Diagnostic Exercise
From The Davis-Thompson Foundation*

Case #: 140 Month: March Year: 2020

Answer sheet

Title: Bovine Respiratory Disease Complex

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Clinical History: Presented for necropsy is a 2-year-old, black and white crossbred heifer (no ear tags), weighing 875 pounds, in good nutritional and postmortem conditions that was found dead. There was no change in diet or environment. The heifer was fed pasture and hay only.

Gross and Microscopic Images:

Figure 1. Lung with distinct line of demarcation between hyperinflated (top) and consolidated (bottom) tissue.
**Figure 2.** Lung (close-up) with fibrinous pleuritis, interlobular edema, and hemorrhage.

**Figures 3 and 4.** Lung (cross section) with consolidation of the parenchyma (Figure 3) and “golf ball” sized abscesses underlying areas of fibrinous pleuritis (Figure 4).
Necropsy Findings: The eyes are sunken (dehydration). There is no external evidence of trauma. There is abundant subcutaneous and visceral fat. The liver, kidneys, and spleen are grossly normal. The uterus is not gravid, although there is a focal circumferential reddening of the mucosa of the right uterine horn (4 cm wide band) and in the lumen within that band there is a small (5 mm diameter) blood clot; the remainder of the uterine horns, uterine body and the ovaries are grossly normal. The urinary bladder is empty. The rumen, reticulum, and omasum contain dry fibrous plant material with no grain, no foreign bodies, and no evidence of poisonous plants. The abomasal mucosa is red and edematous; the abomasum contains brown fluid, black stones and partially digested hay. The small intestines, cecum, colon, and mesenteric lymph nodes are grossly normal. The cranioventral regions of both lungs are very firm and dark red mottled with yellow; the visceral pleural surfaces of the affected lung regions are elevated, with a ground glass appearance and fibrinous adhesions to the parietal pleural surfaces. There is a sharp line of demarcation between the consolidated cranioventral lung and the dorsocaudal hyperinflated, pink (normal) lung fields. Sections from the cranioventral lungs sink in formalin. The heart and great vessels are grossly normal. The tongue, teeth, caudal oral cavity, skull and brain are grossly normal. In five scattered foci around the thoracic inlet and the cranial area of the left shoulder, there is severe red-tinged subcutaneous and intramuscular edema, and scattered foci with acute intramuscular hemorrhage and edema. Axial and appendicular muscles in other parts of the body are grossly normal.
Figure 6. A terminal bronchiole contains fibrinonecrotic exudate. An adjacent blood vessel has a thrombus. Surrounding alveolar lumens contain viable and degenerate neutrophils, pulmonary macrophages, fibrin and edema. 400X, H&E.

Morphologic Diagnosis: Lung: Lobar pneumonia, necrosuppurative, extensive, marked, with thrombi, prominent interlobular edema and fibrinous pleuritis.

Condition: Bovine Respiratory Disease Complex (BRDC), aka. Shipping fever.

Possible Cause(s)\textsuperscript{1,3}:

Bacterial agents:

\textit{Mannheimia haemolytica} (serovar A1)

- Gram-negative coccobacillus, family Pasteurellaceae
- Upper respiratory tract commensal; opportunistic pathogen
- Multiple virulence factors: adhesin, capsular polysaccharide leukotoxin, LPS, transferrin binding protein
- Bacteremia occurs on day 2 post infection
- Causes fibrinosuppurative lobar pneumonia with or without pleuritis
- Histological lesions include coagulation necrosis and fibrinocellular exudate

**Pasteurella multocida**
- Gram-negative coccobacillus, family Pasteurellaceae
- Part of the normal microbiota in the upper respiratory tract; stress or viral infections allow it to infect the lung and cause bronchopneumonia
- Multiple virulence factors: anti-phagocytic capsule, LPS, protein toxin
- A possible sequela is bronchiolitis obliterans or chronic obliterating bronchitis

**Histophilus somni**
- Gram-negative coccobacillus, family Pasteurellaceae
- Upper respiratory and reproductive tract commensal
- Virulence factors and effect on host response: LPS, lipooligosaccharide, apoptosis of endothelial cells, immunoglobulin binding protein
- Associated diseases: thrombotic meningoencephalitis (TME), respiratory disease, myocarditis, polysynovitis, otitis media, mastitis, and reproductive tract diseases

**Mycoplasma bovis**
- Wall-less bacterium, class Mollicutes
- Synergistic with other respiratory pathogens, forms biofilms to facilitate persistence
- Virulence factors include variable surface membrane lipoprotein antigens, adhesins
- Effect on host response: immune modulation (to Th2), inhibits neutrophil respiratory burst
- Causes mastitis, arthritis, otitis media, pneumonia
- Peribronchial lymphoid hyperplasia (lymphofollicular bronchitis and bronchiolitis) with a mixed leukocyte infiltrate is the singular most predominant histological lesion

**Viral agents:**

**Bovine Viral Diarrhea Virus (BVDV)**
- Positive strand RNA virus, family Flaviviridae, two biotypes 1 and 2
- An experimental study of viral aerogenous exposure showed maximal clinical disease 15 days post infection and virus in the ileum
- The classic BVDV lesion in the gastrointestinal tract is lymphoid depletion of Peyer's Patches
• BVDV induced pneumonia is debatable. Nonetheless, acute BVDV infections may be characterized by respiratory signs and respiratory lesions have been proposed as the main lesion in some BVD viral isolates.

• Causes immunosuppression by targeting and depleting lymphoid tissues, spreads in secretions, and causes multiple system disease such as abortion and persistent infection

Infectious Bovine Rhinotracheitis (IBR) virus/Bovine Herpesvirus 1 (BoHV-1)

• DNA virus, family Herpesviridae, subfamily Alphavirinae

• Entry is through respiratory mucosa; causes epithelial cell apoptosis with nasal, laryngeal, and tracheal mucosal erosion and ulceration

• Evades host defenses: depresses interferon 1 responses, causes latency, suppresses CMI by interfering with TAP-dependent peptide transport and intracellular tracking of MHC 1 which disrupts chemokine function

• Histological lesions: fibrinonecrotic (acute) or lymphocytic/plasmacytic (chronic) bronchointerstitial pneumonia with rare intranuclear inclusion bodies that peripheralize the chromatin

• Also causes conjunctivitis, rhinotracheitis, oral ulcers, reproductive tract infection with abortion

Bovine Respiratory Syncytial Virus (BRSV)

• Negative strand RNA virus, family Paramyxoviridae

• Entry is through the respiratory mucosa

• Effect on host response: immune modulation favoring T helper type 2 cytokines, which depress cytotoxic T cell induction

• An experimental study of viral aerogenous exposure showed typical clinical signs, presence of virus in the lung, and histological lesions at seven days post infection

• Causes lung consolidation with histological lesions of bronchiolitis with epithelial necrosis and syncytia

Parainfluenza type-3 (PI-3) virus

• Negative strand RNA virus, family Paramyxoviridae

• Infects exclusively ciliated respiratory epithelial cells

• Fusion protein F on the cell surface mediates fusion of the viral envelope with the cellular membrane to get the viral genome into the cell. Prior to fusion, the virions attach to the cell surface via the hemagglutinin-neuraminidase protein in a sialic acid-dependent manner.

• Causes bronchointerstitial pneumonia with intracytoplasmic inclusion bodies
Comments: The lung of this heifer was culture-positive for Pasteurella multocida. The heifer also had Clostridium novyi induced myositis. Pasteurella is associated with serious diseases in multiple animal species, e.g. fowl cholera in poultry, atrophic rhinitis in swine, hemorrhagic septicemia in cattle and buffalo, and respiratory disease in ungulates and rabbits. The LPS in the outer membrane of the bacteria sets off the innate immune system through Toll-like receptors, which triggers cell-mediated response. This leads to a cytokine storm, which ultimately can kill the host. The clinical disease is based on the host’s response to infection. In addition, the presence of a tetrasaccharide on the surface of P. multocida may allow the bacteria to avoid innate immune detection through molecular mimicry of the host carbohydrate.

Histologically, as demonstrated in Figures 5 and 6, there were characteristic features of infection caused by bacteria of the family Pasteurellaceae. Bacterial cultures must be performed for final diagnosis.

- Exudation of fibrin into alveolar lumens due to LPS/endotoxin effect on endothelial cells
- Increased numbers of pulmonary macrophages that will secrete inflammatory cytokines (TNF-1, IL-1, IL-6) that cause more leakage
- Neutrophils recruitment and lysis by the endotoxin, resulting in “oat cells”
- Thrombus formation due to the activated endothelium that releases procoagulant tissue factors
- Coagulative necrosis due to the thrombosis and direct effects of the endotoxin
- Dense band of degenerate and viable leukocytes (“oat cells”) that encircle the areas of necrosis and release IL-8
- Interlobular septa and pleura distended by fibrinous exudate
- Bronchiolar lumens filled with thick cellular exudate that can lead to bronchiolitis obliterans

Bovine respiratory disease complex (BRDC): The bovine respiratory disease complex (BRDC) occurs when viral pathogens cause infection on cattle having certain bacteria that may or may not be normal inhabitants of the respiratory tract. The exact cost of BRDC to the cattle industry is unknown, but it is reported to be greater than US$500 million per year in beef cattle operations; in the USA alone it is estimated to exceed one billion dollars annually. In the beef cattle industry, increased losses have occurred in 5-year cycles over the last 18 years. Currently prevention measures include vaccination, stress reduction, and prophylactic antibiotic use. Although much research has been done regarding its prevention, there are only a few conclusive findings. Preconditioning offers some benefit (at least to the purchaser), but efficacy is variable. Weaning prior to sale is perhaps the most important component of preconditioning, although vaccination prior to sale may offer benefits. Vaccination after arrival appears to have limited value. The practice with the clearest benefit is metaphylaxis, i.e., mass medication of a group of animals in advance of an expected disease outbreak. Yet the costs, both monetarily and in terms of potential antimicrobial overuse, preclude its routine practice in cattle. Since prevention seems elusive, selective breeding is currently being explored. Selective breeding involves identifying genes involved in the response to each pathogen and selecting for more resistant cattle to the common agents. Each pathogen is unique in its interaction with the immune system.
system of the bovine host and the particular immune responses that are most protective for each pathogen are not necessarily identical.³

A study of 237 fatal cases of BRDC in a Midwestern feedlot in the USA over a one year period showed that 54% of morbidity and mortality was attributed to BRDC (0.7% total of all causes). The agents isolated were the following: Mannheimia haemolytica (25.0%), Pasteurella multocida (24.5%), Histophilus somni (10.0%), Trueperella pyogenes (35.0%), Salmonella sp. (0.5%), and Mycoplasma spp. (71.4%). Viruses recovered by cell culture were BVDV-1a non-cytopathic (NCP; 2.7%), BVDV-1a cytopathic (CP) vaccine strain (1.8%), BVDV-1b NCP (2.7%), BVDV-2a NCP (3.2%), BVDV-2b CP (0.5%), and Bovine herpesvirus 1 (2.3%). Gel-based polymerase chain reaction (PCR) assays were 4.6% positive for Bovine respiratory syncytial virus and 10.8% positive for Bovine coronavirus. Bovine viral diarrhea virus IHC testing was positive in 5.3% of the animals.²

Another study utilized viral metagenomic sequencing to explore nasal swab samples obtained from feedlot cattle in Mexico and the USA. Twenty-one viruses were detected, with bovine rhinitis A (52.7 %) and B (23.7 %) virus, and bovine coronavirus (24.7 %) being the most commonly identified. The emerging influenza D virus (IDV) was significantly associated with disease, whereas other viruses commonly associated with BRDC such as bovine viral diarrhea virus, bovine herpesvirus 1, bovine respiratory syncytial virus and bovine parainfluenza 3 virus were detected less frequently. This study suggests additional pathogens may be involved in BRDC.⁷

References


The material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein
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