NEAR INFRARED SPECTROSCOPY: A POTENTIAL METHOD TO DETECT UNDIFFERENTIATED BOVINE RESPIRATORY DISEASE

by

JEFFRIE THOMAS FOX

B.S., Kansas State University, 1998
D.V.M., Kansas State University, 2003

A THESIS

Submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Approved by:

Co-Major Professor
Dr. Mark F. Spire

Co-Major Professor
Dr. Larry C. Hollis

ABSTRACT

Two studies were undertaken to evaluate the use of Near Infrared Spectroscopy (NIRS) to determine arterial oxygen saturation (StO_2) in cattle with naturally-occurring Undifferentiated Bovine Respiratory Disease (UBRD) and experimentally-induced UBRD utilizing *Mannheimia haemolytica*.

The first study was a natural infection model utilizing 679 beef heifers weighing approximately 227 kg (500 pounds) originating from a southeastern U.S. salebarn. Heifers were evaluated for UBRD upon feedlot arrival, at revaccination, at day 35 on feed, at re-implant time, and two weeks prior to shipment for slaughter. Animals deemed to have UBRD were treated for UBRD and data was collected for 5 days following treatment, while a comparable healthy cohort was also evaluated at the time of treatment. There was a trend for NIRS to be able to predict the incidence of subsequent UBRD when cattle were evaluated on arrival (p=0.0552). However, the ability to detect UBRD in clinically ill cattle was not significantly different (p>0.1690) when compared to healthy cohorts in this model.

When carcass characteristics were evaluated at each time point, NIRS StO_2 values were able to differentiate between yield grades of animals with UBRD and healthy cohorts when evaluated at revaccination, day 35, re-implant, and pre-shipping (p<0.0199). NIRS tended to be able to differentiate yield grades at initial processing (p=0.0513). StO_2 was not a predictor of quality grade at any time point (p>0.1023), nor was there any correlation between lung lesions at slaughter and StO_2 (p>0.2292).

The second study involved 12 head of 181 kg (400 pound) heifers which were subjected to an experimental challenge model of *Mannheimia haemolytica*. Animals were evaluated daily and StO_2 readings recorded 12 hours pre-inoculation, at inoculation, 6, 12 and 24 hours post inoculation and daily for the next 12 days. While NIRS could not definitively differentiate healthy cohort cattle from challenge cattle ($p>0.0713$), there were trends toward challenge cattle having lower StO_2 values than healthy controls.

The authors conclude that while these studies did not provide conclusive evidence of the ability of NIRS to detect UBRD, further studies with a machine that is specifically calibrated and designed for use with cattle should be performed.

Table of Contents

List of Figures   **Page**

List of Tables   **Page**

Acknowledgement

  The author would like to acknowledge Pfizer Animal Health for the generous support of this project. The author would also like to thank the students and staff at the Kansas State University Beef Cattle Research Center for all of their hard work and dedication during this study. Thank you also to Mr. Heath Ritter and Dr Lee Panko for the help and hard work in making this study a success.

Dedication

      This thesis is dedicated to Mrs. Rhonda Fox for her persistence and patience in helping the author accomplish the completion of this project. Without her love and support, this may have never been.

CHAPTER 1

REVIEW OF THE LITERATURE

Undifferentiated bovine respiratory disease, an often insidious malady of young cattle, accounts for nearly 75% of morbidity and greater than 50% of mortalities in feedlot cattle (Edwards, 1996). Bryant *et al* observed cattle at slaughter having lung lesions consistent with cranial ventral bronchopneumonia (CVBP) gained from 0.073 to 0.65 pounds less per day than cattle without observable lung lesions. This study, which observed cattle from birth until slaughter, also found that 42% of animals never diagnosed with undifferentiated bovine respiratory disease (UBRD) had lung lesions at slaughter while 40% of those clinically diagnosed and treated for UBRD had lung lesions (Bryant et al., 1999). Griffin observed that neither clinical respiratory disease nor treatment for UBRD was associated with reduced ADG in the feedlot, although the presence of respiratory lesions at slaughter was associated with reduced ADG. In this study where cattle were randomly assigned to treatment or no treatment after being clinically diagnosed with UBRD, 46% of cattle treated for UBRD had lung lesions, while 58% of cattle not treated had lung lesions at slaughter. Though Griffin stated that lung lesions and ADG were correlated, the amount of reduction in ADG was not stated (Griffin, 1997). Wittum reported lung lesions at slaughter causing a 0.167 pound decrease in ADG compared to cattle without lung lesions. Thirty-six percent of cattle in this study were treated for UBRD between birth and slaughter, while 72% of all animals had respiratory lesions at slaughter. Seventy-eight percent of treated animals in this study had pulmonary lesions and 68% of

untreated animals had lung lesions at slaughter (Wittum et al., 1996). These studies demonstrate the difficulty faced by producers in correctly identifying cattle with UBRD as well as assessing the success of treatment and its impact on future performance.

Undifferentiated Bovine Respiratory Disease has both direct and indirect economic impacts on the industry. Griffin estimates the annual loss of cattle to UBRD at almost $1 billion dollars per year with expenditures on treatments and prevention totaling over $3 billion dollars per year (Griffin, 1997). In a four-year summary of the Texas A&M Ranch to Rail program, healthy steers had a $93.20 advantage over cattle that had been treated for UBRD (McNeill, 2004). Wittum *et al.,* in a review of data from the National Animal Health Monitoring Service (NAHMS) program found 8% of all mortality in calves pre-weaning was due to respiratory conditions. A treatment cost of $263 per calf was incurred for each of these calves, making respiratory disease the most expensive cause of death in pre-weaned calves (Wittum et al., 1993). Bateman *et al*., showed that cattle treated for UBRD during the first 28 days on feed (DOF) were lighter on entry into the feedyard and those requiring retreatment gained significantly less than control cattle during the entire feeding period. Cattle treated for UBRD in the feedlot which did not subsequently relapse after first treatment had significantly lower weight gains during the first 28 days of the feeding period, although this difference did not remain through the entire feeding period (Bateman et al., 1990).

Traditionally, the diagnosis of UBRD is made by clinical observation which may be supported by fever ranging from 103.0° F (39.4° C) to 105.0° F

(40.6° C) depending on the attending veterinarian. Many attempts have been made to increase accuracy of diagnosis and to enhance the determination of clinical outcomes following treatment. Abd-El-Raof *et al.,*, and Reinhold *et al.,* evaluated ultrasonography in combination with other tests to definitively diagnose UBRD (Abd-El-Raof et al., 1999; Reinhold et al., 2002). Abd-El-Raof *et al.* combined ultrasonography with blood gas analysis. Animals with clinical signs of UBRD generally had thickening of the pleura and hypoechoic areas indicative of acute inflammation and exudates on ultrasound examination. In some cases hyperechoic areas indicative of fibrotic lesions in the lungs were demonstrated. Blood gas analysis in these animals indicated significant decreases in blood pH and PO_2, while PCO_2, base excess, and bicarbonate were significantly increased. All parameters of the blood gas analysis returned to normal limits within three weeks of treatment. Reinhold *et al.,* evaluated ultrasonography in combination with clinical and physiological parameters such as respiratory rate, tidal volume, rectal temperature, and respiratory resistance measured by impulse oscillometry. Ultrasound was significantly correlated with respiratory rate, tidal volume and pathologic findings on necropsy. No other clinical parameters were correlated with lung pathology or ultrasound readings. While both studies suggest ultrasonography can be used to detect BRD, the cost and fragile nature of the equipment may limit the utility of this technology in field settings.

Trans-thoracic fine needle aspiration (TTFNA) (Sturgeon et al., 1999), and broncho-alveolar lavage (BAL) (Caldow, 1997) have also been used in attempts to detect UBRD. TTFNA can be used to determine the stage and type of disease

process in the animal. TTFNA serves as a prognostic indicator and also allows for culture of tissue to aid in the identification of pathogens and selection of proper anti-microbial therapy. Sturgeon *et al.*, utilizing TTFNA, cultured only *Actinomyces pyogenes* from three clinical UBRD cases. In discussion with other veterinarians the finding of *A. pyogenes* is generally associated with chronic pneumonia where abscessation is present. In Gagea *et al* 17/99 lung tissue cultures were positive for *A. pyogenes* with all of these animals having pulmonary abscessation .The benefit of utilizing TTFNA was questionable in that the three main bacterial causes of UBRD in cattle are typically *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somnii* (Gagea et al., 2006*)*. BAL can provide insight into the cellular makeup of the lung exudate, help determine pathogens present, and can be diagnostic in lungworm infestations. Both of these techniques are invasive, increase cost due to increased labor requirements and training, and are not frequently used in a field setting.

In another study, a challenge model was used to study the relationship between lung lesions at necropsy and subjective clinical scores of UBRD, as well as objective measurements such as respiratory rate and rectal temperature (Reeve-Johnson, 2001). Respiratory rate was found to be highly correlated to clinical score as well as rectal temperature antemortem. The strongest correlation to lung consolidation was respiratory rate, with rectal temperature and clinical score also being correlated, although not as strongly. While this was a small study with only sixteen experimentally-infected animals, there is the possibility that respiratory rate may be overlooked as a diagnostic tool in UBRD.

The first device to continuously monitor blood oxygen saturation in the body was developed in 1935 (Tremper and Barker, 1998).   By 1970 Hewlett-Packard began marketing the first self-calibrating oximeter which attached to the ear (Tremper and Barker, 1998).   In 1985 Nellcor developed the pulse oximeter and by 1989 it became a standard of therapy in surgery suites and recovery rooms (Tremper and Barker, 1998). Pulse oximetry monitors blood oxygen saturation by measuring arterial oxygen saturation and calculating a value of percent hemoglobin saturation (SpO_2) (Tremper and Barker, 1989).   For all of the benefits of being able to closely monitor the oxygen saturation of patients, pulse oximetry has its limitations as well. In examining the influence of skin pigmentation on the accuracy of pulse oximetry, Emery found that pulse oximetry overestimated the oxygen saturation in infants with darkly pigmented skin as compared to arterial blood gas samples (Emery, 1987). Severinghaus and Kelleher also documented a host of limitations finding probe position, abnormal pulses, influence of electrocautery and skin pigmentation, as well as false alarms and false non-alarms that may influence readings. (Severinghaus and Kelleher, 1992)

Pulse oximetry is also used in the evaluation of oxygen saturation in animals. Hendricks and King showed the usefulness in critically ill small animal patients in an intensive care setting (Hendricks and King, 1993). Whitehair *et al.* showed that pulse oximetry underestimates the level of oxygenation in equine patients when compared to arterial samples. (Whitehair et al., 1990) In evaluating the accuracy of pulse oximetry in neonatal calves, Uystepruyst *et al.* found that

contrary to equine subjects, pulse oximetry overestimates oxygen saturation when compared to arterial sampling (Uystepruyst et al., 2000). Coghe *et al.* evaluated pulse oximetry in the detection of UBRD (Coghe et al., 1999). The ear, tongue, scrotum, vulva, nasal septum, and lip were evaluated as potential sites to obtain readings in this study. The ear, tongue and scrotum were eliminated as possible attachment sites due to the inability to continuously obtain good signals. Coghe *et al.* stratified SpO_2 values into 3 groups consisting of less than 80%, 80 - 90% and those with values over 90%. Of the 149 animals with clinical signs of UBRD, 125 had SpO_2 values above 90%, 15 were between 80% and 90%, and only 9 were less than 80%. All nine cattle with values less than 80% subsequently died of UBRD while the outcome of the rest of the cases was not described. Pulse oximetry had limitations in this study in that all hair had to be removed from the testing site, and readings were unobtainable in black hided cattle. (Coghe et al., 1999)

Near infrared spectroscopy (NIRS) is another technology that has been used to determine tissue oxygenation potential. It performs by using infrared light to measure the amount of oxyhemoglobin in tissues. A reflectance mode fiber optic probe is coupled with a multi-wavelength spectrometer that emits and detects light within 680-800 nm. Silicon optic fibers are utilized in the reflectance probes to capture light intensity measurements. Illuminating and detecting fibers for obtaining a NIRS reading have spacing ranging from 12 mm to 25 mm depending on the probe used. Maximum tissue penetration depth is approximately equal to the spacing between the illuminating and detecting fibers

with average measurement depth being half the probe spacing. Light emitted through tissues is absorbed by oxygenated hemoglobin while light not absorbed is reflected back to the probe. The difference between emitted and received light is calculated in an algorithm to determine hemoglobin saturation. Tissue absorption is measured as -log [Sample Intensity/Reference Intensity]. This value is compared against a calibration curve specific to hemoglobin oxygen saturation to give StO_2 values (Myers et al., 2005).

Near infrared spectroscopy has been studied in many aspects of medicine. Human medicine has found a variety of uses for NIRS. Assessment of compartmental syndrome with NIRS is currently being studied as a means of replacing conventional highly invasive diagnostic methods (Arbabi et al., 1999; Garr et al., 1999; Gentilello et al., 2001). Compartmental syndrome is a sequela to an injury where pressure builds in the intermuscular fascial planes due to inflammation and leading to a decrease in oxygenation of local tissues. Left uncontrolled, compartmental syndrome can lead to impaired blood supply thus damaging muscle and nervous tissue. The current methods to evaluate compartmental syndrome include placing needles in the muscle at risk to obtain a direct measurement. An alternative to placing needles is placing a wick in the affected limb to continuously measure the pressure. In patients who are critically injured and cannot convey their pain with clinicians, NIRS may help to avoid delayed or unnecessary fasciotomies (Gentilello et al., 2001). Peripheral vascular disease is another aspect of human medicine where NIRS is being studied (Casavola et al., 1999; Cheatle et al., 1991; Jarm et al., 2003; Kragelj et al., 2001;

McCully et al., 1994; McCully et al., 1997).   In patients with suspected

peripheral vascular disease, time for muscle saturation to return to normal was on

the order of minutes in diseased patients as compared to seconds in the normal

patient (Casavola et al., 1999). Using NIRS to study post-occlusive reactive

hyperemia has led to new information about the condition of the peripheral

vasculature, confirmation of diagnosis, and evaluation of therapies (Kragelj et al.,

2001). NIRS has also been used to study peripheral vascular disease in the

elderly (Comerota et al., 2003; McCully et al., 1997), traumatic shock (McKinley

et al., 2000), cerebral and myocardial insufficiency (Jobsis, 1977), and muscle

oxygenation during exercise (Nishiyasu et al., 1999). With the widespread use of

NIRS in human medicine, the jump to evaluation of uses in veterinary medicine

was inevitable.

NIRS has been used to study muscle oxygenation in the equine. In 2000,

Pringle *et al* reported that horses under general anesthesia subjected to systemic

hypoxia and limb ischemia had a significant reduction in tissue oxygen saturation

(StO_2) values. Standing horses were subjected to the same treatments and while

limb ischemia induced a significant decrease in StO_2 values, systemic hypoxia

had little effect (Pringle et al., 2000). Laminitis has also been researched using

NIRS as a potential method of diagnosis. Hinckley *et al.* looked at pedal

haemodynamics and oxygenation in normal and laminitic horses. StO_2

differences were estimated by manual digital occlusion of vessels on the palmar

surface of the pastern, requiring the animal to stand on one leg, and application of

a cuff tourniquet. Acutely laminitic horses could not be differentiated from

normal horses, but animals with chronic laminitis had lower StO_2 values in the hoof than normal horses. The difference in acute and chronic laminitis appeared to be indicative of haemostasis in the acute case (Hinckley et al., 1995). These findings lend support to the use of NIRS in the detection of UBRD by showing that hypoxemia can be detected in outlying tissues.

Veterinarians and physicians have been studying various uses of NIRS for several years, although few published studies have evaluated its potential uses in the bovine. In 1998, Pringle *et al.* examined the normal bovine claw and determined that NIRS could potentially be used for detecting ischemia in the bovine hoof when they experimentally occluded the vessels supplying the distal extremities (Pringle et al., 1998). In a 1999 study, Pringle *et al.* also looked at the influence of hair covering and epidermal pigmentation with its respect to optical path length. Black hair covering prevented NIRS from obtaining a reading, although this was overcome by shaving the subject, while white hair covering did not appear to affect the outcome of NIRS readings. Pringle hypothesized cerebral oxygenation could be determined while the animal was in the birth canal if the hair covering was white (Pringle et al., 1999). Based on these two limited studies, NIRS appears to have merit as a tool to be used in assessing tissue oxygenation in the bovine.

CHAPTER 2

INTRODUCTION

The current study was undertaken to evaluate whether near infrared spectroscopy might be a potential tool to evaluate bovine respiratory disease on a real time basis as seen by Fox and Spire (2004). The objectives of the study were to determine (1) if tissue oxygenation observed at initial feedlot processing could serve as a predictor of eventually suffering from or succumbing to UBRD during the feeding period, (2) if StO_2 values upon initial selection for treatment can predict whether an animal will be retreated for UBRD, subsequently die or become chronically ill from the disease, (3) if animals deemed clinically ill had lower tissue oxygenation levels than cattle that were determined as being healthy cohorts, and (4) if tissue oxygen levels are associated with altered growth traits.

MATERIALS AND METHODS

This study was conducted at the Beef Cattle Research Center (BCRC) located at Kansas State University. A total of 691 beef heifers were utilized. This study was approved by the KSU Institutional Animal Care and Use Committee, Animal Monitoring Protocol # 2193.

Near-infrared Spectroscopy (NIRS)

A NIRS device (InSpectra™ Tissue Spectrometer Model 325, Hutchinson Technology, Inc., Hutchinson, MN) with a 20-mm probe was used to perform each study and was calibrated daily using the internal calibration function of the unit. With the coccygeal artery being the target of interest the probe was oriented such that the head of the probe was pointing toward the head of the animal when

placed between the caudal tail folds. This placement allowed the light to be transmitted into the ventral aspect of the tail while minimizing the amount of manure present that had to be removed from the site prior to taking a reading.

Experimental Animals

Six hundred and seventy-nine head of approximately 227 kg (500 pounds) heifers were purchased from various auction markets in the southeastern United States and delivered to the BCRC in June of 2003.   Within 24 hours of arrival at the feeding facility, animals were weighed and processed with a modified live viral vaccine[1], clostridial vaccine[2], implant[3], topical avermectin[4] at 500 mcg/kg, and treated metaphylactically with tilmicosin[5] at 10 mg/kg administered subcutaneously (SQ). An individually-numbered plastic tag was placed in the left ear to uniquely identify each animal. Cattle were randomly (via coin flip at the start of each pen, and then every other animal) vaccinated with a killed endotoxin vaccine as part of a concurrent study. Cattle were randomly divided into 26 pens containing approximately 25 head per pen with two pens having 35 and 36 head based on approximately 206 square feet per animal. Rectal temperature, body weight, hair color and StO_2 values were recorded for each animal on arrival, at revaccination (7 – 10 days post processing), at day 35, at re-implant and approximately two weeks prior to slaughter.

[1] Bovi-Shield® 4, Pfizer Animal Health, New York, NY
[2] Fortress® 7, Pfizer Animal Health, New York, NY
[3] Ralgro®, Schering-Plough Animal Health, Summit, NJ
4 Phoenectin™ Pour-On, Phoenix Scientific, Inc, St. Joseph, MO
5 Micotil, ELANCO Animal Health, Indianapolis, IN

Data Collection

Cattle were assessed daily by trained pen checkers to identify animals that exhibited signs of UBRD. Animals were scored with a Depression Index (Table 1.1).

Table 1.1: Depression Index Criteria		
Index	**Description**	**Clinical Appearance**
0	Normal	Bright, alert and responsive.
1	Mildly Depressed	May be recumbent or stand isolated with head down. Will be brighter, more alert and more responsive after stimulation.
2	Moderately Depressed	May remain recumbent or stand isolated with head down. May knuckle when ambulating. Depression will be evident after stimulation.
3	Severely Depressed	May be recumbent and reluctant to rise, or if standing isolated, be reluctant to move. When animal ambulates, ataxia, knuckling or swaying will be evident. Head will be carried low with ears droopy, eyes dull and salivation and/or lacrimation may be excessive.

Animals with an abnormal Depression Index were subsequently scored with a Respiratory Index (Table 1.2).

Table 1.2: Respiratory Index Criteria		
Index	**Description**	**Clinical Appearance**
0	Normal	Normal pattern and effort.
1	Abnormal	Pulmonary effort or frequency is increased. A cough may be present and may be observed after movement. Breathing may be irregular with an open mouth, and animals may grunt on exhalation.

Any animal identified as having a respiratory index of 1 and/or a depression index of 1 or greater was then assigned an Appetite Index score (Table 1.3).

Table 1.3: Appetite Index Criteria		
Index	Description	Clinical Appearance
0	Normal	Eating, observed as rumen fill by visual interpretation of the left paralumbar fossa indicating that the animal is consuming feed.
1	Mild Anorexia	Slight visual depression of the left paralumbar fossa indicating slight anorexia.
2	Severe Anorexia	Significant visual evidence that the animal is not eating. The left paralumbar fossa will be sunken. The animal may be gaunt and signs of dehydration may be evident.

Pen checkers recorded animal ID, home pen number, animal hair color, and the scores of the three indices on the Pen Rider Observation form and turned this form in to the treatment administrator. At the time an animal was removed from the pen for treatment for UBRD, a clinically healthy cohort was also removed for evaluation. Healthy cohorts were determined to be any animal that was not identified as having UBRD on that day and had not been treated two times for UBRD. Animals treated two times for UBRD were ineligible for further treatment and therefore were excluded from being assigned as healthy cohorts due to the potential of being chronically affected.

Each pair of cohorts was taken to the hospital area where rectal temperature, weight, and a StO_2 reading were recorded. All animals identified as having UBRD were treated according to standard feedyard treatment protocol which consisted of tilmicosin at 10 mg/kg for the initial treatment. All animals removed from the home pen as healthy cohorts were returned to the home pen upon completion of data collection. All animals identified as having UBRD were held in hospital pens where they were observed daily for five additional days with Clinical Scores (Table 1.4) being assigned to determine if further therapy was

Table 1.4: Clinical Index Criteria		
Index	Description	Clinical Appearance
0	Normal	No signs of disease.
1	Noticeable depression	Noticeable depression, altered respiratory rate and depth, signs of weakness not apparent.
2	Marked depression	Marked depression, moderate to labored breathing, moderate signs of weakness may be apparent but without significantly altered gait.
3	Severe depression	Severe depression accompanied by signs of weakness such as altered gait and lowered head, labored breathing.
4	Moribund	Moribund, unable to rise.

required. These animals were handled daily to collect rectal temperature, weight, and StO_2 values. If therapy was deemed successful, animals were returned to their home pens at the end of the 5-day hospitalization period. Animals requiring further therapy were treated with 200 mg oxytetracycline at the rate of 19.8 mg/kg (9 mg/lb) bodyweight. Animals identified as needing a second treatment for UBRD after being returned to their home pen were brought back to the hospital and treated according to standard feedlot treatment protocol following collection of weight, rectal temperature, and StO_2 values.

Carcass Data Collection

Cattle were harvested at an average of 190.75 days on feed (DOF) with a range of 188 – 194 days. Data obtained at harvest included: lung pathology score and hot carcass weight (collected immediately post-harvest). Lung pathology scores were categorically described as 0, 1, or 2. A score of 0 indicated that there was no visible evidence of UBRD. Lungs identified as having atelectasis, slight to moderate adhesions, bulla, or any other abnormality not including abscesses were scored as a 1. Any lungs having abscesses or severe adhesions causing tearing of the lung with pulmonary tissue remaining with the carcass were

classified as a 2. Lung scoring was hampered by United States Department of

Agriculture – Food Safety and Inspection Service (USDA-FSIS) inspectors not

allowing researchers to handle the lungs to observe all aspects of each lung.

Seventy-two hours after slaughter additional carcass data was gathered to include

ribeye area, yield grade, quality grade, marbling score, back fat thickness, and

kidney, pelvic and heart (KPH) fat.

<u>Statistical Analysis</u>

The data was analyzed in two subsets with the first subset consisting of

data from the first 35 days of the feeding period. This data was used to assess

NIRS capabilities of detecting and predicting UBRD. StO_2 values at processing,

revaccination, healthy cohorts, and the six daily assessments in the hospital were

evaluated. The second subset evaluated the five fixed points in time (processing,

revaccination, day 35, re-implant, and two weeks prior to shipping) to determine

if StO_2 values obtained at these times had any predictive value with regard to

carcass data. Individual animal was the experimental unit in this completely

randomized design. Statistical analysis was performed using SAS v.8 with the

Genmod and Proc Mixed procedures[5].

Binomial data was used to record UBRD status of each animal with

animals treated for UBRD designated as 1 and healthy cohorts designated as 0.

The data was analyzed using logistic regression with StO_2 being the dependent

variable and the binomial data acting as the independent variable. The dependent

variable was assessed against initial treatment for UBRD, second treatment for

---

[5] SAS Version 8, SAS Institute, Inc., Cary, NC.

UBRD, total mortality, and mortality due to UBRD to assess the predictive and diagnostic capabilities of the NIRS technology.

Carcass data was analyzed using the Proc Mixed procedure. Quality grade, yield grade, and lung score were the independent variables with StO_2 serving as the dependent variable. StO_2 readings from the five fixed points in time mentioned above were analyzed individually. The mean StO_2 of these five data points was also analyzed to determine if StO_2 readings over time had any correlation with carcass traits.

RESULTS

Thirty-five day receiving study

Of the 679 animals received, 218 (32.1%) were treated for UBRD. StO_2 values at processing had a tendency to be useful in predicting those animals at risk for developing UBRD (p=0.0552). In cattle treated for UBRD, StO_2 values ranged from 68 to 98 with a mean of 89.89 +/-7.18. Of the 218 animals initially treated for UBRD, 77 (35.3%) were retreated for UBRD. Initial StO_2 readings failed to predict those animals subsequently needing re-treatment for UBRD (p=0.4006). Likewise, StO_2 values taken at the initial treatment for UBRD were not useful in predicting animals requiring subsequent re-treatment (p=0.4882). StO_2 values at arrival, revaccination, Day 35 post-arrival, re-implantation, pre-shipping, first treatment for UBRD and re-treatment are listed in Table 1.5.

| Table 1.5: StO2 Data at Critical Times in Trial						
	Arrival	Revaccination	Day 35	Re-implant	Pre-ship	1st Treatment
Mean	91.43	90.84	89.34	85.55	86.13	89.89
Range	60 – 98	61 – 98	6 – 98	35 – 98	46 – 98	68 – 98
S.D.	6.99	6.79	8.46	9.32	7.05	7.18
Median	93	93	92	87	86	92

The range of StO_2 on arrival for all cattle was 60 – 98 with an average of 91.4 and a median of 93. A total of 36 animals (5.3%) died during the course of the thirty-five day study with 17 (47%) of the fatalities having lung lesions of UBRD upon gross necropsy (7.8% case fatality rate). Arrival StO_2 for cattle subsequently dying with evidence of UBRD ranged from 81 – 98 with a mean of 94.4 and a median of 96. StO_2 values from the first day of treatment did not predict animals that would die from UBRD (p=0.1104). Initial StO_2 values of those animals not dying from UBRD and those animals dying from UBRD were statistically different (P=0.0358). The range of StO_2 values for those animals which did not die of UBRD was 60 to 98 with a mean of 91.4 and a median of 93. The mean and median StO_2 values were numerically lower on arrival for animals that did not die from UBRD than for animals that succumbed to the disease.

Cattle that were treated for UBRD and never pulled as healthy cohorts (HC) had StO_2 values at first treatment ranging from 68 to 98 with a mean of 89.92 and a median of 93. All cattle pulled for UBRD had StO_2 values ranging from 68 to 98 with a mean of 89.89 and a median of 92. There was no statistical difference in these two sets of cattle (p=0.8636). Healthy cohorts never treated for UBRD at the time of being pulled as a healthy cohort had a mean StO_2 value of 88.87 (range 59 – 98, median 91) while HC treated for UBRD following

17

utilization as a healthy cohort had a mean StO_2 value of 91.08 (range 71 – 98, median 92). The difference in these two values approached significance at $p=0.0637$ with the cattle being treated having a higher mean StO_2 value than HC. All cattle pulled as HC had a mean StO_2 of 89.26 with a range of 59 to 98 and a median of 92. There was no statistical difference between either HC pulled as sick ($p=0.1127$) or HC never pulled for UBRD ($p=0.5705$) and the HC group as a whole. There was no statistical difference in any of the remaining groups with the p-values ranging from 0.1690 to 0.4274.

Hospitalized cattle that were evaluated daily for 6 days showed very little change in StO_2 values over this period although day 3 was the lowest average reading for all days. The average StO_2 value for day 1 was 89.91, day 2 was 89.96, day 3 was 88.79, day 4 was 89.41, day 5 was 89.71 and day 6 was 89.88. Figure 1.1 depicts these values. There was no statistical difference in average values over the six days ($p=0.6362$).

Figure 1.1: Average StO$_2$ values for all animals each day in the hospital system during the 35 day receiving study.
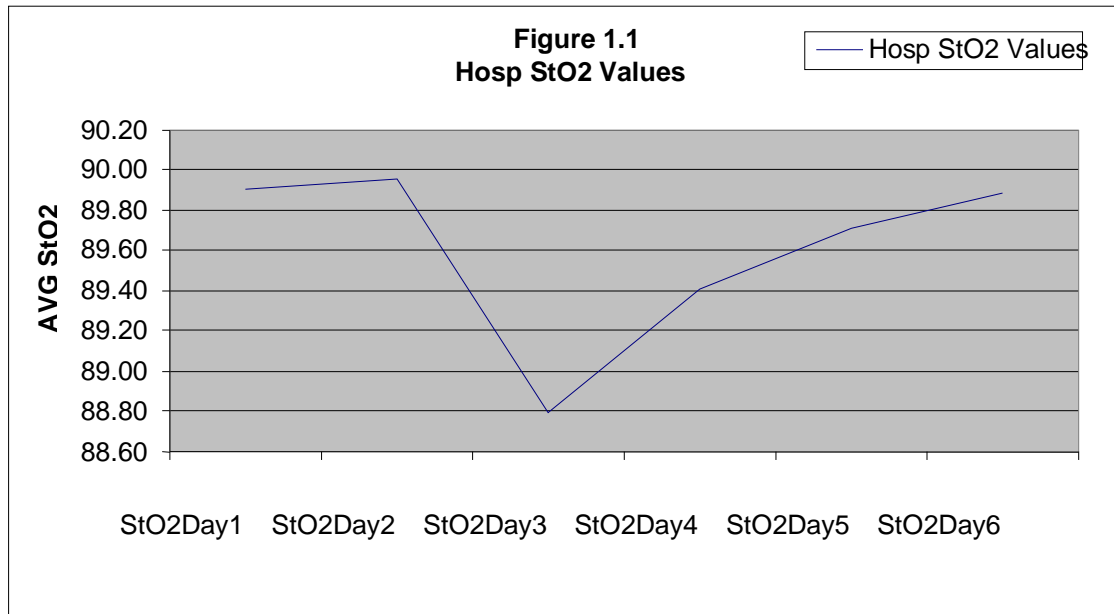
Hair color was evaluated against arrival StO$_2$ readings to determine if there was an impact of black hided cattle. There were a total of 291 black cattle with a mean arrival StO$_2$ value of 89.18 and 387 cattle with hair color other than black which had a mean StO$_2$ value of 93.13. The black cattle had a statistically lower StO$_2$ reading on arrival than did non-black hided cattle ($p<0.0001$).

Carcass Data

Lung Lesions:

StO$_2$ at the five main collection times and mean StO$_2$ over all time periods were compared to lung scores, quality grade, and yield grade.

Lung Scores: When lung scores were measured against StO$_2$, there were no statistical relationships found. Mean StO$_2$ compared to lung score had a p value range from 0.2960 to 0.4911. StO$_2$ values at processing compared to lung scores at slaughter were not different (p value range from 0.3860 to 0.9118). StO$_2$

values at revaccination compared to lung scores at harvest were not different (p

value range from 0.5255 to 0.8535). StO_2 values at day 35 was not different for

the various lung lesions seen (p value range from 0.6123 to 0.9401). StO_2 values

at re-implant did not differ in lung scores (p value range from 0.2920 to 0.6236).

Pre-shipping StO_2 did not relate to a measurable difference in lung scores (p value

range from 0.0875 to 0.2292). Overall there were 348 (54%) animals with no

lung lesions and 295 (46%) with lung lesions. Data was not recorded on 4

animals. Of the 295 with lung lesions there were 13 (4.4%) with a lung lesion

score of 2. Descriptive data for all lung lesion groups are listed in table 1.6 – 1.8.

Table 1.6: StO_2 Values for Cattle with Lung Lesion Score 0						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	91.11	90.67	89.30	85.29	85.76	88.43
Range	64 – 98	65 – 98	6 – 98	35 – 98	46 – 98	69.4 – 97
S.D.	7.31	6.84	8.50	9.52	7.27	4.55
Median	93	93	92	86	86	89
Count	348	348	348	347	348	348

Table 1.7: StO_2 Values for Cattle with Lung Lesion Score 1						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	91.55	90.98	89.29	85.57	86.38	88.73
Range	60 – 98	61 – 98	24 – 98	41 – 98	62 – 98	72.6 – 97
S.D.	6.66	6.82	8.61	9.12	6.79	4.20
Median	93	93	92	87	86	89.40
Count	282	282	281	283	283	283

Table 1.8: StO$_2$ Values for Cattle with Lung Lesion Score 2						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	91.46	91.85	88.54	88.31	89.46	89.92
Range	73 – 98	81 – 98	70 – 98	67 – 98	80 – 98	84 – 97
S.D.	7.05	6.44	8.01	8.61	6.13	4.13
Median	94	93	91	90	92	91
Count	13	13	13	13	13	13

Quality Grade:

Mean StO$_2$ over the entire feeding period was not a useful indicator of quality grade (p value range for quality grade was 0.1023 to 0.2245). StO$_2$ readings were not statistically correlated to quality grade when compared at receiving, revaccination, day 35 post-arrival, re-implantation or at shipping, with p value ranges of 0.672 to 0.7549, 0.3492 to 0.5194, 0.2742 to 0.9406, 0.6411 to 0.8213 and 0.1989 to 0.4510, respectively.

Yield Grade:

Yield grades (YG) at slaughter showed different results from either lung scores or quality grade. Overall there were 142 YG1, 375 YG2, 125 YG3 and 4 YG4. Tables 1.9 – 1.12 detail the descriptive data concerning StO$_2$ within yield grade.

Table 1.9: YG 1 StO$_2$ Values at Each Time Point						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	92.03	92.62	92.62	87.31	87.42	90.10
Range	60 – 98	67 – 98	70 – 98	41 – 98	63 – 98	72.6 – 97
S.D.	6.92	5.24	5.24	8.22	6.51	3.69
Median	94	94	94	89	88	90.6
Count	142	141	141	142	142	142

Table 1.10: YG 2 StO$_2$ Values at Each Time Point						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	91.55	90.64	89.58	85.31	86.30	88.68
Range	60 – 98	65 – 98	24 – 98	52 – 98	53 – 98	76 – 97.2
S.D.	6.80	6.90	7.92	9.14	6.73	4.23
Median	93.5	93	92	86	86	89.4
Count	374	375	375	374	375	375

Table 1.11: YG 3 StO$_2$ Values at Each Time Point						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	89.71	89.48	86.13	83.94	84.22	86.68
Range	66 – 98	61 – 98	6 – 98	35 – 98	46 – 98	69.4 – 97.2
S.D.	7,59	7.65	11.25	10.76	7.98	4.87
Median	93.5	92	89	85.5	84	87.6
Count	124	124	123	124	124	124

Table 1.12: YG 4 StO$_2$ Values at Each Time Point						
	Processing	Revaccination	Day 35	Re-implant	Pre-shipment	Mean
Mean	92.25	86.75	89.75	85.25	81.75	87.15
Range	84 – 98	81 – 96	86 – 92	77 – 93	62 – 91	79.2 – 91.2
S.D.	6.55	7.23	2.63	7.14	13.53	5.50
Median	93.5	85	90.5	85.5	87	89.1
Count	4	4	4	4	4	4

The break down in these numbers with respect to cattle with lung lesions is

detailed in table 1.13.

Table 1.13: Lung Lesion Status by Yield Grade		
	With Lung Lesions	No Lung Lesions
YG1	65	76
YG2	169	205
YG3	60	65
YG4	2	2

At many points measured, there were statistical differences in yield grade related

to StO$_2$ values. Differences in StO$_2$ values between YG 1 and YG2 animals were

significant when measured at revaccination (p=0.0199) and with the mean StO$_2$

reading over the entire feeding period (p=0.0152). Differences in StO$_2$ values

between YG1 and YG3 animals were significantly different at all time points

measured with the exception of processing StO_2 (p=0.0513). The mean of StO_2 values were significantly different at $p < 0.001$ over the feeding period, at $p = 0.0015$ at revaccination, at $p<0.001$ at day 35 of the study, at p=0.0117 at re-implant, and at p=0.003 at pre-shipment   Yield grade 1 and 4 animals showed no difference in StO_2 values with a range of p values from 0.1072 to 0.8114.

Hot Carcass Weight:

Hot carcass weight (HCW) showed no difference with respect to lung lesion (p=0.2897). The mean HCW of cattle with no lung lesions was 645.46 +/- 51.18 and mean HCW of cattle with lung lesions was 641.06 +/- 53.90. When looking at HCW within lung lesions scores there was no difference detected, although the mean for lung lesion score 1 cattle was 642.16 +/- 50.21 while cattle with lung lesion score 2 had a mean of 624.14 +/- 106.15.   HCW was not analyzed against StO_2.

DISCUSSION

The purpose of this study was to determine if NIRS could accurately predict UBRD on arriving cattle or predict outcomes of cattle subsequently diagnosed with UBRD. The study was performed using calves at high risk of developing UBRD in a research feedyard. NIRS readings were obtained at several times throughout the feeding period including arrival processing, when cattle were pulled, and other fixed times.

Several issues arose during the course of the study. The primary issue was returning hospital cattle to the working facility for six consecutive days. Forty-four animals suffered from either cellulitis in the scapular region or abscessation

of the phalanges. Mortality from these injuries represented over 50% of the death loss experienced in this study. In retrospect, repeated returns to the working facility were not the best design for this study.

Along with handling animals through the chute multiple times, pulling "healthy" cohorts was an issue. Several animals with a propensity for being easily agitated were used as healthy cohorts on consecutive days. Also, there were more animals pulled as healthy cohorts than pulled as sick cattle due to more animals leaving the pen than was necessary when pulling cattle. Rather than further upsetting the cattle by sorting, all animals which left the home pen were taken to the treatment facility and data was collected on all cattle. Healthy cohorts were paired with sick animals from the same pen on the same day and extra readings for healthy cohorts were removed from the analysis. The selection of which healthy cohort to use was performed randomly by the statistician. Of the 280 healthy cohorts utilized, 49 were used more than once. There were also 41 healthy cohorts that were subsequently pulled as having UBRD. Eight of the 49 that were used multiple times as healthy cohorts were later pulled for signs of UBRD. A better system of cattle handling and identifying repeat healthy cohorts would have been beneficial in this study.

Coghe *et al*. demonstrated that animals with SpO_2 levels below 80 while concurrently exhibiting clinical signs of UBRD subsequently died (Coghe et al., 1999). In preliminary testing with the NIRS device, arterial blood samples obtained from the coccygeal artery were consistent with the StO_2 values obtained with the NIRS unit just prior to sample collection. Based on the findings of

Coghe *et al.*, any animal exhibiting a StO_2 reading below 80 had three repeat readings taken with the highest value being recorded.

This study did not provide the same outcomes as Coghe *et al.* with regards to animals with low readings. As stated above, all animals that had pulse oximetry readings below 80 died in Coghe's study. The same finding was not observed in this study. Of the cattle which died of UBRD, 3 had a StO_2 reading below 80 when evaluated at the hospital, while 7 had readings above 90. There were also 25 calves that did not die which had NIRS readings below 80. When comparing these finding to Coghe's, the question must be asked as to the validity of both techniques. If NIRS is grossly erring in regards to true StO_2 values this would explain the difference in outcomes. Conversely, this study was done in a real world setting and clinical physical exams were not performed to determine severity of UBRD. Coghe *et al* state that a clinical exam was performed in ill cattle and moderate to severe cases were selected. Neither the components of the physical exam nor the criteria for determining the severity were listed to compare each technique in a similar manner.

Cattle with hair color other than black had statistically higher StO_2 values than did black-hided cattle in this study. This is in contrast to work performed by this lab in preliminary studies. When evaluating StO_2 values vs. arterial blood oxygen saturation, there was no difference. Work performed by Coghe *et al* with pulse oximetry stated that readings could not be obtained on black-hided cattle. Pringle's work in 1999 confirmed that NIRS readings could not be obtained through black hair, but shaving alleviated this issue. The target of interest in this

study was a hairless area, thus it was assumed that hide color would not affect readings.   This may be another area of potential new research to be pursued.

In the assessment of health outcomes in this study only StO_2 values derived at processing appeared to be a statistically significant prognostic tool. StO_2 readings obtained at this time suggest a predictive value in determining animals subsequently dying as a sequela to UBRD.   The data shows that cattle eventually dying from UBRD had significantly higher StO_2 values at processing than did those that survived UBRD or never became ill. While there is a statistical difference in these numbers, and thus the appearance of prognostic value, the overlap of value ranges coupled with StO_2 values of cattle eventually dying of UBRD being greater than those that did not die, indicate that StO_2 is not useful in the prediction of death due to UBRD when evaluated at processing. While this measurement may not be useful, it still bears looking at potential reasons that cattle subsequently dying had a higher arrival StO_2 value.

The most logical explanation for why difference occurred at processing between cattle that subsequently died or lived is primarily a function of numbers. The difference in the means of the these two groups was 3, while a difference in means of just greater than one approached significance in another analysis of processing StO_2 values which is explored in the next paragraph. Another issue is that there were only 17 cattle dying of respiratory disease and there were 662 which lived regardless of treatment history. The dead cattle had two (12%) processing StO_2 readings below 90 and both of these were in the 80's while cattle not dying from UBRD had 179 head (37%) below 90 with several of those

26

readings in the 60's. There is no physiological reason that can be found as to why cattle that died had higher StO_2 values.

There was a trend for StO_2 values at time of processing to be predictive for animals diagnosed with UBRD at the 0.0552 level. Cattle never pulled for UBRD had mean StO_2 values at processing ranging of 91.1 and a median of 93. Cattle pulled for UBRD had a mean StO_2 value at processing of 89.9, which is over two points lower than those never pulled, but the median was 92. Even though cattle deemed subsequently to have UBRD did have a lower average StO_2 reading at processing, these cattle would not be differentiated from the others based on the overlap of ranges and the closeness of the median exhibited for both classes of cattle. Being able to detect these small differences between the means suggests that the study had excellent power.

When looking at the healthy cohort data, some interesting trends emerged. While there was no difference in most of the data analyzed, one analysis did approach significance. Healthy cohorts that had not been treated for UBRD at the time of being utilized as a cohort had a lower mean StO_2 value than those that had been treated. As with the data above, there was a great amount of overlap in the data from these two groups and the cattle that had been treated had a tighter range of values thus giving them a higher mean StO_2 value.

Bryant et al. reported on a more intense system for recording lung lesions than used in the current study. The use of the system described by Bryant where lungs were actually handled by the researcher and evaluated for lesion size and texture may have provided a more quantitative estimate of actual percentage of

lung pathology. (Bryant et al., 1999) Due to slaughter plant regulatory enforcement, the inability to perform a complete examination of the lungs at harvest necessitated the development of a less intensive visual scoring system that allowed observations from a distance of approximately three feet. As examples, several animals with lesions of 2.5 cm or less received a score of 1 as did several animals with significant consolidation estimated at over 50% of the visible lung fields but without abscesses or adhesions. The wide range in the amount of infected lung could have had an influence at final data analysis as, intuitively, cattle with a large amount of the cranial lung lobe involvement should tend to have a lower capacity for blood oxygenation compared with cattle having smaller or milder lesions. With the trends that were evident from this study, the differences may have been greater if the observers had been allowed access to the lungs for critical, detailed assessment. The differences may have also been more robust if there had been a larger number of severe lesions noted, or if the lung scores of 1 could have been split into moderate and mild subgroups.

While closer assessment may have been of value, in actuality, no previous research was found in the literature dealing with the adaptive capabilities of the bovine to compensate for respiratory function lost due to severe respiratory disease. The quantity of consolidated lung may have had no bearing on the ability to detect UBRD with NIRS due to the adaptive capacity of the bovine and the methodology used in obtaining readings for the study. Cattle are very adept at living with lung lesions even though performance in the feedyard may suffer. Compensation may be a factor for animals having the ability to oxygenate the

blood to levels that make detecting respiratory disease improbable with NIRS. In cattle with consolidated lung changes, blood flow and gas exchange are altered in the consolidated areas; as a result, blood flow and oxygen may be diverted to other areas of the pulmonary system. When a group of cattle are brought to the working facility, factors such as exercise and increased adrenaline output will increase blood flow to the lungs. This increased blood flow will potentially improve oxygenation transiently and possibly mask any insufficiencies that exist in tissue perfusion. Belardinelli *et al.* showed that in humans following maximal exercise, StO_2 levels rapidly reached levels that were higher than pre-exercise resting values (Belardinelli et al., 1995). Sahlin also noted that following artificial, temporary arterial occlusion, StO_2 values returned to higher than resting values (Sahlin, 1992). A potential fault in the current system may be due to artificially high StO_2 values resulting from the exertion of being moved through the working facility.

Yield grade analysis revealed results that were not expected with this study. Van Beekvelt *et al* shows that humans with thicker adipose layers had significantly lower StO_2 readings(van Beekvelt et al., 2001) . This is more comparable to yield grade, which is a function of fat cover on the boneless closely trimmed retail cuts. This difference could be detected as early as arrival, although when comparing yield grade 1 and 3 animals, there were significant differences at every data collection point with the exception of processing. Essentially, NIRS could detect animals that would yield better as early as arrival when comparing yield grades 1 and 3.

Unlike with determining morbidity or mortality, where some results were inverted, at all points in the feeding period better yielding cattle had higher StO_2 values; however, a technical error of not using a probe that would penetrate deep tissues may have contributed to this finding. The downfall is that, like in the morbidity and mortality data, the range of StO_2 readings in the cattle overlap dramatically. If one was to further evaluate this technology, day 35 of the feeding period should be the optimal measuring point, as this was the time when the greatest differences were seen. With further investigation, NIRS may be a technology that would allow the cattle feeder to better predict yield grades.

The work done by van Beekvelt *et al* leads one to question why StO_2 would be able to detect cattle of essentially the same general body condition on arrival and differentiate them at slaughter (van Beekvelt et al., 2001). Breed may have an impact on this, as it is general knowledge that continental breeds such as Limousin and Charolais tend to have a leaner carcass at a common body weight. In this study, 292 of the 680 head that arrived had black hides. The balance of the cattle had either yellow or red hides with 14 gray and 3 blue roan animals. While there were numerically more cattle that could have come from an exotic cross, this cannot be verified based on hair color alone. No statistical analysis was performed to examine the relationship between hair color and yield grade. In a review of the literature, nothing was found indicating that cattle from the continental breeds have fewer adipocytes than do the English breeds. Another issue is this set of cattle was harvested approximately four weeks prior to their predicted physiological growth end point, thus predisposing them to leaner

carcasses. In our preliminary NIRS work, a 25 mm probe was selected from

probes measuring 15-30mm. For 227 kg weight cattle, this probe seemed to work

the best. In retrospect as cattle grew larger, the use of the 25 mm probe may not

been of sufficient strength to penetrate the tissues effectively to give repeated,

accurate StO2 readings as the cattle increased in size or were dark hided.

CHAPTER 3

INTRODUCTION

UBRD is a costly disease that has few if any good scientific methods of diagnosis that are practical in a field setting. In fact, the current methods of diagnosing UBRD are crude at best and can lead to a failure to detect animals that need treatment, unnecessary use of antimicrobials in animals not needing treatment, and unnecessary labor and expense. When looking at UBRD diagnosis in the field, the work by Wittum and Bryant (Bryant et al., 1999; Wittum et al., 1996) reveal that a better method is necessary. The current method of observing an animal that is not well and broadly categorizing it as UBRD is insufficient. Currently any animal that is off feed, depressed or has any other symptom that cannot be placed into a specific systemic category is deemed to have UBRD. When only 40% of the animals treated for UBRD are diagnosed as having lung lesions at slaughter, a better *in vivo* diagnostic method is needed.

The monetary value of misdiagnosis must not be overlooked. Extrapolating the data from Wittum and Bryant (Bryant et al., 1999; Wittum et al., 1996) it is estimated that 60% of animals selected for treatment for UBRD are misdiagnosed. Griffin stated that UBRD may cost the industry $1 billion per year in death losses with an additional $3 billion per year in preventatives and treatments (Griffin, 1997). When one looks at the estimated $2 billion per year in treatment and diagnostics, the beef industry may be spending up to $1.2 billion per year on misdiagnosed cattle based on an estimated misdiagnosis of 60% of

cattle. The ability to correctly diagnose UBRD in the field with a novel diagnostic tool could help alleviate a majority of that cost.

As the industry grapples with UBRD, public perception is another issue that needs to be taken into account. The issue of antibiotic resistance is at the forefront of mainstream media, and misdiagnosis and potential overuse of antibiotics plays to this concern. As long as the issue of antibiotic resistance in food animals being transferred to humans is a topic of debate, the cattle industry must police itself to ensure this does not happen. Being able to properly diagnose and apply antibiotic therapy will aid in the industry's public perception. By better diagnosing animals with UBRD, the beef industry will be seen as on industry that cares about both animal and human health and well being.

MATERIALS AND METHODS

This study was conducted at the Biosecurity Level 2 (BL-2) Unit of the Animal Resources Facility located at Kansas State University. A total of 12 beef heifers were utilized. This study were approved by the KSU Institutional Animal Care and Use Committee, Animal Monitoring Protocol # 2193 and the Institutional Biosafety Committee, IBC Registration # 427

Experimental Animals

Twelve 181 kg (400 pound) heifers of unknown serological or lung pathology status were purchased from a local auction in September, 2003 and housed at the BL-2 Unit. Upon arrival at the BL-2 Unit, all animals were uniquely identified by placing individually-numbered plastic ID tags in the left ear. Rectal temperature, individual weights and StO_2 values were obtained and

the animals were randomly assigned to four groups of three animals each. The groups were then randomly assigned to four pens with three of the pens being designated to hold cattle to be challenged with *Mannheimia haemolytica* Type A1. Control animals were separated from challenged animals by a natural buffer zone between pens which did not allow for any form of cross-contamination. Prior to the challenge date each animal was assessed twice daily for seven days for UBRD and assigned a clinical illness score, as described in the materials and methods for the aforementioned BCRC trial.

Challenge Procedure

The challenge inoculum was prepared as described in Mosier *et al.* (Mosier et al., 1995). *M. haemolytica* was grown in a brain heart infusion agar containing 5% bovine blood for 18 hours at 37° C in a 7% CO_2 atmosphere. Colonies were then recovered and incubated in brain-heart infusion broth for 6 hours at 37° C in a rotary shaker bath. Centrifugation at 3,000 rpm for 30 minutes at 5° C resulted in pellet formation in the centrifuge tube. This pellet was re-suspended in 20 ml of phosphate buffered solution to obtain a final concentration of approximately 1×10^9 colony forming units (CFU). Twelve hours prior to challenge each animal had individual weight, rectal temperature, and StO_2 values recorded. At hour 0, challenge animals received 20 ml of phosphate buffered sterile saline (PBSS) containing 1×10^9 CFU of *Mannheimia haemolytica* type A (OSU Strain) via nasobronchial instillation utilizing a 33 Fr broncho-alveolar lavage tube. The tube was passed through the external nares via the ventral meatus and into the trachea to a point where resistance was met and then

34

withdrawn slightly before instillation of the inoculum. Following deposition of the inoculum approximately 20 ml of air was introduced into the catheter to complete delivery of the challenge dose, whereupon the tube was removed.

Data Collection

StO_2, weight, and rectal temperature were recorded at 0 hrs, 6 hrs, 12 hrs, and 24 hrs post-challenge for each animal, and daily thereafter until day 12 post-challenge. After collection of data on day 12, the animals were humanely euthanized via captive bolt. A general necropsy was performed by a trained veterinarian, with a board certified pathologist recording gross descriptions of the pathology. Based upon visible lesions and palpation of the lung tissue, a percent involvement of each lobe was recorded. Estimated percent lung involvement was reported as described in Jericho and Langford (Jericho and Langford, 1982).

Experimental Design and Analysis

The data from the challenge model was categorized into two distinct groups (One and Two) for statistical analysis. Group One consisted of a direct comparison of challenged animals vs. controls. Analysis was performed on StO_2 values using the readings obtained on arrival, one day prior to inoculation, and immediately before inoculation to ensure there was no pre-treatment difference between challenge and control animals. StO_2 values from immediately prior to inoculation (0 hour) to the end of the study (day 12) were analyzed to ensure no pretreatment bias existed. A secondary analysis using the first reading after inoculation (6 hours) until the end of the study (12 days) was used based on the working hypothesis that calves pre-treatment should have a higher StO_2 than

calves post-treatment. By excluding readings before treatment, the ability to find differences between the groups post-challenge should be enhanced. A final analysis of all readings obtained from the animals was performed. There was no analysis made of pretreatment vs. post treatment readings.

The data in Group Two was broken down into three categories based on lung lesions found at harvest and challenge status. Control calves were labeled as category C (n=3), challenge calves without lung lesions were labeled category NLL (n=4), while challenge calves with lung lesions comprised category WLL (n=5). Analysis was performed in the same manner as Group One.

Rank transformation of the StO_2 values was performed in both groups due to non-normality. Repeated measures analysis using Proc Mixed in SAS was used to evaluate StO_2 by treatment on each day.

RESULTS

In the *M. haemolytica* challenge model, a comparison of challenge calves to control calves revealed no difference in pre-challenge StO_2 values (p=0.4285). From hour 0 through day 12 post-challenge there were tendencies towards lower StO_2 values (p=0.0713) as well as a trend from hour 6 to day 12 (p=0.0859) for lower StO_2 readings to occur in challenged animals (Figure 2.1). Analysis that encompassed all of the readings from pretreatment to day 12 (p=0.0994) found trends in StO_2 readings as well.

Based on gross lung lesions (0= control cattle no lesions, 1= challenge cattle with no lesions, 2=challenge cattle with gross lesions) no differences were found in comparisons of StO_2 at different time points (Table 2.1). Although there

Table 2.1: p Value for StO2 of Different Time Points in Challenge Model			
	0 vs. 1	0 vs. 2	1 vs. 2
Pretreatment	0.5805	0.4386	0.8235
Hour 0 – Hour 12	0.1802	0.0802	0.6351
Hour 6 – Hour 12	0.2204	0.0862	0.5650

was no statistical difference, a tendency did exist for control cattle to have a

higher StO_2 value than challenged cattle with lesions. An assessment of

aggregate reading points found no difference in StO_2 values relative to lung

lesions (0 vs. 1 p=0.2488, 0 vs. 2 p=0.0958, 1 vs. 2 p=0.5500), with a trend in

the control to have higher StO_2 values than challenge cattle with lesions.

Figure 2.1 shows the mean values of each lung lesion group over time with a

polynomial trendline to demonstrate the change in StO_2 over time.   The trend

lines in figure 2.1 show a tendency for challenged cattle to have lower

oxygenation values.

Figure 2.1: Mean StO$_2$ values for the challenge model of control cattle, cattle with no lung lesion and animals with lung lesions at each time point. Trendlines demonstrate change over time for each group.

Figure 2.1
Mean StO2 Values by Lung Lesion
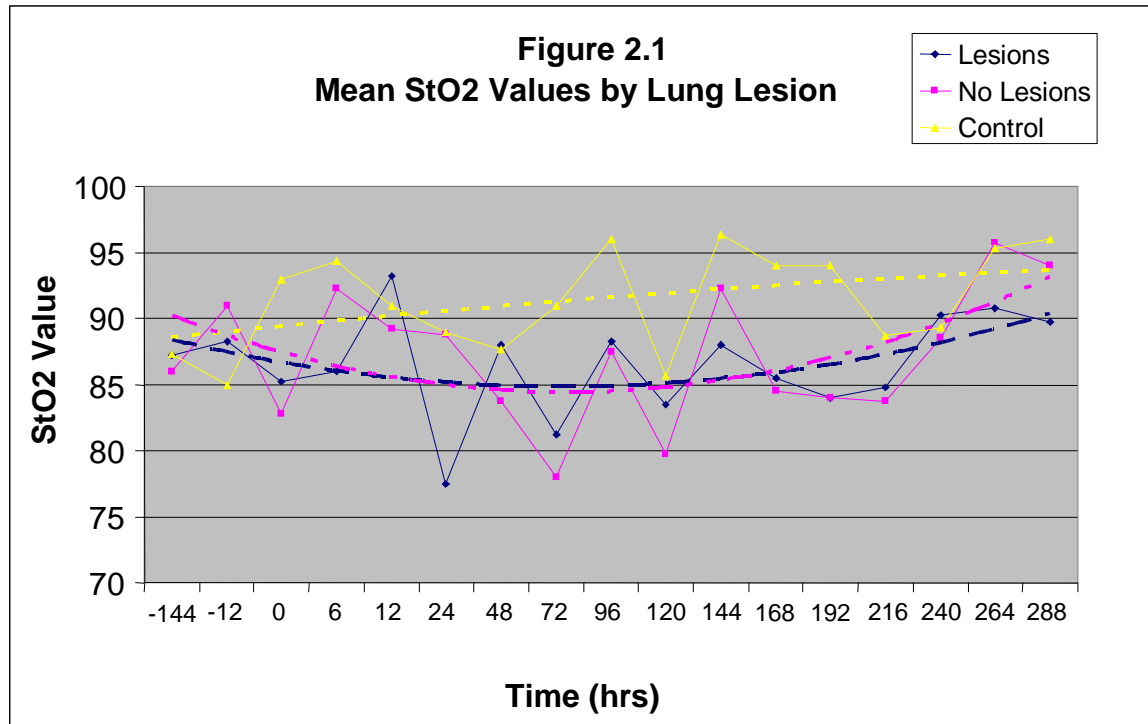
Figures 2.2 – 2.4 show individual StO$_2$ levels for each lung lesion category with Figure 2.2 representing challenged cattle showing no gross lung lesions, Figure 2.3 demonstrating challenged animals with lesions and Figure 2.4 depicting control cattle. These figures show that the values for StO$_2$ are not consistent day to day.

Figure 2.2: StO$_2$ values for each animal in the challenge model that was exposed but did not exhibit lung lesions.
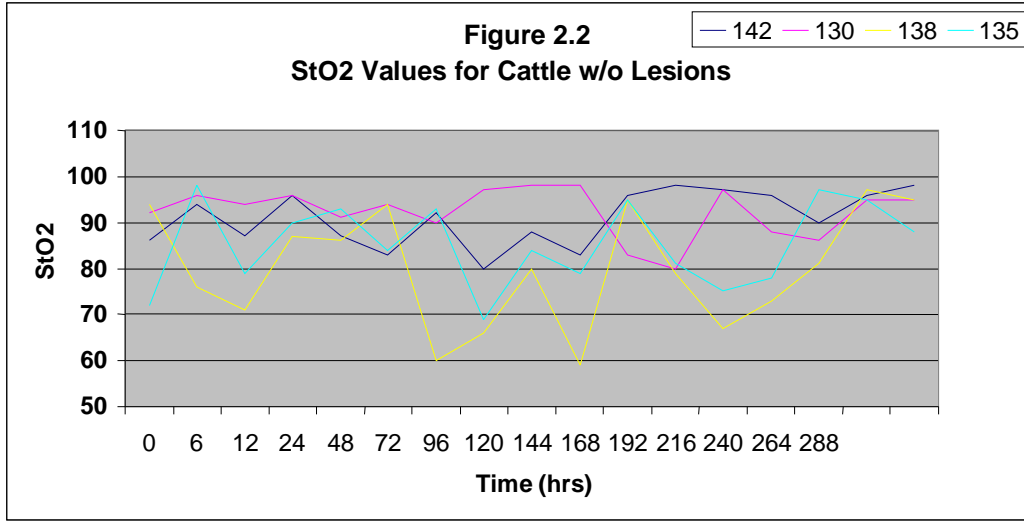

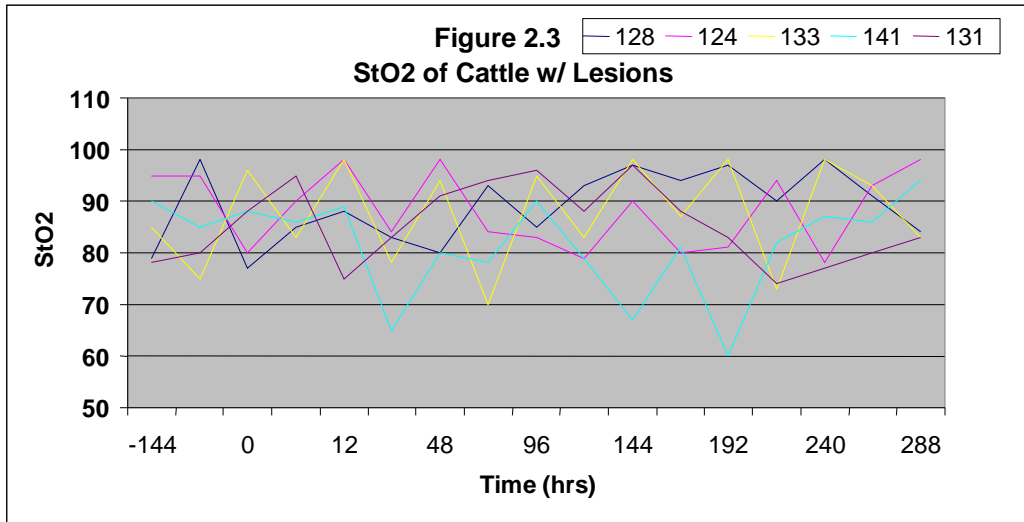Figure 2.3: StO$_2$ values for each animal in the challenge model that was exposed and exhibited lung lesions.

Figure 2.4: StO$_2$ values for each negative control animal in the challenge model.
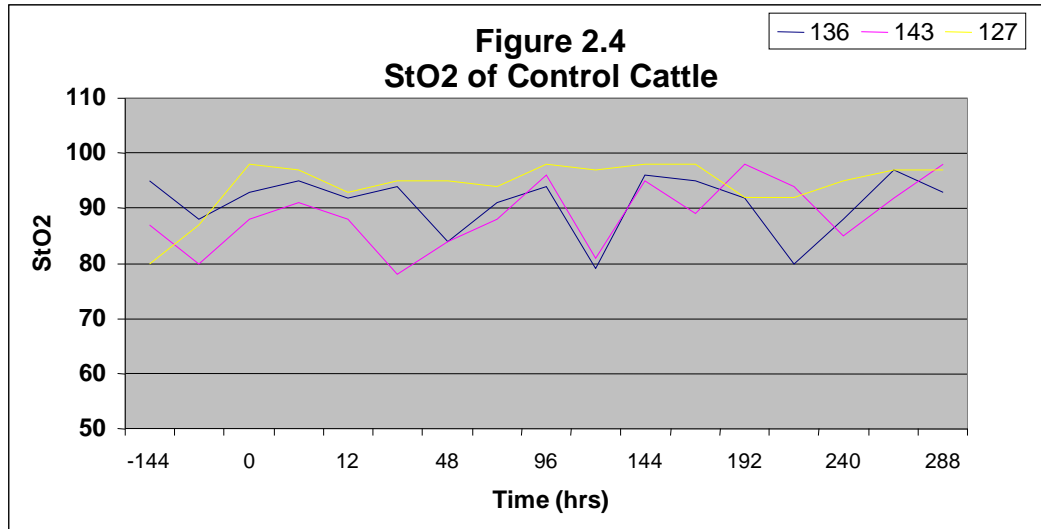

Figure 2.4
StO2 of Control Cattle

Table 2.2 demonstrates the estimated lung consolidation percentage of each animal involved in the challenge model. Only calf 124 had a significant amount of consolidation with 14.25% of the total lung being involved. The other three animals from the challenged group showing consolidation had between 1.75% and 5.25% involvement.

Table 2.2: Percent Lung Consolidation by Lung Lobe (Challenge Model)

Calf ID	Right Apical Cranial	% of Total Lung	Right Apical Caudal	% of Total Lung	Right Middle Lobe	% of Total Lung	Right Diaphragm	% of Total Lung	Left Apical-Caudal	% of Total Lung	Left Diaphragm	% of Total Lung	Accessory	% of Total Lung	All Lobes % Affected
		6%		5%		7%		35%		32%		6%		4%	
124	70%	4.20%			50%	3.50%	5%	1.75%	10%	3.20%	20%	1.20%	10%	0.40%	14.25%
127															0.00%
128							15%	5.25%							5.25%
130															0.00%
131							5%	1.75%							1.75%
133	30%	1.80%									10%	0.60%			2.40%
135															0.00%
136															0.00%
138															0.00%
141	30%	1.80%	5%	0.25%											2.05%
142															0.00%
143															0.00%

Table 2.2: Lung consolidation table demonstrating amount of lung consolidation for individual animals following challenge with *M. haemolytica*. Percent of total lung represents the amount of lung that the lobe listed to the left encompasses. The number listed below each individual lobe represents the percent of that lobe affected, while the number below "% of Total Lung" represents the amount of total lung volume that is affected by this one lobe being affected. The left apical-cranial lung lobe is not included in this chart as there were no lung lesions found in this area. This lobe encompasses 5% of total lung volume and was included in calculations.

DISCUSSION

This study was performed to determine if tissue oxygenation levels are altered by using a controlled challenge model for *M. haemolytica*. Changes in StO_2 levels between challenged and control cattle were looked at as an absolute over the entire study, as well as over time using repeated measures and with respect to lung lesions found at necropsy

While there were no statistical differences in StO_2 values found between the challenged and control animals throughout the course of this study, there were some trends towards significance. StO_2 data collected from time 0 to day 12, shows challenged cattle had a tendency to have lower StO_2 values ($p = 0.0713$). A trend towards significance was seen in StO_2 readings from hour 6 to day 12 ($p = 0.0859$) and from the pretreatment reading to day 12 with challenged cattle tending to have lower StO_2 readings than control cattle ($p = 0.0713$). This trend can best be seen in Figure 2.1 which graphs the change in StO_2 over time for control cattle, cattle without lung lesions on necropsy and those with lung lesions at necropsy. While this graph plots the average score for each time point, it shows that control cattle had a linear increase in StO_2 over time while the other two groups had a slight drop in StO_2 that occurred between days three and four. This drop in StO_2 could correlate with inflammation as a result of the insult caused by bacterial infection. Infection with *M. haemolytica* causes an increase in vascular leakage which leads to an influx of white blood cells, fluids, and other immune system responses which cause inflammation, edema, and fibrin deposition. This inflammatory response will lead to an inability to oxygenate

blood in the affected lung tissue and lead to the decreased values observed at day 3. Although there were no statistical differences in StO_2 values, oxygen tissue saturation levels did have a tendency to decrease in animals challenged with *M. haemolytica*.

The relationship between lung lesions and StO_2 values tended toward significance. Based on statistical power calculations performed using a combination of pulse oximetry data and a pilot study performed in this research facility, 12 cattle should have been a sufficient number to detect differences if they existed. While there were no statistical differences in challenged cattle with lesions and those without lesions, or in control cattle and those without lesions, cattle that had lesions at necropsy did tend to show a decrease in StO_2 readings compared to controls. The presence of more extensive lung lesions may have given more consistent readings, resulting in the technology being able to detect significant differences. The sensitivity of this technology may not be adequate to assess minor differences that sometimes occur.

Considerable variation existed in StO_2 at each daily reading post-challenge, however composite analysis reveals a clear decrease in variation in StO_2 values in the control cattle (Figure 2.1). There were no consistent readings on consecutive days even though readings were taken at approximately the same time each morning. As would be expected, challenged cattle with lesions had the most variation in StO2 values, challenged cattle without lesions had less variation, and control cattle had the least variation. The significance of the variation in these data is not understood at this time, but the relation to more variation and

lung lesions leads one to believe that NIRS may be able to detect animals with UBRD that have more extensive lung lesions than those found in this study. Coghe *et al* (Coghe et al., 1999) reported precision as an estimate for standard deviation that was much smaller than any standard deviations seen in this study using NIRS.

The lack of lung lesions in the challenged cattle leads one to believe that the challenge was not strong enough to induce disease. The method of challenge is documented in several publications (Desmecht et al., 1996; Reinhold et al., 2000; Sustronck et al., 1995; Yoo et al., 1995). In previous studies conducted at this facility using the same procedures, lesions were present in challenged cattle. Potential reasons for a suboptimal challenge could include a diminished virulence in the challenge organism, lower total colony forming units (CFU) than anticipated and/or inoculant placement error, and lung lesion resolution resulting from waiting 12 days post-inoculation to euthanize the animals.

CHAPTER 4

Conclusion

These studies were performed in an effort to develop a better system of differentiating and managing UBRD. While there are no hard and fast conclusions that can be drawn at this time, there are some ideas that have arisen as a result of these efforts. Further studies should include a closer look at yield grade detection ability, more challenge model work, a comparative study to ultrasound, and the development of a bovine specific NIRS model.

The trend of NIRS being able to detect cattle with lung lesions versus negative controls assures that the ideas have merit and warrant further investigation. The issue arises in differentiating the overlap in ranges. Also when looking at the results of the yield grade data, the evidence is overwhelming that NIRS can detect leaner cattle. With both of these findings, further study may benefit the cattle industry.

A study comparing this technique to ultrasound may yield interesting results. In the current studies, NIRS was compared to the typical method of visually assessing cattle and determining a diagnosis of UBRD. As stated in the discussion in Chapter 2 and introduction of Chapter 3, this method is crude at best. Ultrasound has been shown to be able to diagnose UBRD as well as categorize it as acute or chronic. The ability to correlate NIRS findings to a confirmed ante mortem diagnosis should provide interesting results.

It is believed that if NIRS is to have any future in beef production that a bovine specific model must be developed. With the uses that have been

documented in the literature, and the potential to use this device in UBRD diagnosis, a bovine-specific model is the next logical step. The ability to detect small differences in StO_2 changes may simply hinge on a better device. The potential ability to determine better yielding carcasses would provide the economic incentive for a company to develop this technology further.

These novel studies using near infrared spectroscopy as a diagnostic technique, while not dramatic, do raise expectations that improved experimental design, increased numbers of animals used in the challenge model and increased severity of the challenge model could have significant influences on the outcomes of all phases of this work. Development of a bovine specific NIRS device may allow for more accurate readings and better diagnosis of animals suffering with UBRD.

REFERENCES:

Abd El Raof, Y. M., H. Y. Hassan, S. A. El Amrousi, M. S. Youssef, and A. A. Aamer. Ultrasonography and other aids for calf pneumonia diagnosis. *Proceedings of the Fifth Scientific Congress Egyptian Society for Cattle Diseases, Assiut, Egypt, 28-30 November 1999.* Faculty of Veterinary Medicine, Assiut University; Assiut; Egypt.
Ref Type: Conference Proceeding

Arbabi, S., S. I. Brundage, and L. M. Gentilello. 1999. Near-infrared spectroscopy: a potential method for continuous, transcutaneous monitoring for compartmental syndrome in critically injured patients. J. Trauma 47:829-833.

Bateman, K. G., S. W. Martin, P. E. Shewen, and P. I. Menzies. 1990. An evaluation of antimicrobial therapy for undifferentiated bovine respiratory disease. Canadian Veterinary Journal 31:689-696.

Belardinelli, R., T. J. Barstow, J. Porszasz, and K. Wasserman. 1995. Changes in skeletal muscle oxygenation during incremental exercise measured with near infrared spectroscopy. Eur. J. Appl. Physiol Occup. Physiol 70:487-492.

Bryant, L. K., L. J. Perino, D. Griffin, A. R. Doster, and T. E. Wittum. 1999. A method for recording pulmonary lesions of beef calves at slaughter, and the association of lesions with average daily gain. *Bovine Practitioner* 33:163-173.

Caldow, G. 1997. Broncho alveolar lavage in the investigation of bovine respiratory disease. *Cattle Practice* 5:39-40.

Casavola, C., L. A. Paunescu, S. Fantini, M. A. Franceschini, P. M. Lugara, and E. Gratton. 1999. Application of near-infrared tissue oximetry to the diagnosis of peripheral vascular disease. Clin. Hemorheol. Microcirc. 21:389-393.

Cheatle, T. R., L. A. Potter, M. Cope, D. T. Delpy, P. D. Coleridge Smith, and J. H. Scurr. 1991. Near-infrared spectroscopy in peripheral vascular disease. Br. J. Surg. 78:405-408.

Coghe, J., C. Uystepruyst, F. Bureau, and P. M. Lekeux. 1999. Non-invasive assessment of arterial haemoglobin saturation in cattle by pulse oximetry. Veterinary Record 145:666-669.

Comerota, A. J., R. C. Throm, P. Kelly, and M. Jaff. 2003. Tissue (muscle) oxygen saturation (StO_2): A new measure of symptomatic lower-extremity arterial disease. J. Vasc. Surg. 38:724-729.

Desmecht, D., A. Linden, H. Amory, and P. Lekeux. 1996. Hemodynamic responses to Pasteurella haemolytica inoculation in calves given type 2 serotonergic antagonist. Can. J. Physiol Pharmacol. 74:572-579.

Edwards AJ. 1996. Respiratory diseases of feedlot cattle in central USA. *Bovine Practitioner* 30:5-11.

Emery, J. R. 1987. Skin Pigmentation as an Influence on the Accuracy of Pulse Oximetry. Journal of Perinatology VII:329-330.

Fox, J. T., M. F. Spire. 2004. Near infrared spectroscopy as a potential method to detect bovine respiratory disease. *The AABP Proceedings,* Fort Worth, TX, 37:175-176.

Gagea, M.I., K.G. Bateman, T. van Dreumel, B.J. McEwen, S. Carman, M. Archambault, R.A. Shanahan, J.L. Caswell. 2006. Diseases and pathogens associated with mortality in Ontario feedlots. J. Vet. Diagn. Invest. 18:18 - 28.

Garr, J. L., L. M. Gentilello, P. A. Cole, C. N. Mock, and F. A. Matsen, III. 1999. Monitoring for compartmental syndrome using near-infrared spectroscopy: a noninvasive, continuous, transcutaneous monitoring technique. J. Trauma 46:613-616.

Gentilello, L. M., A. Sanzone, L. Wang, P. Y. Liu, and L. Robinson. 2001. Near-infrared spectroscopy versus compartment pressure for the diagnosis of lower extremity compartmental syndrome using electromyography-determined measurements of neuromuscular function. J. Trauma 51:1-8.

Griffin, D. 1997. Economic impact associated with respiratory disease in beef cattle. *Veterinary Clinics of North America, Food Animal Practice* 13:367-377.

Hendricks, J. C. and L. G. King. 1993. Practicality, usefulness, and limits of pulse oximetry in critical small animal patients. Veterinary Emergency and Critical Care 3:5-12.

Hinckley, K. A., S. Fearn, B. R. Howard, and I. W. Henderson. 1995. Near infrared spectroscopy of pedal haemodynamics and oxygenation in normal and laminitic horses. Equine Vet. J. 27:465-470.

Jarm, T., R. Kragelj, A. Liebert, P. Lukasiewitz, T. Erjavec, M. Preseren-Strukelj, R. Maniewski, P. Poredos, and D. Miklavcic. 2003. Postocclusive reactive hyperemia in healthy volunteers and patients with peripheral vascular disease measured by three noninvasive methods. Adv. Exp. Med. Biol. 530:661-669.

Jericho, K. W. and E. V. Langford. 1982. Aerosol vaccination of calves with pasteurella haemolytica against experimental respiratory disease. Can. J. Comp Med. 46:287-292.

Jobsis, F. F. 1977. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 198:1264-1267.

Kragelj, R., T. Jarm, T. Erjavec, M. Presern-Strukelj, and D. Miklavcic. 2001. Parameters of postocclusive reactive hyperemia measured by near infrared spectroscopy in patients with peripheral vascular disease and in healthy volunteers. Ann. Biomed. Eng 29:311-320.

McCully, K. K., C. Halber, and J. D. Posner. 1994. Exercise-induced changes in oxygen saturation in the calf muscles of elderly subjects with peripheral vascular disease. J. Gerontol. 49:B128-B134.

McCully, K. K., L. Landsberg, M. Suarez, M. Hofmann, and J. D. Posner. 1997. Identification of peripheral vascular disease in elderly subjects using optical spectroscopy. J. Gerontol. A Biol. Sci. Med. Sci. 52:B159-B165.

McKinley, B. A., R. G. Marvin, C. S. Cocanour, and F. A. Moore. 2000. Tissue hemoglobin O2 saturation during resuscitation of traumatic shock monitored using near infrared spectrometry. J. Trauma 48:637-642.

McNeill, J. 4-Year Health Summary. Texas A&M Dept. of Animal Science Ranch to Rail Summary. 2004. http://animalscience.tamu.edu/ansc/publications/rrpubs/ASWeb026-4yrhealth-sum.pdf

Mosier, D. A., K. R. Simons, and J. G. Vestweber. 1995. Passive protection of calves with Pasteurella haemolytica antiserum. AJVR 56:1317-1321.

Myers, D. E., L. D. Anderson, R. P. Seifert, J. P. Ortner, C. E. Cooper, G. J. Beilman, and J. D. Mowlem. 2005. Noninvasive method for measuring local hemoglobin oxygen saturation in tissue using wide gap second derivative near-infrared spectroscopy. J. Biomed. Opt. 10:034017.

Nishiyasu, T., N. Tan, N. Kondo, M. Nishiyasu, and H. Ikegami. 1999. Near-infrared monitoring of tissue oxygenation during application of lower body pressure at rest and during dynamical exercise in humans. Acta Physiol Scand. 166:123-130.

Pringle, J., C. Roberts, T. Art, and P. M. Lekeux. 2000. Assessment of muscle oxygenation in the horse by near infrared spectroscopy. Equine Veterinary Journal 32:59-64.

Pringle, J., C. Roberts, M. Kohl, and P. M. Lekeux. 1999. Near infrared spectroscopy in large animals: Optical pathlength and influence of hair covering and epidermal pigmentation. The Veterinary Journal 158:48-52.

Pringle, J., C. Uystepruyst, T. Art, and P. Lekeux. 1998. Near infrared spectroscopy of the normal bovine claw. The Veterinary Journal 156:155-158.

Reeve-Johnson, L. 2001. Relationships between clinical and pathological signs of disease in calves infected with Mannheimia (Pasteurella) haemolytica type A1. Veterinary Record 149:549-552.

Reinhold, P., G. Becher, and M. Rothe. 2000. Evaluation of the measurement of leukotriene B4 concentrations in exhaled condensate as a noninvasive method for assessing mediators of inflammation in the lungs of calves. AJVR 61:742-749.

Reinhold, P., B. Rabeling, H. Gunther, and D. Schimmel. 2002. Comparative evaluation of ultrasonography and lung function testing with the clinical signs and pathology of calves inoculated experimentally with Pasteurella multocida. Veterinary Record 150:109-114.

Sahlin, K. 1992. Non-invasive measurements of O2 availability in human skeletal muscle with near-infrared spectroscopy. Int. J. Sports Med. 13 Suppl 1:S157-S160.

Severinghaus, J. W. and J. F. Kelleher. 1992. Recent developments in pulse oximetry. Anesthesiology 76:1018-1038.

Sturgeon, B., M. Doherty, A. Healy, and H. Larkin. 1999. Use of trans-thoracic fine needle aspiration in the investigation of chronic bovine respiratory disease. Irish Veterinary Journal 52:673-679.

Sustronck, B., P. Deprez, E. Muylle, H. Vermeersch, G. Vandenbossche, and J. P. Remon. 1995. Evaluation of the nebulisation of sodium ceftiofur in the treatment of experimental Pasteurella haemolytica bronchopneumonia in calves. Res. Vet. Sci. 59:267-271.

Tremper, K. K. and S. J. Barker. 1989. Pulse Oximetry. Anesthesiology 70:98-108.

Uystepruyst, C., J. Coghe, F. Bureau, and P. M. Lekeux. 2000. Evaluation of accuracy of pulse oximetry in newborn calves. The Veterinary Journal 159:71-76.

van Beekvelt, M. C., M. S. Borghuis, B. G. van Engelen, R. A. Wevers, and W. N. Colier. 2001. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin. Sci. (Lond) 101:21-28.

Whitehair, K. J., G. G. Watney, D. E. Leith, and R. M. Debowes. 1990. Pulse Oximetry in Horses. Veterinary Surgery 19:243-248.

Wittum, T. E., M. D. Salman, K. G. Odde, R. G. Mortimer, and M. E. King. 1993. Causes and costs of calf mortality in Colorado beef herds participating in the National Animal Health Monitoring System. JAVMA 203:232-236.

Wittum, T. E., N. E. Woollen, L. J. Perino, and E. T. Littledike. 1996. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. JAVMA 209:814-818.

Yoo, H. S., S. K. Maheswaran, S. Srinand, T. R. Ames, and M. Suresh. 1995. Increased tumor necrosis factor-alpha and interleukin-1 beta expression in the lungs of calves with experimental pneumonic pasteurellosis. Vet. Immunol. Immunopathol. 49:15-28.