titer.

Table 6.1—Mean serum BRSV antibody concentrations on day 0 and 28 post entry. The ELISA was thought to measure antibodies directed against both neutralizing and non-neutralizing epitopes, while the serum neutralizing assay was thought to only measure neutralizing antibodies. Seroconversion was determined using the serum neutralizing assay. Row values with different superscript letters are statistically different (P<0.05). SN:ELISA ratio = average ratio of antibody concentrations determined using the ELISA and serum neutralizing assays.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-SN (day 0)</td>
<td>0.1541</td>
<td>0.0959</td>
<td>1.16*</td>
<td>0.98 - 1.43*</td>
<td>0.0899</td>
</tr>
<tr>
<td>3MI*</td>
<td>0.0539</td>
<td>0.0056</td>
<td>1.05</td>
<td>0.84 - 1.33</td>
<td>0.6410</td>
</tr>
<tr>
<td>Entry weight</td>
<td>-0.0048</td>
<td>0.0018</td>
<td>0.61*</td>
<td>0.43 - 0.87*</td>
<td>0.0049</td>
</tr>
<tr>
<td>Lot number</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>0.2882</td>
</tr>
</tbody>
</table>

*OR per 2-fold decrease in serum neutralizing antibody titer. *Maximum serum 3-methylindole concentration (μg/ml) on day 0 or 3. *OR per 100 lb. increase in entry weight.

Table 6.2—Final logistic regression model for estimating adjusted effects of 3-methylindole and serum neutralizing antibody against bovine respiratory syncytial virus (BRSV-SN) on treatment for clinical respiratory disease during the first 3 days after feedlot entry.
Table 6.3—Final logistic regression model for estimating adjusted effects of 3-methylindole and total serum BRSV ELISA antibody (BRSV-ELISA) and vaccination against BRSV on treatment for clinical respiratory disease in feedlot cattle after day 3 post entry.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-ELISA (day 0)</td>
<td>0.0273</td>
<td>0.0137</td>
<td>1.31*</td>
<td>1.04 - 1.82*</td>
<td>0.0164</td>
</tr>
<tr>
<td>3MI*</td>
<td>0.3450</td>
<td>0.1890</td>
<td>1.41</td>
<td>0.97 - 2.06</td>
<td>0.0682</td>
</tr>
<tr>
<td>Vaccine</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.1286</td>
</tr>
<tr>
<td>MLV*</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>...</td>
<td>....</td>
</tr>
<tr>
<td>KV*</td>
<td>0.9632</td>
<td>0.6383</td>
<td>2.62</td>
<td>0.79 - 10.28</td>
<td>....</td>
</tr>
<tr>
<td>control</td>
<td>1.1480</td>
<td>0.6212</td>
<td>3.15</td>
<td>1.00 - 12.08</td>
<td>....</td>
</tr>
<tr>
<td>Gender</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.0268</td>
</tr>
<tr>
<td>Lot number</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.1774</td>
</tr>
</tbody>
</table>

*OR per 10 unit decrease in ELISA. *Maximum serum 3-methylindole concentration (μg/ml) on day 0 or 3. *MLV = BRSV modified-live vaccine, KV = BRSV inactivated vaccine.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Least-squares mean</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-ELISA (day 0)</td>
<td>0.0000</td>
<td>0.0004</td>
<td>....</td>
<td>0.00</td>
<td>0.9735</td>
</tr>
<tr>
<td>3MI*</td>
<td>-0.0217</td>
<td>0.0093</td>
<td>....</td>
<td>5.27</td>
<td>0.0201</td>
</tr>
<tr>
<td>Vaccine</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>2.29</td>
<td>0.1039</td>
</tr>
<tr>
<td>MLV*</td>
<td>0</td>
<td>....</td>
<td>0.95</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>KV*</td>
<td>0.0308</td>
<td>0.0267</td>
<td>0.98</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>control</td>
<td>-0.0259</td>
<td>0.0265</td>
<td>0.92</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>Gender</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>13.15</td>
<td>0.0004</td>
</tr>
<tr>
<td>Feedlot</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>4.22</td>
<td>0.0158</td>
</tr>
</tbody>
</table>

*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6. F = test statistic.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Least-squares mean</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-ELISA (day 0)</td>
<td>0.0000</td>
<td>0.0004</td>
<td>....</td>
<td>0.00</td>
<td>0.9735</td>
</tr>
<tr>
<td>3MI*</td>
<td>-0.0217</td>
<td>0.0093</td>
<td>....</td>
<td>5.27</td>
<td>0.0201</td>
</tr>
<tr>
<td>Vaccine</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>2.29</td>
<td>0.1039</td>
</tr>
<tr>
<td>MLV*</td>
<td>0</td>
<td>....</td>
<td>0.95</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>KV*</td>
<td>0.0308</td>
<td>0.0267</td>
<td>0.98</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>control</td>
<td>-0.0259</td>
<td>0.0265</td>
<td>0.92</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>Gender</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>13.15</td>
<td>0.0004</td>
</tr>
<tr>
<td>Feedlot</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>4.22</td>
<td>0.0158</td>
</tr>
</tbody>
</table>

*Maximum serum 3-methylindole concentration (μg/ml) on day 0, 3, or 6. F = test statistic.

Table 6.4—Final ANOVA model for estimating adjusted effects of 3-methylindole and total serum BRSV ELISA antibody (BRSV-ELISA) and vaccination against BRSV on mean daily weight gain (kg) for the overall feeding period (x̄ = 269 days) in feedlot cattle (n = 244).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-ELISA (day 0)</td>
<td>-0.0066</td>
<td>0.0097</td>
<td>0.94*</td>
<td>0.76 - 1.11*</td>
<td>0.4803</td>
</tr>
<tr>
<td>3MI*</td>
<td>0.2553</td>
<td>0.1648</td>
<td>1.29</td>
<td>0.92 - 1.78</td>
<td>0.1344</td>
</tr>
<tr>
<td>Vaccine</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>0.1963</td>
</tr>
<tr>
<td>MLV*</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>KV*</td>
<td>1.0215</td>
<td>0.6228</td>
<td>2.78</td>
<td>0.87 - 10.66</td>
<td>....</td>
</tr>
<tr>
<td>control</td>
<td>0.8639</td>
<td>0.6304</td>
<td>2.37</td>
<td>0.73 - 9.19</td>
<td>....</td>
</tr>
<tr>
<td>Feedlot</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>0.9254</td>
</tr>
</tbody>
</table>

*OR per 10 unit increase in ELISA. *Maximum serum 3-methylindoIe concentration (μg/ml) on day 0 or 3. *MLV = BRSV modified-live vaccine, KV = BRSV inactivated vaccine.

Table 6.5—Final logistic regression model for estimating adjusted effects of 3-methylindoIe and total serum BRSV ELISA antibody (BRSV-ELISA) and vaccination against BRSV on lung consolidation detected on post mortem examination.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (β)</th>
<th>Standard Error (β)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV-ELISA (day 0)</td>
<td>0.0061</td>
<td>0.0054</td>
<td>1.06*</td>
<td>0.96 - 1.18*</td>
<td>0.2602</td>
</tr>
<tr>
<td>3MI*</td>
<td>0.0874</td>
<td>0.1232</td>
<td>1.09</td>
<td>0.85 - 1.39</td>
<td>0.4819</td>
</tr>
<tr>
<td>Vaccine</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>0.6020</td>
</tr>
<tr>
<td>MLV*</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>KV*</td>
<td>-0.3382</td>
<td>0.3655</td>
<td>0.71</td>
<td>0.35 - 1.46</td>
<td>....</td>
</tr>
<tr>
<td>control</td>
<td>-0.0434</td>
<td>0.3530</td>
<td>0.96</td>
<td>0.48 - 1.92</td>
<td>....</td>
</tr>
<tr>
<td>Feedlot</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>....</td>
<td>0.1592</td>
</tr>
</tbody>
</table>

*OR per 10 unit increase in ELISA. *Maximum serum 3-methylindole concentration (μg/ml) on day 0 or 3. *MLV = BRSV modified-live vaccine, KV = BRSV inactivated vaccine.

Table 6.6—Final logistic regression model for estimating adjusted effects of 3-methylindole and total serum BRSV ELISA antibody (BRSV-ELISA) and vaccination against BRSV on lung fibrosis detected on post mortem examination.
6.8 References


12. Martin SW, Bateman KG, Shewen PE, et al. The frequency, distribution and effects of antibodies, to seven putative respiratory pathogens, on respiratory


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CHAPTER 7
CONCLUSIONS

7.1 Young cattle, similar to those entering feedlots, are susceptible to the pneumotoxic effects of 3MI. Clinical respiratory disease was induced in calves in both experimental challenges with 3MI. Serum 3MI concentrations in cattle entering feedlots are much lower when compared to serum 3MI concentrations in cattle experimentally challenged with 3MI. However, they may be high enough to cause subclinical lung damage, either alone or synergistically with common pulmonary pathogens.

7.2 Cattle entering the feedlot produce measurable amounts of serum and rumen 3MI. Mean serum 3MI concentrations were highest on day 0 in cattle entering the feedlot in both spring and fall. Rumen 3MI concentrations in some cattle were similar to those reported in experimental inductions of naturally occurring acute bovine pulmonary edema and emphysema.

7.3 Differences in management prior to entering the feedlot or differences in ration quality may influence serum 3MI concentrations in feedlot cattle. Cattle entering
a feedlot in the fall had access to better quality forages and had higher maximum serum 3MI concentrations when compared to cattle entering a feedlot in the spring. Cattle entering commercial feedlots which are given access to better quality rations than those found in these feedlots may produce higher concentrations of serum 3MI than those reported here.

7.4 Controlling the effects of 3MI metabolism may be important in feedlot cattle. Increased serum concentrations of 3MI were associated with decreases in mean daily gain in cattle entering the feedlot in both spring and fall. However, neither of these associations were statistically significant. An increase serum 3MI concentration was also associated with treatment for respiratory disease after day three in cattle entering a feedlot in the fall. However, serum 3MI concentration was not associated with treatment for respiratory disease in cattle entering a feedlot in the spring. Rumen 3MI concentrations on day 3 were associated with increased pleural fibrosis at post mortem examination.

7.5 Combined exposure to both 3-methylindole (3MI) and bovine respiratory syncytial virus (BRSV) causes more severe clinical respiratory disease in cattle when compared to either agent alone. Cattle challenged with both 3MI and BRSV had earlier onset of clinical symptoms of respiratory disease, greater mortality, increased lung weight and lung displacement volume, and increased gross and
histologic lung lesions than what would have been expected from the additive
effects of either agent alone.

7.6 Control of pulmonary damage caused by exposure to 3MI and BRSV may be
important in controlling a proportion of respiratory disease in feedlot cattle which
may be attributed to this synergy. Feedlot cattle are commonly exposed to BRSV
and produce measurable quantities of 3MI when entering the feedlot.

7.7 Synergy between BRSV and 3MI either did not occur, was not detected, or was
not mitigated by humoral immunity against BRSV in the cattle in our field trial.
Humoral immunity against BRSV, both at feedlot entry and vaccine induced, did
not mitigate the effects of 3MI.

7.8 Management practices of the feedlots which participated in the field trials may
have had an affect on our ability to detect gross post mortem lung lesions. There
was no association between serum 3MI, humoral immunity to BRSV, or aspirin
and chronic post mortem lung lesions in cattle entering the feedlot in either spring
or fall. Cattle in the two field trials reported here were fed conservatively over an
extended period of time. This may have affected the formation and appearance of
lung pathology in these cattle.
7.9 Aspirin and vitamin E may partially abrogate the pneumotoxic effects of 3MI in feedlot cattle. Cattle treated with both aspirin and vitamin E had delayed onset of tachypnea, dyspnea, and lethargy, lower mortality, lower lung weight and lung displacement volume, and decreased gross and histologic lung lesions when compared to cattle in the control, aspirin, and vitamin E treatment groups after subsequent challenge with 3MI.

7.10 Cattle pre-treated with aspirin or vitamin E alone and subsequently challenged with 3MI were not protected against the pneumotoxic effects of 3MI. Clinical signs, mortality, and post mortem pathology in cattle treated with aspirin or vitamin E alone were similar to those observed in the control group.

7.11 A single dose of aspirin given to cattle upon feedlot entry may improve growth performance. Mean daily gain in cattle treated with a single dose of aspirin was higher for both the backgrounding unit and overall feeding period when compared with untreated cattle. This improvement mean daily gain was independent of a mitigation of a 3MI effect.

7.12 Humoral immune status against BRSV in cattle at entry into the feedlot may be an important factor in the subsequent risk of respiratory disease. Lower BRSV serum neutralizing antibody on day 0 was associated with an increase in the risk of treatment for respiratory disease in cattle during the first 3 days after entry.
Similarly, lower BRSV ELISA antibody an day 0 was associated with an increase in the risk of treatment for respiratory disease in cattle after 3 days in the feedlot.

7.13 There was no evidence that the disproportionate amounts of non-neutralizing antibody or low serum neutralizing antibody to non-neutralizing antibody ratio produced by the inactivated BRSV vaccine had a deleterious effect on the respiratory health and growth performance in feedlot cattle. There was no difference in treatment rates for respiratory disease and mean daily gain between BRSV vaccine groups.

7.14 An inactivated BRSV vaccine administered to cattle upon feedlot entry and on day 14 may increase mean daily gain compared to unvaccinated control cattle. Cattle administered the inactivated BRSV vaccine gained 0.06 kg more per day compared to cattle unvaccinated for BRSV.
BIBLIOGRAPHY


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Martin, S. W. and J. G. Bohac (1986). “The association between serological titers in infectious bovine rhinotracheitis virus, bovine virus diarrhea virus, parainfluenza-3 virus,
respiratory syncytial virus and treatment for respiratory disease in Ontario feedlot calves.”


