Lesions Associated with Endotoxin Release and Subsequent Mediator Shock

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Abstract

Gram-negative organisms are responsible for many diseases of ruminants, including neonatal coliform septicemia, coliform mastitis, salmonellosis, pneumonias caused by *Pasteurella* spp and *Actinobacillus* spp, brucellosis, metritis, campylobacteriosis, infections of the cornea and sclera, and thromboembolic meningoencephalitis. Bovine pneumonic pasteurellosis (BPP) is a common respiratory disease in many age groups of cattle. Although the precise mechanism responsible for inducing the exudative fibrinous pleuropneumonia is not understood, other microbial agents and stress predispose cattle to respiratory disease by allowing overgrowth of the normal nasal flora with virulent *Pasteurella hemolytica*. Although experimental infections with *P hemolytica* alone can be accomplished, infecting cattle with one of several agents (infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, parainfluenza-3 virus, and *Mycoplasma bovis*) increases subsequent to infection with *P hemolytica*. The pathophysiology of endotoxemia and the subsequent mediator shock will be briefly reviewed as background information for discussing the lesions associated with this disease process.

Importance of Gram-negative disease in the bovine species

Gram-negative organisms are responsible for many diseases of ruminants. The clinical disease entities include neonatal coliform septicemia, coliform mastitis, Salmonellosis, Pasteurella and Actinobacillus pneumonias, brucellosis, metritis, campylobacteriosis, infections of the cornea and sclera, and thromboembolic meningoencephalitis. Studies in food animal neonates document that the majority of the clinical infections and mortality are the result of Gram-negative organisms, specifically *Escherichia coli (E. coli)* and *Salmonella spp.*[1-6] The mortality rates vary among farms and production units. However, estimates of dairy calf losses during the first 8 weeks of life may range from 2.5% to 29%.[7] Exposure to invasive serotypes of *E. coli* and low serum immunoglobulin concentration are two major determinants for development of colisepticemia.[3,8-11] *E. coli* septicemia (colisepticemia) generally affects calves <1 week of age and produces a rapidly fatal course of the disease process. Death losses from bovine salmonellosis are most severe in confinement-raised dairy calves at 1-10 weeks of age.[5,6] Salmonellosis is described as confined to the gastrointestinal tract where most calves develop bacteremia with spread of infection to the liver, lung, bone marrow, and central nervous system.[6] Shipping fever pneumonia (SFP) occurs in feedlot cattle, with 75% of the cases developing during the first 45 days of their term in the feedlot facility. This disease process appears to be a complex interaction among stressors, viruses, and bacteria. The primary bacterial isolates of SFP are *Pasteurella hemolytica, Pasteurella multocida*, and some *Pseudomonas spp.*[12] Coliform mastitis is another disease process initiated by Gram-negative opportunists and can be devastating to the dairy production unit. This disease process, primarily occurs during the first 100 days of lactation and can manifest itself as a clinical event encompassing all degrees of severity between peracute to subclinical.[13] Gram-negative environmental opportunists are an important group of organisms that cause mastitis. The most frequent isolates from this form of bovine mastitis include *E. coli, Enterobacter aerogenes,* and *Klebsiella pneumoniae.* Less common isolates include *Pseudomonas aeruginosa, Pasteurella multocida,* and *Serratia marcescens.*

Description of clinical signs in ruminants associated with gram-negative disease

The widespread effects of endotoxins are well chronicaled[14] and are reported as a general depression, respiratory distress, the induction of a transitory elevated body temperature, followed by hypothermia, shock-like reductions in systemic blood pressure (increased heart rate followed by decreased cardiac output), diarrhea, changes in the peripheral blood count, and alterations in the blood coagulation system. Some of the more detailed physiological and pathological reactions include: a) lymphopenia followed by a lymphocytosis, b) polymorphonuclear leukopenia followed by leukocytosis, c) hyperglycemia followed by hypoglycemia, d) depletion of liver glycogen, e) anabolic and catabolic responses in protein metabolism, f) local and generalized Shwartzman reactions, g) induction of tolerance and altered reproductive performance. The Complement system will have the Classical and Alternate pathways activated, anaphylatoxin generation, and

many secondary effects. The clotting system will respond by activating the intrinsic and extrinsic pathways, enhanced tissue factor expression, and DIC. The platelets will aggregate and sequester in various capillary locations and secrete their mediators, while the neutrophils will respond by producing inflammatory mediators (prostaglandins/leukotrienes) and increased oxygen radicals. The macrophages will be induced to reach a state of enhanced function and secrete cytokines (GM-CSF, IL-1, TNF).

Investigating "endotoxemia" has resulted in a number of proposed mechanisms of action of the endotoxin molecule in many different hosts. It is notable that the most unique feature of endotoxin is that it possesses relatively little direct toxic effect. Because endotoxin molecules interact with a variety of host cells and induces the synthesis and/or secretion of many soluble mediators, the broader term of "mediator shock" will best apply when contemplating a therapeutic course of action (Table 1).

Table 1. Compounds Associated With Inflammation During Mediator Shock and Endotoxemia

Arachidonic acid metabolites

- Cyclooxygenase pathway
  a. PGE_2 - vasodilator
  b. PGF_2 - vasoconstrictor
  c. Thromboxane A_2 - vasoconstrictor and promoter of platelet aggregation
  d. PGI_2 - vasodilator and inhibitor of platelet aggregation
- Lipoxygenase pathway (Noncyclic products: 5-HETE, LTA_4, LTB_4, LTC_4, LTD_4): These compounds are potent bronchoconstrictors, vasoconstrictors, elicit plasma exudation, chemotatactic for leukocytes, and are involved in microthrombi formation.

Interleukin-1
Interferon
Complement cascade
Colony-Stimulating Factors
Tumor Necrosis Factor
Procoagulent activity
Platelet Activating Factor
Serotonin
Histamine
Bradykinin
Myocardial Depressant Factor
Catecholamines
ß-endorphins

Experimental Infections and Endotoxin Challenges: What Can We Learn?

Salmonellosis Oral Challenge Model

Salmonellosis is one of the few serious infectious diseases which is increasing in incidence in the world today.15,16 The most common serotypes involved are *Salmonella dublin* and *Salmonella typhimurium*.[17,18] Salmonellae cause diseases most commonly in calves under 12 weeks of age; however, adult cattle may be affected as well. Fever and diarrhea, often containing mucus, fibrin, and flecks of blood, is usually observed as common clinical manifestations. Septicemia, arthritis, and pneumonia[19] have also been associated with *Salmonella dublin* infection in calves, and mortality rates may be high in many cases. *S. dublin* often presents as acute pneumonia and deaths, with red meaty lungs observed at postmortem. Many calves remain unthrifty and develop poorly after having contracted salmonellosis and abortion may occur in affected adult cows.[19] The mode of entry of the organism into the host is normally considered to be oral; thus, the oral challenge is thought to be desirable to evaluate the protective capabilities of any immunogen.

Smith, *et al.*[6] investigated and reported on a *S. typhimurium* oral challenge model in calves. The challenge doses examined ranged from 10^4 to 10^{11} organisms. Clinical signs varying from a transient elevated rectal temperature with fecal shedding of small numbers of organisms for 2 days, to severe acute diarrhea and septicemia with death in a few days, to acute disease followed by weight loss or by recovery were reported. The higher challenge dose levels in younger calves resulted in higher mortality. Varying degrees of illness occurred and paralleled the challenge dose of bacteria administered, and were inversely related to age of the calf. Almost all calves exhibited the fever peak, developed diarrhea, and then appeared to improve for a few days before suddenly dying. All calves that died were terminally septicemic. All tissues examined via bacteriological culture were positive for the challenge organism, while blood cultures performed at the initial temperature increase were negative for the presence of the challenge organism. An age difference of 2-3 weeks in the calves appeared to alter susceptibility, but could be overcome to some degree by a corresponding increase in the level of the challenge dose of bacteria. The authors reported that this model appeared to accurately reflect the clinical presentation of bovine salmonellosis.

Intravenous Challenge: Purified Endotoxin

The pathophysiological manifestations by either the intravenous bolus injection of endotoxin, or the smaller doses of endotoxin employed in the slow infusion intravenous models follow similar patterns. As the

challenge dose of endotoxin increases, latency time decreases, the peak clinical effect becomes more pronounced, and the duration of the clinical effect is more protracted. In addition, individual susceptibility, age, pregnancy, charge of the endotoxin, and route of administration significantly influence the degree of clinical distress observed.

The typical intravenous challenge model employed in the bovine has been the bolus inoculation or rapid infusion of various endotoxins.[21-24] The common theme with this model is that clinical manifestations of shock can be observed in minutes. Whereas, those employing the live organism oral challenge will not observe the same clinical picture for 2-3 days. Tennant, *et al.*[21], for example, employed an intravenous injection bolus of *Escherichia coli* endotoxin in neonatal calves at a dose of 0.1 mg/kg to 0.2 mg/kg body weight. This challenge model produced abnormal clinical signs within 5 minutes of endotoxin administration and the calves became recumbent within 15 minutes. In this study, 8 calves died (mean survival time of 6.2 hours), and 4 calves survived the event and returned to clinical normalcy by 12-18 hours after the challenge.

The rapidity with which this category of challenge model creates the mediator shock event does not reflect the observations of Gram-negative disease in the field situation. Most of the models will require the injection of hundreds of micrograms (i.e. 0.07 mg/kg) to milligrams (i.e. 2.0 mg/kg) of endotoxin intravenously within a matter of seconds. It seems clear this rapid fashion and level of purified endotoxin dumping does not occur in the live animal.

The emerging technique in the bovine is the "low dose, slow infusion" method of creating the pathophysiological events that accompany Gram-negative disease. For instance, in 8 week old Holstein calves, a total challenge dose of 0.05 μg/kg of a purified *Salmonella typhimurium* lipopolysaccharide was infused continuously intravenously in a final volume of 250 ml nonpyrogenic saline over a 5 hour period. This model produced signs of endotoxemia during the first few hours of the challenge in 100% of the subjects, with no mortalities.[24] The calves exhibited depression, developed a fever, had increased heart and respiratory rates, alterations in the clotting factors, developed a leukopenia during the infusion and then a mild leukocytosis at the end of the test period (72 hours post-infusion). Other variations of this technique employ various endotoxins at dosages of 4 μg/kg/hr for 5 hours,[25] 24 ng/kg/min for 500 minutes[26], 0.1 μg/kg of body over 30 minutes,[27] and intravenously at 20 μg/kg the first hour, followed by a continuous infusion at 10 μg/kg/hour the following 4 hours.[32]

The most comprehensive reports on the pathophysiological and metabolic consequences of intravenous endotoxin challenge in neonatal calves have recently appeared in the literature.[27,28] In this series of investigations, *Escherichia coli* (055:B5) endotoxin was infused intravenously at a dosage of 0.1 μg/kg of body weight for 30 minutes. The hemodynamic changes observed included large decreases in cardiac index, stroke volume, maximal rate of change of left ventricular pressure, femoral and mesenteric arterial blood flow, glomerular filtration rate, urine production, and mean aortic pressure. Additionally, severe pulmonary arterial hypertension and increased pulmonary vascular resistance were evident at the cessation of endotoxin infusion. This challenge model also elicited severe respiratory effects with marked hypoxemia and increases in arterial-alveolar O_2 gradient, physiologic shunt fraction, and physiologic dead space tidal volume ratio. The alterations in renal function observed in this model were distinguished by a decrease in free-water reabsorption and osmotic clearance, as well as a decrease in sodium and phosphorous excretion.

Respiratory Tract Challenge Model

Bovine pneumonic pasteurellosis (BPP) is a common respiratory disease in many age groups.29 Although the precise mechanism for producing the exudative fibrinous pleuropneumonia is not understood, stress and other microbial agents predispose the bovine to respiratory disease by stimulating overgrowth of the normal nasal flora by virulent *Pasteurella hemolytica*.[30] Although experimental infections with *P. hemolytica* alone can be accomplished, preinfecting bovine lungs with one of several agents (infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, parainfluenza-3 virus, and *Mycoplasma bovis*) increases susceptibility to challenge with *P. hemolytica*.[31]

The combination challenge model, virus followed by bacteria, has been successfully employed for investigating the pathogenesis of BPP.[32] In this model, BPP is induced by sequential inoculations of study subjects with bovine herpes virus-1 (BHV-1, 3×10^7 tissue culture infectious disease 50/nostril ($TCID_{50}$/nostril), followed 2-3 days later by challenge with *P. hemolytica* (15×10^9 cfu intratracheally). This model was performed by Emau *et al.*, in a study designed to elucidate portions of the pathogenic mechanisms of the disease. They examined the alterations in plasma prostaglandins (PG), thromboxane B_2 (TxB_2), histamine, serotonin, and long-chain fatty acids (LCFA) during subsequent to BHV-1 infection alone and after challenge exposure to *P. hemolytica*. At 24-72 hours after the BHV-1 inoculation, the calves developed clinical signs of upper respiratory disease and an elevated rectal temperature. The data indicated that the initial BHV-1 infection conspicuously increased plasma PGE, and nominally elevated plasma $PGF_{2\alpha}$, TxB_2, and arachidonic, oleic and palmitic acids. Three

hours following the scheduled *P. hemolytica* challenge and manifestation of clinical signs of BPP in which the respiratory rate and rectal temperature achieved their maximal values, the levels of plasma arachidonic, oleic, and palmitic acids, along with the PGE and 6-keto-$PGF_{1\alpha}$ were remarkably elevated. In this model, the BHV-1 infection alone produced clinical respiratory disease which was exacerbated by *P. hemolytica.* Consequently, this model made it possible to examine the biochemical and metabolic changes during the BHV-1 infection and during the interaction between BHV-1 and *P. hemolytica.*

An experimental challenge model attempted to study the interaction of *Pasteurella haemolytica* with an attenuated bovine herpesvirus 1 in aerosol-exposed calves.[34] Low titre of the virus culture used for aerosol exposure did not produce measurable clinical interaction. The experiment did provide the an opportunity to study the light-microscopic changes in lungs of calves (n = 3) to a low-dose exposure (5-min aerosol) of *P. haemolytica* A1 from a fresh 5-h log-phase culture. The histopathological study was confined to tissue exposed to only *P. haemolytica.* A limited macroscopic pneumonia was produced in the ventral parts of the cranial lobes. Four days after exposure, a typical reaction featured zones of representative lesions. Zone 1a, at the center of the lesion, featured acute inflammatory processes and necrosis of phagocytic cells that was surrounded by a broad band of compacted, largely necrotic macrophages and polymorphonuclear leukocytes (PMNL) in alveoli of zone 1b. Tissue necrosis was confined to zone 1. Zone 2a frequently occupied the remainder of the lobule with irregular distribution of congestion, edema with a fibrinous component, and infiltration by numerous PMNL, macrophages and other mononuclear inflammatory cells. The narrow zone 2b was located between zones 1b and 2a and had edema with a fibrinous component, numerous fibrocytes, few inflammatory cells and empty capillaries. It is suggested that zone 2 served to isolate zone 1 by surrounding it with nonfunctional tissue.

Brief Summary of Feed Lot Pathology: Gram-Negative Disease and/or Mediator Release

Bronchial pneumonias

Bronchial pneumonia is a common form of bovine respiratory disease, and includes the pathology observed when cattle get "shipping fever." The early tissue pathology usually occurs in anterior and ventral locations in the lung and progresses, through the stages of red and gray hepatization. The lesions can occur within a matter of hours and significant damage can occur within 1-2 days after the initial disease insult. There are small necrotic foci that progress to larger coalescing lesions characterized by gray or yellow discolored areas, as the tissue reactions continue to develop and mature. The conclusion of the bronchial pneumonia inflammatory process is frequently abscessation of the areas that were initially necrotic.

Interstitial pneumonias

Interstitial pneumonias comprise a significant proportion of acute cattle respiratory diseases, and mediator release is part of the pathophysiology of the disease process.[35] The lesions are typically different from bronchopneumonia in that they are more diffusely distributed throughout the lung. The dramatic presentation of the lesions may include edema, emphysema, and areas of dark red discoloration with tissue firmness. Known by different names, such as acute bovine pulmonary emphysema and edema (ABPE), fog fever, atypical interstitial pneumonia (AIP) and cow asthma, the condition seems to occur predominantly in late summer or fall. However, depending on the etiology, cases have occurred throughout the year. Interstitial pneumonia often begins with acute respiratory distress in animals that were clinically normal 12 hr earlier. Animals are observed breathing very rapid and shallow with their mouths open. If disturbed, death may occur rapidly from hypoxia. Etiologies of interstitial pneumonia are quite varied ranging from parasitic, viral and bacterial to toxic. Toxic agents constitute the most economically important cause of this condition in cattle. The primary toxin is the amino acid L-tryptophan in lush pasture grasses, a compound which is converted to 3-methylindole by rumen microorganisms. Other leading toxic causes of interstitial pneumonia are perilla mint and moldy sweet potatoes. Although treatments are mainly symptomatic and ineffective, preventive measures will reduce the occurrence of interstitial pneumonia. Prevention consists of denying animals exposure to known pneumotoxic agents, eliminating certain rumen microflora that break down the toxic compounds to reactive metabolites, and supplying ample good forage so that cattle will not as likely consume toxic plants.

Metastatic pneumonias

This pneumonia type is characterized by multiple inflammatory and sometimes abscessed lesions scattered throughout the lungs. This presentation is suggestive of a disease process with hematogenous origins, and liver abscesses may be a frequent source.

Mediator-induced pulmonary edema

Endotoxin-mediated vascular damage in pulmonary capillaries and other shock-like states can lead to acute pulmonary edema with occasional sudden death in the bovine. Many of the toxic or shock-like etiologies causing the edema that accompanies acute pulmonary

injury may be sufficiently severe to create a set of lesions that appear identical to acute interstitial pneumonia.

Edematous lungs are wet, heavy, and do not collapse completely when the thorax is opened. There is frequently excess fluid in the thoracic cavity. There is an accentuated pattern of the septa because of edematous subpleural and interstitial tissues. Air can be mixed with edema and grossly visible distended, tortuous, and beaded lymphatics are commonly observed. Foam is discharged from the nostrils in severe cases, and is often present in the trachea and intrapulmonary airways.

In general, the histologic presentation of edema fluid is an acidophilic, homogeneous, or faintly granular material filling alveoli. The postmortem seepage of fluid into the alveoli of animals euthanized with barbiturate solutions can easily be mistaken for edema.

The current knowledge base of endotoxin-induced pulmonary injury is that there is an early severe, transient pulmonary hypertension followed several hours later by an increased vascular- and alveolar-wall-permeability phase. Molecules from the cyclooxygenase pathway of the arachidonic acid metabolism (thromboxane and prostaglandin F_2) are responsible for the initial hypertension. The subsequent increased vascular permeability phase is associated with aggregation of leukocytes in pulmonary capillaries, most likely in response to the presence of hyperoxides and leukotrienes derived from the lipoxygenase pathway of the arachidonic acid metabolism cycle.[36]

Discussion

Clinical disease is the result of dynamic interactions between critical factors that exist among pathogens, the environment, and host defense capabilities. Environmental contamination, weather factors that create stress and impaired host defenses may all contribute in tipping the balance between health and disease. In fact, typical definitions of health and disease may not be adequately descriptive in food animal production any longer, because the primary goal is optimal productivity at low input costs, rather than clinical normalcy.

The interest of medical, biological, and chemical investigators in endotoxins has persisted throughout our century. Gram-negative bacteria are responsible for many clinical conditions in animals that range from transient episodes of scours to life threatening meningoencephalitis or sudden death. Multiple organ systems are often involved in these clinical entities and current therapeutic modalities and management practices remain only moderately successful in adequately addressing these diseases. Therefore, a better understanding of the pathophysiology of this disease process and enhanced diagnostic capabilities is imperative.

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