ALVMA’S 28TH ANNUAL CONFERENCE FOR
FOOD ANIMAL VETERINARIANS
PROCEDINGS

FORWARD
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Presented by Soren Rodning, DVM, MS, DACT of AUCVM
Practice Management Rotation and How Your Clinic Can Benefit
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1. Overview
   a. Win-Win Opportunity for all
   b. Practice Management Rotation
      ▪ How it works
      ▪ Hospital Assessment
      ▪ How it benefits your clinic

2. Auburn Practice Management Rotation
   a. Veterinary Practice Management encompasses the study, understanding and application of pertinent business disciplines guiding the decision-making responsibilities of practice owners, veterinarians, veterinary technicians and practice managers that seek to improve profitability and efficiency allowing for a competitive advantage and long-term success.

3. Rotation Goals
   a.Expose students to different business practices, disciplines and strategies which include accounting, economics, finance, hospital design, marketing, management, human resources or labor relations, law and taxation.
   b. Provide veterinary clinic owners a practice evaluation

4. How the rotation works
   a. General overview

5. How the rotation works
   a. Hospital visits

6. How the rotation works
   a. CVM Campus work

7. How your clinic can benefit
   a. Hospital Assessment
      i. Work flow
      ii. Financial analysis
      iii. Fee analysis / comparison
      iv. Communications
      v. Inventory Management

8. The Assessment
   a. Area Observations
   b. Case Observations
   c. Financial Analysis
   d. Major Themes
   e. Demographics

9. What’s needed from you and your clinic
   a. Tax returns
   b. Profit and loss
   c. Balance Sheets
   d. Revenue by category or service
e. Revenue by doctor
f. Inventory
g. Payroll
h. Invoices

10. Your cost
   a. Over 350 dedicated hours form the rotation
   b. Your time and effort
   c. Your bill - $0.00
   d. Travel cost
   e. Any donations or feedback to the program

11. Contact info:
   a. Glen Sellers – 334-844-6732
   b. Dr. Dan Givens, Assoc Dean of Academic Affairs – 334-844-2685

12. What’s up right now
   a. USDA – NIFA Grant
      i. Recruitment and Support of Veterinarians in Underserved Rural Areas of Kentucky
      ii. Student Learning Assessment Improvement Project

13. Question?
Pregnancy loss can be divided into three categories: embryonic death, abortion, and stillbirth. Embryonic death is defined as loss of the conceptus before organogenesis which occurs around day 42 of gestation in cattle. Once organogenesis has occurred the conceptus is considered a fetus. Pregnancy loss after organogenesis is defined as abortion. Abortions in cattle are of great economic concern to producers as an abortion of mid-gestation to term fetus results in a loss of $600-1000. An acceptable average abortion rate in beef cattle is 1-2%, and reported to be as high as 10% in dairy herds. There is cause for concern when the abortion rate rises above 3-5% in beef herds. Discovering the cause of abortion in cattle can be one of the most frustrating problems a practitioner will face, as less than 50% of cases submitted to a diagnostic laboratory are accurately diagnosed. A thorough history and proper sample submission, along with collaboration with the diagnostic laboratory, will increase the success of diagnosing the inciting cause. Infectious agents are usually responsible for about 50% of abortions in cattle. Non-infectious causes of bovine abortion include toxins, genetic conditions, nutritional deficits or extremes, hormonal imbalances, and heat stress.

Key words: Bovine abortion, pregnancy loss

It is crucial to develop a protocol with producers on how to handle abortions on their farm. Many abortifacient agents are also zoonotic and precaution should be taken during sample collection. The female should be identified and isolated. Both fetus and placenta (when available) should be obtained and refrigerated until submitted to the diagnostic laboratory. Producers should contact their referring veterinarian as soon as possible. Helping the producers to maintain accurate herd records will aid in the diagnosis. It is important to manage the producer’s expectations. Make sure they understand that accurate diagnosis is not possible for every case. The process can be time consuming, expensive, and often unrewarding. This does not mean that diagnosis should not be attempted. It is difficult, if not impossible, to resolve the problem without knowing the inciting cause, but the producer should understand the limitations before the process starts.

Important questions to consider when taking a herd history

1. Number abortions/abortion rate
2. Gestation age
3. Age of the dam
4. Duration
5. Number of females at risk
6. Weak calves
7. Clinical signs
8. Females sick/retained placenta
9. Previous abortions in herd
10. Vaccination protocol
11. Breeding program
12. Nutritional program
13. Herd recently worked
14. Weather
15. New additions
16. Abortions at neighbors
Pathognomonic gross lesions are uncommon, and when present, may not be apparent in cases of tissue autolysis. Gross examination might not help in determining which diagnostic test to select. Contact the diagnostic laboratory to ensure you are submitting the correct samples in the appropriate manner. Optimal samples for submission usually include an intact placenta, fetus, and serum samples. The placenta is a better diagnostic sample than the fetus. In cases where the placenta is not available, a caruncle can be removed from the uterus and submitted. It should be divided into two with half being placed in formalin and half sent fresh chilled. If the placenta is fresh and normal, the cotyledons will be bright red and the intercotyledonary areas translucent. As autolysis occurs, the cotyledons become a pale brown color and the intercotyledonary areas become less translucent. Changes due to autolysis can be hard to distinguish from abnormalities. Opacity to the intercotyledonary spaces can also indicate edema, fibrosis, and inflammation. Exudate on the chorioallantoic surface is also indicative of inflammation. Fibrin can be present as yellow, friable material on the cotyledons. Often maternal blood provides limited information, especially if it is a single sample taken the day of the abortion. A titer response to an organism only indicates exposure, and might not distinguish natural exposure or vaccination. In many cases, antibodies levels are high weeks prior to abortion but may be within normal range at the time of abortion. Maternal blood samples are most helpful in animals that have not been vaccinated. Additional information can be gained by submitting serial maternal blood samples taken at least 3 weeks apart and submitting blood samples from unaffected females in the herd for comparison (ideally at least 10). Inspection of the fetus is important to determine gestational age and condition of tissues. If the entire fetus cannot be submitted, both formalin fixed and fresh chilled samples should be taken and include:

**Formalin Fixed**
- Lung
- Liver
- Kidney
- Spleen
- Heart
- Brain
- Skeletal muscle
- Thymus
- Eyelid
- Abnormal tissue

**Fresh Chilled**
- Lung
- Liver
- Kidney
- Spleen
- Heart
- Brain
- Abomasal contents
- Thoracic fluid
- Ocular fluid
When the fetal necropsy is performed on farm, care should be taken to observe for signs that the fetus was alive at the time of parturition. This includes inflation of lungs, thrombosis of umbilical vessels, hemorrhage around the vessels, and milk in the abomasum. The changes to the umbilical vessels are seen due to tearing and thrombosis which occurs during parturition. Also, take note of fluid within the body cavities. A recently deceased fetus will have clear amber fluid within the body. Corneal edema present in a recently expelled fetus indicates that death occurred 6-12 hours prior. Evaluating the liver is very important because organisms which reach the fetus by way of the umbilical veins often induce lesions in the liver. *Listeria monocytogenes*, BHV-1, *Yersinia pseudotuberculosis*, and *Salmonella enterica* are known to induce liver necrosis. Evaluation of the dermis is also important. Fungal infections commonly cause thickened, raised skin lesions. An additional test that can be helpful is fetal IgG concentration. Bovine IgG can be measured in fetal blood or thoracic fluid and if elevated (>20mg/dL) suggest an active fetal immune response to an infectious organism. Serology should be performed to test for BHV-1, BVD, *Leptospira*, *Neospora*, *Brucella*, bluetongue virus, and PI3.

Abortifacient organisms invade the dam by different routes: skin (*Leptospira*), conjunctiva (*Brucella*), respiratory tract (IBR, BVD), vaginal (*Campylobacter, Tritrichomonas*, and *Ureaplasma*), and mouth (BVD, *Listeria, Leptospira*). The placenta is infected by either hematogenous or venereal routes. Placental inflammation/infection puts stress on the fetus with potential spread of the organism to the fetus. The result depends on the stage of gestation. In the first trimester, placental and/or fetal infection induces fetal death, resorption, mummification, maceration, or abortion. During the second trimester, infection causes fetal death, abortion, or mummification. Fetal and/or placental infection during the third trimester results in fetal death, abortion, maceration, mummification, emphysema, stillbirth, or birth of weak nonviable calves. The dam might develop endometritis, metritis, or retained placenta following pregnancy loss due to placental/fetal infection.

**Infectious Causes of Abortion**

**Bacterial**

There are some opportunistic organisms which can come overgrown the uterus resulting in sporadic abortion. These include *Trueperella pyogenes, E. coli*, and *Bacillus sp.* As these organisms are ubiquitous to the environment or part of the normal flora in the reproductive tract, contamination of the placenta or fetus can occur during delivery or sample collection thus caution must be taken when interpreting laboratory results. The sporadic abortions due to *T. pyogenes* usually happen in the second half of gestation. Typically, lesions are not present on the fetus, however small white foci have been reported in the lungs of feti aborted in the first half of gestation. *Bacillus sp.* causes sporadic abortion in the last trimester and the fetus has pericarditis.

*Brucella abortus* is a zoonotic, reportable bacterial disease in cattle. This disease is uncommon in the United States due to stringent eradication programs. Transmission most commonly occurs via contact with aborted tissues or fluids, but can also be spread through the milk or in utero. The organism has an affinity for the erythritol present in placental trophoblast. Abortion rates can reach as high as 80% in unvaccinated herd. The most common time for *Brucella* to induce abortion is from six months on to term. Placentitis is a consistent finding. The membranes will be thickened with yellow exudate, and greyish yellow debris is present around the cotyledons. The placenta has a leathery appearance, causing it to be referred to as ‘Moroccan leather placenta’. The fetus is usually autolyzed, due to being expelled 1-4 days after death. Pneumonia is commonly found in the aborted fetus, with a characteristic cobblestone texture to the lung surface with small white foci. Metritis and retained placenta are common sequelae to *Brucella* abortions. The gold standard of diagnosis for *Brucella* is isolation of the organism from placenta or fetus (lung, spleen, abomasal contents). Serology is the best method of test and is usually performed by card agglutination test. *Campylobacter fetus* subspecies *venerealis*, also known as Vibrio, is a bovine venereal disease causing early embryonic death, infertility, and occasionally abortion. It is an obligate pathogen of the bovine reproductive tract.
The organism is deposited in the vagina after coitus with an infected bull and ascends to the uterus within two weeks. Embryonic death occurs within the first 2 months of gestation. The conceptus is usually lost after maternal recognition of pregnancy resulting in a prolonged interestrus period. Therefore, the most common indications seen in the herd are repeat breeders and infertility. Conception rates have been reported as low as 10% during a herd outbreak. Although uncommon, abortions can also occur between 4-6 months of gestation. Fetal lesions include fibrinous pleurisy, pericarditis, and peritonitis. Placental lesions are similar to those seen with Brucella (leathery intercotyledary areas) however the placenta is typically expelled. Diagnosis can be achieved by multiple methods. Organisms can be seen in abomasal fluid by dark field microscopy or cultured from aborted tissues. If submitting for culture, it is best to ship samples in Amies transport media, with or without charcoal.

*Chlamydia* is an intracellular gram negative bacteria. Clinical signs include abortion, polyarthritis, encephalomyelitis, keratoconjunctivitis, pneumonia, enteritis, hepatitis, vaginitis, and infertility. *Chlamydia abortus* is the most common *Chlamydia* species to induce abortion in cattle, however it is still an unlikely cause of bovine abortion. *Chlamydia abortus* abortions are more sporadic in cattle than small ruminants. Abortions typically occur during 6-8th month of gestation and are accompanied with a necrotizing placentitis. The fetus may have edema, ascites, pleuritic, or peritonitis. Histologic examination of the cotyledons can be diagnostic as the organisms multiple in the cotyledons. Isolation of the organism is gold standard for diagnosis, but this requires specialized media. Members of the *Chlamydia* family are found in the gastrointestinal tract of cattle, so false positives can occur in cases of fecal contamination on aborted tissues. Serial titers can be helpful, as the level is usually normal at the time of abortion but rises 2-3 weeks later.

*Leptospira interrogans* serovar *hardjo* and *Pomona* are the most likely to cause leptospirosis abortion in cattle. The *hardjo* strains are host adapted to cattle whereas cattle are an incidental host for *Pomona*. The organism is transmitted by urine, transplacental, venereally, orally, and across the conjunctiva. *Leptospira* localizes to the reproductive and urinary tracts. Bacteremia occurs within two weeks after inoculation. Abortion occur during the last trimester, and infection can also induce delivery of weak infected calves. Abortion rates due to *Pomona* are much higher than *hardjo*, 50% versus 10-30% respectfully. The aborted fetus is usually expelled autolyzed and may be icteric. Intercotyledary edema is present in the placenta and occasionally yellowish brown fluid and necrotic cotyledons are present. This is similar to what is seen with BHV-1 abortions. Good samples to submit for isolation of the organism include kidney, pericardial fluid, and abomasal fluid. A diagnosis can be made by serology using microscopic agglutination test. Immunohistochemistry (IHC) for the organism can be performed on formalin-fixed tissues.

*Listeria monocytogenes* causes meningoencephalitis, neonatal septicemia, and abortion in cattle. It most common route of transmission is by ingestion of contaminated silage. The organism can spread to the placenta via the blood in 5-12 days inducing fetal septicemia and death. Abortions most commonly occur in the winter months, when cows are in the last trimester of pregnancy. The dam typically is pyrexic and anorexic prior to abortion. The fetus is expelled a few days after death resulting in autolysis of tissues. This masks any lesions that may be present on the fetus. The placenta will have cotyledonary necrosis and intercotyledary placentitis characterized by greyish white to reddish brown exudate. Often the placenta is retained. Other clinical signs seen in the cow include circling and blindness due to encephalitis. The dam can be clinical ill pre-, para-, or post-abortion. The dam can also become re-infected and abort again if continues to be feed contaminated silage. Diagnosis is commonly made by organism isolation, although culture can be slow (up to one month). Gram staining of abomasal fluids or impression smears of tissues can also reveal the presence of the organism.

*Ureaplasma diversum* is part of the normal flora of the bovine reproductive tract. In cases of overgrowth, this organism can cause early embryonic death, late term abortion, stillborn/weak calves, and neonatal pneumonia. Typically, the dam will not have clinical signs of illness but will retain the placenta. It induces a characteristic hemorrhagic amnionitis. The membranes will be thickened and opaque with ecchymotic fibrin, necrosis, and fibrosis. Diagnosis is usually by culture of the organism from abomasal fluid, placenta, or lung.
Viral
Bovine herpes virus 1 (BHV-1) is an alphaherpes virus, causing abortion, genital disease, respiratory disease, and encephalomyelitis in cattle. BHV-1.1 and 1.2a are more associated with abortion and 1.2b with genital disease. Adult cattle have mild clinical signs including conjunctivitis and respiratory signs. Abortion most commonly occurs in the second half of gestation, weeks to months after clinical disease. The virus spreads to the placenta then the fetus. The fetus is expelled autolyzed with pin-point white foci on the liver. Placentitis is similar to that seen with Leptospirosis. Histopathology of fetal lung, liver, spleen, kidney, and placenta can be diagnostic, but should be confirmed by IHC, PCR, fluorescent antibody detection, or virus isolation.

Bovine viral diarrhea virus (BVD) is a pestivirus that causes significant reproductive loss in cattle. Effects on reproduction include genital infection, embryonic death, birth defects, abortion, delivery of small calves, and persistently infected calves. Transplacental infection occurs during the viremic state in the dam. Infection will have a different outcome depending on the stage of gestation at time of infection. Fetal infection in the first trimester causes early embryonic death and resorption. In the second trimester, infection results in abortion and mummification. Abortions most commonly occur 4 months to term. Persistently infected calves develop when infection with the noncytopathic strain occurs before day 125 in gestation. Infections occurring between days 100-150 of gestation can develop birth defects such as hydrocephalus, cerebellar hypoplasia, microphthalmia, retinal dysplasia, cataracts, and brachygnathism. Diagnosis can be made by PCR, IHC, antigen capture ELISA, fluorescent antibody test, and virus isolation. Sample types needed depend on the test being submitted. An ear notch is the most common sample taken to test for persistently infected animals and can be submitted for immunohistochemistry, antigen capture ELISA, or PCR.

Bluetongue is an arthropod spread orbivirus with a worldwide distribution. The disease may appear seasonal, depending on the density of the Culicoides midge. Clinical signs in adult cattle typically manifest as ulcers in the mouth and tongue. Hyperemia and ulceration may also occur at the coronary band, progressing to sloughing of hooves in severe cases. Bluetongue virus replicates in endothelial cells, macrophages, and lymphocytes, resulting in cell death. Reproductive signs associated with infection are congenital abnormalities, abortion, mummification, and stillborn calves. Infection between 70-130 days of gestation can cause abortion and hydranencephaly. After day 150 of gestation, encephalitis or premature delivery occurs but not malformations. Diagnosis can be difficult as the virus may no longer be present at time of abortion, but virus isolation from blood, spleen, or lymph nodes is the best test.

Protozoal
Neospora caninum is a protozoal parasite of canines. Cattle serve as an intermediate host by ingesting canine feces. Vertical transmission occurs in cattle and can pass through multiple generations of females via infection in utero. Abortion may be the only clinical sign present in the herd, typically occurring between 5-6 months of gestation. Calves can be stillborn, mummified, malformed, or born alive. Aborted fetus are usually autolyzed. The placenta may have cotyledonary necrosis with soft areas of dark discoloration. The parasite causes lesions in the fetal brain. IHC can be used to identify the parasite in tissues. Interpretation of maternal titer can be complicated as levels fluctuate or may decline over time and some cows become persistently infected. Pre-colostral serum sample of calves can help to interpret infection in suspected cows, as calves infected in utero will have high titers.

Tritrichomonas foetus is a flagellated protozoal parasite that causes bovine venereal disease. The organism is deposited into the vaginal during coitus with an infected bull. The organism ascends to the uterus, inducing early embryonic death after maternal recognition of pregnancy. Cows show a prolonged inter-estrus period before returning to estrus. Rarely pyometra occurs in infected cows. Late term abortion occasionally occurs, with fetal lesions of bronchopneumonia and enteritis. Diagnosis is made by culture and/or PCR of vaginal fluids or fetal abomasal fluid. Commercially available media is available for shipment and culture.
**Fungal**

The most common mycotic abortions are due to *Aspergillus fumigatus*, other fungal agents that are routinely involved include *Mucor* and *Rhizopus*. Clinical sign are absent in the dam. Placentitis develops slowly, disrupting fetal nutrition leading to death. Abortions occur in the last trimester. The placenta is usually retained and has a leathery appearance, similar to *Brucella*. Fungal organism form lesions along the periphery of the placenta and tend to run with blood vessels. Irregular tan plaques develop on fetal skin. Diagnosis is made by culturing the fungi from aborted tissues or abomasal fluid, or by fungal identification on KOH wet mounts.

**Rickettsial**

*Anaplasma marginale* induces a rickettsial disease in cattle and other hoofstock. The organism is transmitted by arthropods or fomites (such as reusing needles). It is able to cross the placenta to the fetus. The organism invades erythrocytes inducing extravascular hemolysis. Clinical signs seen in adult cattle include icterus, anemia, fever, weakness, and inappetence. Fetal loss occurs due to the maternal systemica illness, including severe anemia, stress, hypoxia, and pyrexia. Abortion can occur at any point in gestation. The aborted fetuses will have splenic enlargement, as well as lung and liver petechiation. Giemsa stained blood smears will reveal the presence of the organism on erythrocytes in acute cases, however this is not the case in carrier animals. Serology and PCR are available to aid in diagnosis.

**Noninfectious Causes of Abortion**

**Nutritional**

A wise farmer once told me “you can’t starve a profit out of a cow”. This is especially true for the pregnant cow. In most cases, starvation will result in termination of pregnancy, as the body prioritizes. Nutritional deficiencies are associated with infertility and neonatal mortality. Nutritional excesses have also been reported to cause reproductive issues.

**Deficiencies**

- Protein – premature calves, dystocia, and high neonatal mortality of poorly muscled calves (known as 'weak calf syndrome')
- Vitamin A – later term abortion, abnormal musculoskeletal and neural development, and weak, blind calves
- Iodine – hyperplastic goiter and hairless, weak calves
- Selenium/Vitamin E – premature calves, stillbirths/abortions, and weak calves

**Excesses**

- Iodine – abortion
- Selenium/Vitamin E - abortions

**Heat Stress**

- Embryos are extremely sensitive to heat stress induced by high ambient temperatures.
- Can induce fetal hypotension, hypoxia, and acidosis

**Hormonal abnormalities**

- Endotoxemia and metritis induce inflammation which stimulates the release of endogenous PGF2α causing lysis of the corpus luteum.
- Estrogen
  - Silage, Legumes, Poultry litter → Abortion
**Genetics**
- Pregnancy loss before day 90 of gestation
- Chromosomal abnormalities
- Lethal genes

**Toxins**
- Nitrates
  - Methemoglobin → Hypoxia → EED & Abortion
- Mycotoxins – zearalenone
- Abortions
- Ergot alkaloids
  - Abortions

Investigation of pregnancy loss in a herd can be a labor intensive, frustrating process. Since there are numerous noninfectious and infectious causes, diagnosis can be complicated. A detailed history and appropriate sample collection are key to success. It is important to submit the fetus, placenta, and maternal serum for laboratory testing and work with the diagnostic laboratory for optimal sample handling and appropriate test selection. The clinical findings should be combined with laboratory test results and herd investigation findings to determine if the presumptive diagnosis fits the clinical picture. An infectious cause is identified less than 50% of the time, in such cases noninfectious causes should be explored. As a bovine practitioner, investigating pregnancy loss in a herd can be challenging, but important undertaking with the goal of improving herd health and ultimately profitability.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Infectious Agent</th>
<th>Abortion Rate</th>
<th>Abortion Time</th>
<th>Recurrence</th>
<th>Lesions</th>
<th>Samples</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella abortus</td>
<td>Up to 80%</td>
<td>6-9 months</td>
<td>Rare</td>
<td>Placenta – retained, necrotic cotyledons, red-yellow color, thickened intercotyledonary areas</td>
<td>Fetus (lung, abomasal fluid), placenta, maternal blood</td>
<td>Maternal serology, FAT for antibodies in placenta, Bacterial culture</td>
<td></td>
</tr>
<tr>
<td>Campylobacter fetus ss venerealis</td>
<td>&lt;10%</td>
<td>Early embryonic death, 5-8 months</td>
<td>Rare</td>
<td>Placenta – mild placentitis, hemorrhagic cotyledons, intercotyledonary edema</td>
<td>Fetus (lung, abomasal fluid), placenta, vaginal fluid</td>
<td>IHC, Darkfield microscopy, Bacterial culture</td>
<td></td>
</tr>
<tr>
<td>Campylobacter fetus ss fetus</td>
<td>Sporadic</td>
<td>Second and third trimester</td>
<td>Rare</td>
<td>Same as above</td>
<td>Same as above</td>
<td>Same as above</td>
<td></td>
</tr>
<tr>
<td>Chlamyphila abortus</td>
<td>Sporadic</td>
<td>Last trimester</td>
<td>Rare</td>
<td>Placenta – placentitis, thickened yellow-brown exudate</td>
<td>Fetus – pneumonia, hepatitis</td>
<td>Placenta, fetus</td>
<td>Acid-fast staining, IHC, FAT, PCR</td>
</tr>
<tr>
<td>Leptospiira interrogans serovars pomona, grippotyphosa, hardjo, icterohaemorrhagiae, canicola</td>
<td>5-40%</td>
<td>Last trimester</td>
<td>Susceptible to other serovars</td>
<td>Placenta – diffuse placentitis, avascular pale tan cotyledons, yellow edematous intercotyledonary areas</td>
<td>Placenta, fetus (kidney)</td>
<td>FAT, PCR, IHC</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Sporadic to &gt;50%</td>
<td>Last trimester</td>
<td>Possible</td>
<td>Placenta – retained, white necrotic foci in cotyledons</td>
<td>Placenta, fetus (brain, lung)</td>
<td>Bacterial culture,</td>
<td></td>
</tr>
</tbody>
</table>
### Protozoal

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Abortion Rate</th>
<th>Abortion Time</th>
<th>Recurrence</th>
<th>Lesions</th>
<th>Samples</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tritrichomonas foetus</strong></td>
<td>Sporadic</td>
<td>Early embryonic death</td>
<td>Immunity developed but not life long</td>
<td>Placenta – retained, mild placentitis with hemorrhagic cotyledons and thickened intercotyledan areas</td>
<td>Placenta, fetus (lung, abomasal contents), vaginal or uterine fluid</td>
<td>Culture, PCR, IHC, Bodian’s silver stain</td>
</tr>
<tr>
<td><strong>Neospora caninum</strong></td>
<td>Up to 30%</td>
<td>5-6 months</td>
<td>Possible but decreases with parity</td>
<td>No gross lesion on placenta or fetus</td>
<td>Placenta, fetus (brain, kidney, lung, liver, skeletal muscle), maternal blood</td>
<td>Histopathology (brain), IHC, PCR, ELISA</td>
</tr>
</tbody>
</table>

### Viral

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Abortion Rate</th>
<th>Abortion Time</th>
<th>Recurrence</th>
<th>Lesions</th>
<th>Samples</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bovine Viral Diarrhea Virus (BVD)</strong></td>
<td>Low</td>
<td>Up to 4 months</td>
<td>Rare</td>
<td>Placenta – retained</td>
<td>Placenta, fetus (lung, liver, skin, heart), maternal blood</td>
<td>FAT, IHC, Virus isolation, Antigen-capture ELISA, PCR</td>
</tr>
<tr>
<td><strong>Bovine Herpesvirus type 1 (Infectious Bovine Rhinotracheitis virus – IBR)</strong></td>
<td>5-60%</td>
<td>4 months to term</td>
<td>Rare</td>
<td>Usually no lesions in placenta or fetus</td>
<td>Placenta, fetus (adrenal, kidney, liver, lung), maternal blood</td>
<td>IHC, Virus isolation, FAT</td>
</tr>
<tr>
<td><strong>Bluetongue virus</strong></td>
<td>Low</td>
<td>Variable</td>
<td>Rare</td>
<td>Fetus – autolyzed, hydrocephaly, arthrogryposis, dwarfism, excessive gingival tissue</td>
<td>Placenta, fetus (brain, spleen), maternal blood</td>
<td>PCR, Virus isolation</td>
</tr>
</tbody>
</table>

### Fungal
<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Abortion Rate</th>
<th>Abortion Time</th>
<th>Recurrence</th>
<th>Lesions</th>
<th>Samples</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>Sporadic, up to 5-10%</td>
<td>4 months to term</td>
<td>Possible</td>
<td>Placenta – severe necrotizing placentitis, enlarged cotyledons, leathery thickened intercotyledonary areas</td>
<td>Placenta, fetus (abomasal contents, lungs, skin,)</td>
<td>Fungal culture, H&amp;E, KOH</td>
</tr>
<tr>
<td>Mucor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizopus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Abortion Rate</th>
<th>Abortion Time</th>
<th>Recurrence</th>
<th>Lesions</th>
<th>Samples</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma marginale</td>
<td>Low</td>
<td>Variable</td>
<td>Possible</td>
<td>Fetus – splenic enlargement, lung and liver petechiation</td>
<td>Blood, Fetus</td>
<td>Organism identified on blood smear, PCR</td>
</tr>
</tbody>
</table>

*ELISA=enzyme-linked immunosorbent assay
*FAT=fluorescent antibody test
*IHC=immunohistochemistry
*KOH=potassium hydroxide test
*PCR=polymerase light chain

References:
11. Larson R. Field investigation of abortion

Ectoparasite Control in Cattle Herds
Kelly Palmer, BS Animal Science, PhD Entomology
Animal Science & Forages Alabama Cooperative Extension System
Bay Minette, AL

NOTES
Management of Equine Ophthalmology Cases in the Field
Jennifer Taintor, DVM
JT Vaughan Large Animal Teaching Hospital AUCVM

Slide 1

Equine Ophthalmology
Managing Cases in the Field

Slide 2

History
• When did the problem start?
• Have they treated it?
• Has it happen previously?
• What is the horse used for?
Exam

- Eyelash angle
- Ocular discharge
- Blepharospasm
- Surface topography
- Visual

Exam

- Cranial nerve and reflexes
  - Optic (CN2)
  - Oculomotor (CN3)
  - Trochlear (CN4)
  - Trigeminal (CN5)
  - Abducens (CN6)
  - Facial (CN7)
  - Vestibulocochlear (CN8)

Exam

- Vision exam
Exam

• Exam location
• Exam aids
  • Sedation
    • Xyalzine
    • Detomidine
    • Butorphanol

Exam

• Exam aids
  • Local nerve blocks
    • Auriculopalpebral
    • Supraorbital
  • Topical anesthesia
    • Corneal
    • Proparacaine
    • Tetracaine
  • Pupil dilation
    • Tropicamide

Exam

• Gross examination
  • Eyelids
  • 3rd eyelid
  • Conjunctiva
  • Cornea
  • Iris
  • Anterior chamber
  • Nasolacrimal
Exam

• Direct ophthalmoscope/ light source
• Cornea
• Anterior chamber
• Fundus
  • Optic disc
  • Tapetal and nontapetal zones
• Lens

Diagnostics

• Fluorescein stain
• Rose Bengal stain
• Cytology
• Culture
• Ultrasound
• Photography

Differential diagnosis (common)

• Corneal Ulcer
  • Bacterial
  • Fungal
• Uveitis
• Conjunctivitis
• Immune mediated
• Neoplasia
Corneal ulcer

- **Bacterial**
  - Most common
  - Strep
  - Staph
  - Pseudomonas

- **Fungal**
  - Most common
  - Aspergillus
  - Fusarium

---

Corneal ulcer

- Stain uptake
- Vascularization +/-
- Cytology
  - Neutrophilic infiltration, bacterial organisms
  - Fungal hyphae

---

Corneal ulcer

- Stromal abscess
  - Nonstaining opacity
  - Asymmetric vascularization
- Indolent ulcer
  - No infectious agents present
Slide 15

Uveitis

- Acute uveitis
- Variety of causes
- Damage to the uveal tract
- Inflammation
- Recurrent uveitis
- Aka moon blindness
- Bouts of inflammation
- Environmental factors, genetics

Slide 16

Conjunctivitis

- Primary
  - Allergic
  - Viral
  - Parasitic
  - Trauma
- Secondary
  - Corneal disease
  - Uveitis
  - Glaucoma

Slide 17

Immune mediated

- Nonulcerative corneal disease
- Etiology unknown
- Corneal opacity
  - Without uveitis present
  - Minimal ocular discomfort
Neoplasia
- Orbital
- Lymphosarcoma
- 3rd eyelid
- Squamous cell
- Corneal
- Squamous cell
- Melanoma

Other
- Blindness
- Cataracts
- Eyelid laceration
- Glaucoma
- Corneal opacity
- Iris or corpus nigra abnormality

Treatment Options
- Topical medications
- SPL systems
- Subconjunctival injections
Slide 21

Treatment options
- Antimicrobial
  - Topical
    - BNP
    - Chloramphenicol
    - Tobramycin
    - Sulfacetamide
- Solutions (SPL)
  - Cefazolin
  - Ofloxacin
  - Ciprofloxacin

Slide 22

Treatment options
- Anti-fungal
  - Miconazole
  - Voriconazole
  - Natamycin

Slide 23

Treatment options
- Corticosteroids
  - BNP with desamethasone
  - Prednisolone
  - Desamethasone
- NSAIDS
  - Flurbiprofen
  - Diclofenac
  - Cyclosporine
Slide 24

Treatment options

• Therapeutic mydriatic/cycloplegic
• Atropine
• Antiproteases
• Serum

Slide 25

Standing field surgery

• Tarsorrhaphy
• Corneal debridement
• Superficial keratectomy
• Mass removal or adjunctive therapy
• 3rd eyelid removal
• Enucleation

Slide 26

Standing field surgery

• Tarsorrhaphy
  • Protection of cornea
  • Facial nerve trauma
**Slide 27**

Standing field surgery

- Corneal debridement
  - Blocks
  - Swabs

**Slide 28**

Standing field surgery

- Superficial keratectomy
  - Blocks
  - Procedure

**Slide 29**

Standing field surgery

- Mass removal/ adjunct therapy
  - Surgical excision
  - Cryotheraphy
  - Intrallesional chemotherapy
Standing field surgery

- 3rd eyelid removal
- Blocks
- Procedure

Standing field surgery

- Enucleation
- Blocks
  - Retrobulbar
  - Supraorbital
  - Zygomatic
  - Lacrimal
  - Intraocular
- Method
  - Closed transpalpebral
  - Trans conjunctival
Update from the AL Dept of Agriculture & Industries

Tony Frazier, DVM
Alabama State Veterinarian
Alabama Department of Agriculture and Industries

Slide 1

Animal Disease Traceability

Overview and current updates
Tony Frazier, DVM
State Veterinarian

Slide 2

- Beef cattle 18 months of age and older must be officially ID’ed when moving interstate and at Change of Ownership in Alabama
- All rodeo stock, dairy cattle and show animals must have official ID
- Exemptions: moving directly to slaughter, commuter herds in agreeing states
- Other species: sheep and goats-scrapie rule
Slide 13

Next Steps
• Advance electronic ID
• Work with Market to help implement
• Work with Accredited labs to advance eCVI’s
• Get official electronic tag to producers
USDA Goals for Increasing Traceability

1. Advance the electronic sharing of data among federal and state animal health officials, veterinarians and industry; including sharing basic animal disease traceability data with the federal animal health events repository (AHER).
2. Use electronic ID tags for animals requiring individual identification in order to make the transmission of data more efficient;
3. Enhance the ability to track animals from birth to slaughter through a system that allows tracking data points to be connected; and
4. Elevate the discussion with States and industry to work toward a system where animal health certificates are electronically transmitted from private veterinarians to state animal health officials.
Slide 18

Points of Interest

- USDA will not dictate the use of specific tag technology
- Low vs. ultra high frequency
- Ending free metal tags program
- January 2019
- Instead, offering some cost-share for electronic tags or possibly infrastructure during transition

Slide 19

Slide 20

Questions?
Mycoplasma: Diagnostics and Prevention
Jason Shumaker, DVM, MS
Newport Laboratories
662-598-5755

*Mycoplasma* species is the smallest sized bacterial pathogen that we face in the cattle industry, but has a significant impact on the profitability of producers. *Mycoplasma* species lacks a cell wall and this makes it difficult to treat and challenging to prevent with vaccines. There are several species of *Mycoplasma* in cattle such as: *M. bovis*, *M. alkalescens*, *M. arginini*, *M. bovoculi*, and *M. bovirhinis*. This paper will focus on *Mycoplasma bovis* and *Mycoplasma bovoculi*.

*Mycoplasma bovis*

Primary *Mycoplasma* infections in cattle are usually mastitis, otitis, arthritis and sometimes pneumonia. *Mycoplasma bovis* presents as pneumonia, otitis media, or polyarthritis in young stock of both dairy and beef facilities. More mature cattle typically present with mastitis, especially in dairy herds. However, it is much more common in the Southeast to see *Mycoplasma* as a secondary pathogen of BRD, bovine respiratory disease. *Mycoplasma bovis* is often associated with a viral component such as (BVD) bovine viral diarrhea virus, or bovine coronavirus in the BRD process. *Mycoplasma bovis* has also be implicated in pneumonia cases that persist in a group of animals for 10-20 days past the initial BRD outbreak.

*Mycoplasma bovis* is spread through nasal secretions. This occurs in nose to nose interactions, feedbunks, waterers, mineral blocks, etc. *Mycoplasma bovis* is well adapted to our environment in the Southeast. It can survive for up to six months in liquids at any temperature. It can survive for up to four months in warm and dry environments, but can only survive for one month in cold, dry environments. Although *Mycoplasma* can survive in the environment, it is susceptible to most common chlorine, chlorhexidine, acid and iodine disinfectants (1). There is a significant amount of strain variation inside the *Mycoplasma bovis* family. This can lead to antigenic variability in VSPs, variable surface proteins, which are one of the main antigenic portions of the pathogen. These are encoded by multiple divergent genes which undergo a high rate of DNA change (2). *Mycoplasma bovis* can present like any other BRD case. However, the disease can present differently on necropsy. *Mycoplasmsa bovis* necropsies will usually present as bronchopneumonia with multi-focal caseous necrosis.

There are several options when trying to diagnose *Mycoplasma bovis* in a herd or group of animals. Joint swabs or exudate from otitis media are good options for non-BRD diagnostics. When investigating BRD, deep naso-pharyngeal swabs or bronchoalveolar lavage is typically preferred over submitting tissues. Post-mortem tissue can inhibit *Mycoplasma* recovery if not submitted to a diagnostic lab within hours of collection. It is also important to select an appropriate media when submitting *Mycoplasma* samples. Most laboratories will provide swabs that are validated for fastidious organisms such as BD Universal Viral transport media or the Copan eSwab. Common Aimes media may limit *Mycoplasma* detection and recovery. It is also important to avoid wooden shaft or cotton tipped swabs, as these swabs can inhibit *Mycoplasma* growth and interfere with PCR testing. Serological testing is also available for *Mycoplasma* bovis. Antibodies can be detected 6-10 days after exposure and the titers can persist for months (1). The limitation is that serology only indicates exposure and not disease. However, serology can be predictive of *Mycoplasma bovis* on a herd basis. Diagnostics have also advanced with the introduction of MLST, multi-locus sequence typing. MLST evaluates nucleotide sequences at specific genes and can identify changes in specific lipoproteins, adherence factors, virulence factors and variable surface proteins (3). MLST testing can only be performed on *Mycoplasma bovis* isolates. MLST can be used to help identify strain variation in *Mycoplasma bovis* within a region or within a herd.

Practitioners should consider utilizing the MLST technology into a prevention program in the form of vaccines. While there are commercial *Mycoplasma bovis* vaccines on the market, their success is dependent on how similar they are
genetically to the actual patient pathogen. Practitioners can incorporate multiple farm specific *Mycoplasma bovis* isolates into a custom-made vaccine using the MLST technology. A *Mycoplasma bovis* surveillance program will help the practitioner identify strain variation and include multiple different and emerging strains into new vaccines.

*Mycoplasma bovoculi*

*Mycoplasma bovoculi* is a mycoplasma associated with keratoconjunctivitis in cattle. Historically, *Moraxella bovis* was considered the primary pinkeye pathogen. However, as diagnostics have improved and expanded, *Moraxella bovoculi* and *Mycoplasma bovoculi* are increasingly identified in keratoconjunctivitis diagnostic cases. The prevalence of *Mycoplasma bovoculi* is difficult to determine because few diagnostic laboratories are able to test for *Mycoplasma bovis*. This mycoplasma appears to cause an infection of the superficial corneal epithelium and results in “dark cells”, which increases the risk of secondary bacterial infections. Studies have found that *Mycoplasma bovoculi* is more common in the eye microbiota of pinkeye cases than the *Moraxella* species (4). Herds with high *Mycoplasma bovoculi* prevalence are more predisposed to infectious keratoconjunctivitis outbreaks (5). *Mycoplasma bovoculi* has also been associated with infectious keratoconjunctivitis in the absence of *Moraxella* species (6).

Sampling early in the disease process from eyes exhibiting only epiphora, blepharospasm and without significant corneal changes usually have more diagnostic value. Diagnostics are very similar to *Mycoplasma bovis*. Eye swabs should be taken with swabs that are validated for fastidious organisms such as BD Universal Viral transport media or the Copan eSwab. Common Aimes media may limit *Mycoplasma* detection and recovery. It is also important to avoid wooden shaft or cotton tipped swabs as these swabs can inhibit *Mycoplasma* growth and interfere with PCR testing.

There are currently no commercial vaccines that include *Mycoplasma bovoculi*. The only way to include this organism into a pinkeye prevention program is to make a custom-made vaccine. Custom-made vaccines are becoming commonplace in bovine practice to address other pinkeye pathogens like *Moraxella bovis* and especially *Moraxella bovoculi*. *Mycoplasma bovoculi* is being included into custom-made pinkeye vaccines at an increasing rate due to practitioner demand. Practitioners should at least include *Mycoplasma bovoculi* as a pinkeye rule-out and consider a custom-made vaccine if they want to address it in their client’s pinkeye prevention program.

References:


NOTES
Trace Minerals in Beef Cattle – Diagnostics and Supplementation

Dan Tracy, DVM, MS
Technical Services Veterinarian
Multimin USA

2/12/2019

Trace Minerals in Beef Cattle - Diagnostics and Supplementation

Trace Minerals: of importance
- Cobalt: Co
- B12
- Iron: Fe
- Iodine: I
- Zinc: Zn
- Copper: Cu
- Manganese: Mn
- Selenium: Se

Co-factors that are incorporated into functional enzymes and proteins.

Nutrition
- Energy
- Protein
- Fats
- Minerals
  - Macronutrients
  - Micronutrients (trace minerals)

Raw Materials
Nutrition

- Energy
- Protein
- Vitamins
- Minerals
  - Macrominerals
  - Microminerals (Trace minerals)

Immune System

- Innate/ Cell mediated Immune Response
  - Immediate Response
- Acquired/ Adaptive Immune Response
  - Takes Time
Reactive Oxygen Species (ROS)

- Normal byproduct of essential oxidative processes
- Also called Reactive Oxygen Molecule (ROM), oxygen radicals, radicals or Free radicals
- Increased production in disease and stress conditions
- Inhibits with exercise and heat stress
- Neutrophils specialize in producing ROS, which are used in host defense to kill invading pathogens
- Act as mitogens, induction of mitosis

How to Control Oxidation

- Electron Donors
  - Vit E, B-carotene, and Vit C
- Enzymatic
  - SOD (Cu, Zn)
  - GPX (Cu, Mn)
  - Catalase (Fe)

- Prevention of Oxidative Stress

Trace Mineral Metabolism

- Absorption
- Transport
- Oxidation
- Excretion
- Storage & Release

- Calcium
- Iron
- Zinc
- Copper
- Protein

[Image of a cow with various mineral absorption points]
Selenium Regulation by FDA

- How it is mixed and premixed
- What levels are allowed
  - Free Choice
    - (b) Bovine: At a level not to exceed an intake of 3 milligrams per head per day.
  - Mixed in feed
    - (1) In complete feed for chickens, swine, turkeys, sheep, cattle, and dikes at a level not to exceed 0.3 parts per million.
Zinc

- "Zinc is required for the structural and functional integrity of over 2000 transcription factors and almost every signaling and metabolic pathway is dependent on one or more zinc-requiring proteins.” (Wurz and Knip, 2006, Science, 2006)

- Zinc-dependent enzymes: 6, 37, 39
- Zinc-binding proteins: 3, 12, 16
- Tumor suppression: 46
- Epithelial Integrity: 2
- Essential for the Rapid Proliferation of cells: 48
- Heat shock proteins: 33
- Copper Zn SOD: 50
- Zinc-regulating proteins: 30

Manganese

- Glutathione peroxidase: 30
- Superoxide dismutase: 30
- Metallothionein: 30
- Peroxidase, catalase, and reductase: 30
- Metallothionein: 30
- Manganese superoxide dismutase: 30

Fig. 1: This figure shows a schematic representation of manganese transport and metabolism. Manganese is transported in the diet and is absorbed in the small intestine, where it is transported to the liver and other tissues. Manganese is involved in various metabolic processes, including antioxidant defense and detoxification. The figure highlights the importance of manganese in human health.
2/12/2019

Changes in TM Status

Challenges

Oral Supplementation
Variation in Intake, Absorption and Antagonists

Trace Mineral Status Not Static

Pregnancy/Trace Mineral Relationship

- 2001 Dairy NRC
  - Gestating Dairy Cow: Reduced from 40 mg Mn/kg DM to 17.8
- Study: control (15.8, trt 50 mg/kg DM)
  - Weaned less at birth
  - Seizures, brachygnathism
  - Prematurity, disproportionate dwarfism
  - Swollen joints
Pregnancy/Trace mineral relationship

Liver selenium depletion

WHEN DOES TRACE MINERAL SUPPORT FROM THE COW END?

- For the calf it has already ended, at birth
  - Calves are born with 2-5 times the level of the cow (liver)
  - Everything the dam will give this calf is already complete
- Except for a small amount in milk

<table>
<thead>
<tr>
<th>MINERAL</th>
<th>MLC (ppm)</th>
<th>NRC RECOMMENDED (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPPER</td>
<td>.012</td>
<td>10</td>
</tr>
<tr>
<td>MAGNESIUM</td>
<td>.013</td>
<td>30</td>
</tr>
<tr>
<td>zinc</td>
<td>3.16</td>
<td>20</td>
</tr>
<tr>
<td>selenium</td>
<td>0.001</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Deficiencies

- Primary
  - Deficient area, Low levels in forage
  - No oral supplementation
- Secondary
  - Free-choice minerals vs. supplemented feed
  - Mineral "tie-up"
    - Sulfur: DDG, CGF, water
    - Iron, Calcium
cattle grazing endophyte-infected fescue exhibited decreased copper status as opposed to cattle grazing endophyte-free fescue. However, the magnitude of this decrease was greater than the difference between the forages. This demonstrates that the endophyte not only decreases the total amount of copper present in the fescue forage, but also negatively affects bioavailability of copper for the animal.

Factors That Influence Dietary Trace Minerals

- Intake
  - Variable
- Absorption
  - Bioavailability/Absorption
  - Organic
- Antagonism
  - Negative Interaction

% OF CALVES EATING AFTER ARRIVAL AT FEEDYARD

<table>
<thead>
<tr>
<th>Reg</th>
<th>Calves eating, %</th>
<th>Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71.7</td>
<td>0-90</td>
</tr>
<tr>
<td>2</td>
<td>55.7</td>
<td>0-90</td>
</tr>
<tr>
<td>4</td>
<td>65.7</td>
<td>0-90</td>
</tr>
<tr>
<td>8</td>
<td>80.5</td>
<td>0-90</td>
</tr>
<tr>
<td>7</td>
<td>71.1</td>
<td>0-90</td>
</tr>
<tr>
<td>9</td>
<td>74.2</td>
<td>0-90</td>
</tr>
<tr>
<td>10</td>
<td>85.0</td>
<td>0-90</td>
</tr>
</tbody>
</table>

Bioavailability VS Absorption

<table>
<thead>
<tr>
<th>Substances</th>
<th>Copper</th>
<th>Zinc</th>
<th>Iron</th>
<th>Selenium</th>
<th>Zinc Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Meal organic</td>
<td>0.7%</td>
<td>0.7%</td>
<td>0.2%</td>
<td>0.5%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Fish Meal inorganic</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Flaxseed Oil</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

Compounding factors

- **Antagonism: Negative Interaction “tie up”**
  - Calcium: >2.5% DM reduces Se absorption
  - Sulfur (Water, DMS, molasses): reduces Se, Cu absorption
  - Iron (5%): reduces Cu absorption or gut solubility
- Cu-Mo 5: Thiomolydate: reduces copper bioavailability

Sources of High Sulfur

- Requirement 0.13%
- Trace Mineral Tie-Up, marginal 0.25%, full 0.3%
- Distillers grains: 0.4%
- Molasses: 0.3-0.5%
- Alfalfa: 0.2-0.5%
- Brassicas (Turnips and Radishes): 0.5-0.6%
- Fungi fertilized with sulfur

**Effect of Sulfur Content of Supplemental Feeds On Liver Copper Accumulation in Sheep**

- Mouse (30 ppm)
- Corn (20 ppm)
- Soy (10 ppm)
Interactions between Mo, S and Mo + S on Cu absorption

<table>
<thead>
<tr>
<th>% Reduction in Cu Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Diet (0.1% S, 0.5 ppm Mo)</td>
</tr>
<tr>
<td>+ 4 ppm Mo</td>
</tr>
<tr>
<td>+ 0.3% S</td>
</tr>
<tr>
<td>+ 4 ppm Mo and 0.3% S</td>
</tr>
</tbody>
</table>

1) Cu and Mo = Formed in diet or metabolized
2) Cu and S = Formation of Cel
3) Cu-Mo interaction = Enhanced release formation (formal, direct and indirect)


Cu Zn SOD and antibody titers to porcine RBC

Although no differences were apparent in plasma or liver Cu concentrations among calves fed the control, Mo, and Cu + Mo diets, calves fed the Mo diet had a more severe Cu deficiency based on depressed humoral immune response and superoxide dismutase activity.

Don’t overlook WATER!!

< 600 ppm: Safe
600-1000 ppm: Generally Safe
Slight reduction in Performance may be indicated (on dry feed)
Slight reduction in Cu availability

1,000 - 2,000 ppm: Severe Cu deficiency likely indicated
Performance may be reduced (on dry feed)
May cause minor Cu deficiency

2,000 - 3,000 ppm: Severe Cu deficiency likely indicated
Performance likely reduced (on dry feed)
May cause substantial reduction in Cu availability

3,000 - 4,000 ppm: All classes of cattle affected
4,000 ppm and greater: TOXIC
Total DM Sulfur Intake with 1000 ppm in Water

Depletion Period
- Steers blocked by BW to one of two diets fed for 95 d:
  - Control diet (CON) supplemented with Cu, Mn, Se, and Zn at NRC recommendations (n = 30)
  - Antagonist diet (ANT) with 5 mg of Mn/kg DM and 0.3% S (as CaSO₄) added, and not supplemented with Cu, Mn, Se, or Zn (n = 30)
- 40% corn stage, 33% DDGS, 25% corn, 2% supp
- Liver samples were collected at the start and end of depletion

Repletion Period
- Within depletion diets steers assigned to one of three TM repletion strategies:
  - Multirepletion injection and 100% of NRC recommended dietary TM supplemented from inorganic sources (ITM)
  - Sulfur injection and 100% of NRC dietary TM supplemented from only inorganic sources (INS)
  - Sulfur injection and 25% of NRC dietary TM supplemented from both inorganic (75%) and organic (25%) sources (BLEND)
- Depletion diets continued through the repletion

Effect of trace mineral repletion strategy on status of steers fed supplemental Ca and S

- Seventy-two Red Angus steers (354 ± 14 kg) were utilized in a 334 d trial consisting of a 140 d depletion period lasting 95 d, and a 62 d TM repletion period
- Steers were housed in pens (n = 6 steers per pen) equipped with GrowSafe bunkers to collect individual feed intake data

S. J. Hartman, O. Goedert-Schreuder, and S. L. Hansen
Mineral testing: The approach depends on what you want to find out

- Clinical: Poor Performance/Disease vs. Deficiency.
- Sub-Clinical: Deficiency.
- Serum vs. Liver Tissue
- Sensible Reference Range

Influenced by stress or stage of production
Poor Performance (Clinical)

- Sample:
  - Correlates with affected sites systemically.
  - Blood (Serum or Plasma)
- Sample Time:
  - Affected Animals
  - Test at the time of the problem
- Results:
  - Mean or average
  - Individual results

Diagnostic Purpose

- Homeostasis maintains serum/blood trace minerals within a relatively constant range regardless of storage status.
- Deficiencies and toxicities are not manifested in serum until stores are very low or very high.
- Selenium is the most dynamic and blood levels are of value.
  - Short term for serum
  - Longer for whole blood

Farm Deficiency (Sub-Clinical)

- Sample: Blood (serum, plasma), Liver Tissue
- Sample Time:
  - Before anticipated deficiency
  - Late pregnancy, early lactation, or growing calves (>6 months).
- Results:
  - Mean or average and individual results

When to Biopsy - Cow

Figure 3: Changes in Liver and Acrea Copper Concentrations for Beef Cows

<table>
<thead>
<tr>
<th>Time of Sampling</th>
<th>Preparturition</th>
<th>Lactating</th>
<th>Breeding</th>
<th>Postparturition</th>
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<tbody>
<tr>
<td>Days</td>
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<td>0.1</td>
<td>0.7</td>
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<tr>
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<td>0.1</td>
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<td>0.4</td>
<td>0.7</td>
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</tr>
</tbody>
</table>
WHEN TO BIOPSY

• Liver Biopsy at Pregnancy Test Time Should Correspond to the "High Point" in the Cow’s Annual Storage
  – Evaluation Prior to Cow Entering the Last Trimester When Stores Will Begin to Mobilize and Drop
• Anytime Diagnostic Value May be Achieved

WHEN TO BIOPSY - CALF

• From Birth to Weaning, Calves have Demonstrated in Multiple Studies that Liver Stores are Depleted and May Drop to Deficient or Critical Low Levels
• Biopsy Prior to Weaning
• Biopsy Anytime Disease Levels in Herd Indicate Suboptimal Immunity and Health
  – Pinkeye, Footrot, Scours, BVD

OTHER LIVER SAMPLES

• Liver Samples Should be Collected from any Dead Cow, Calf or Bull
• May be Used for Herd Monitoring of Trace Minerals and Vitamins

• Number: 10-12 samples
  – Blood or Liver Tissue
• Serum or Whole blood
  – Royal blue-top Vacutainer® tubes
Liver Biopsy

- Steve Ensley DVM, PhD
  - Clinical Toxicologist
  - Kansas State University Veterinary Diagnostic Laboratory
  - https://www.youtube.com/watch?v=xypF500wR0M
- Utah State University Extension: http://www.youtube.com/watch?v=3ZlazzywN6U

INTERCOSTAL SPACE

- 10th or 11th intercostal space
- Between ribs 10 and 11 or ribs 11 and 12
- Needle needs only to penetrate the skin, intercostal muscles, and diaphragm before entering the liver

BIOPSY PROCEDURE

- Site should be prepared as if for surgery
- 2 to 5 ml of 2% lidocaine
- #15 Bard Parker blade is of convenient size for making a stab incision
- Insert the needle approximately parallel to the ground and angled slightly forward toward the left shoulder of the animal
- Needle needs only to penetrate the skin, intercostal muscles, and diaphragm before entering the liver, a distance of only about two and one half inches in most adult cows
BIOPSY PROCEDURE

- notice a distinct "popping" sensation as the needle punctures the diaphragm.
- You will also notice that the needle moves with the animal's respirations after it is through the diaphragm.
- When the needle is in this position proceed with taking the biopsy
- When notch is completely full, that is usually about 10 to 12 mg of liver tissue need 15 mg for best result - get two

<table>
<thead>
<tr>
<th>Reference Number</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Heart</th>
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<tr>
<td>100 g</td>
<td>400</td>
<td>300</td>
<td>200</td>
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<td>200</td>
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<td>50</td>
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<td>25 g</td>
<td>100</td>
<td>75</td>
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</tr>
</tbody>
</table>

Questions???
A Brief Review of Anaplasmosis
Daniel Scruggs DVM, Diplomate ACVP

Overview
Although Anaplasmosis has been recognized clinically and well described for over a century, there is surprisingly little and conflicting information regarding innate immunity and endemic stability to explain the waning and waxing prevalence of disease in endemic areas. Anaplasmosis is an extremely complex disease in terms of the host immune response, innate immunity of young cattle, and the assumed level of protection conferred by chronic infection. Because of these uncertainties there is a lack of consensus among practicing veterinarians and some researchers regarding the best practices for control of anaplasmosis in cattle herds. Over the years, veterinarians have tried to capitalize on the derived benefits of allowing some level of infection to occur in young cattle, coupled with the control benefits from the use of Aureomycin® in susceptible older cattle, and the therapeutic use of oxytetracycline in clinical cases of Anaplasmosis to achieve suitable prevention of disease in herds depending on their geographic prevalence of disease and the goals of the operation.

The Disease: Transmission, and Clinical Presentations
*Anaplasma marginale* is transmitted predominantly by insect vectors. Anaplasma will multiply and concentrate in the salivary glands of some species of ticks making them effective amplification vectors (Kocan 2010, Kocan 2004). Ticks are regarded as the primary vectors in the central and western US. In the eastern US, multiple species of biting flies serve as mechanical vectors transmitting blood from infected cattle. Transmission by mechanical means such as castration, ear tagging, implanting, dehorning, etc. where blood contaminated instruments transmit the organism from one animal to another are well documented and are often responsible for large scale outbreaks. Anaplasmosis has an incubation period of between 7 and 60 days depending on the number of organisms transmitted (Kocan 2003).

Cattle under 6 months of age are usually relatively resistant to development of disease. Cattle older than 2 years are much more susceptible, and there are varying levels of disease manifestations in cattle between 9 months and 2 years. Anaplasma organisms attach to the red blood cells and multiply, roughly doubling in number every 24 hours. Cattle usually do not show clinical signs until 15% or more of the red cells are infected. Depending on the strain, up to 70% of the red blood cells may become infected. The infected cells are removed predominantly by the spleen resulting in an acute onset of profound anemia that may result in mortality rates over 40%. ((Richey 1991; Kocan et al. 2003). The removal of infected erythrocytes by the spleen results in rapidly developing and profound anemia without hemoglobinemia or hemoglobinuria. Affected cattle develop anemia, icterus, weakness, lethargy, hyperexcitability. Abortion subsequent to acute infection is common. Bulls seem to be more prone to develop clinical disease and seem more prone to prolonged effects from acute disease. Bulls experiencing acute or subclinical anaplasmosis show reduced libido and reduced fertility due to testicular degeneration perhaps associated with the febrile response, profound hypoxia or both (*Theriogenology* vol 11 (4) April 1979 pp 277-290) Cows in late gestation are prone to abortions due to anaplasma-induced anemia, and congenital transmission to calves has been described.

On necropsy, the affected cattle have thin watery blood, and blood clots may be difficult to find. The tissues are yellow discolored, the liver is often swollen and yellow discolored, and the gallbladder is often distended. The spleen is greatly enlarged, dark red colored and meaty. There is no blood in the urine, but the urine may be discolored brownish yellow due to the bilirubin in the urine. Blood smears taken at necropsy or from acutely affected animals stained with Wright-Giemsa or Diff quick Stain have minute spherical bodies along the margins or erythrocytes. They are not subtle or difficult to detect in acutely infected cattle.

Cattle infected early in life are usually clinically unaffected, remain chronically infected and generally do not develop acute anemic disease except under unusual circumstances. This is the usual disease course when cattle are infected under a year of age. The exact mechanism of this age-related protection is not known but could be due to the young
animal’s ability to regenerate red blood cells effectively and possibly because of maternal antibodies. These cattle infected early in life and those recovering from clinical or subclinical disease develop a chronic low level infection that generates waves of rickettsiaemia that peak over intervals of 10 to 14 days (Kieser, S. 1990). These parasitemic waves are due to variation in the surface proteins expressed by the anaplasma organism, creating new antigenic variants which transiently evade immune detection. Usually these parasitemic waves are mild and below the clinical threshold of disease, but they are important in the epidemiology of disease transmission from carrier cattle by insect vectors or by transmission due to surgical procedures. While the literature indicates that chronically infected cattle rarely develop disease, some researchers suggest that sufficient immune suppression resulting in imbalance between the host and parasite in these chronically infected cattle could be responsible for outbreaks of clinical disease, especially in highly stressed cattle (Coetzee personal communication)

Diagnostics
The competitive ELISA (cELISA) is now the most frequently used serologic test for anaplasmosis, largely replacing the older and less sensitive complement fixation test. The older CF test has been responsible for some erroneous conclusions regarding anaplasma clearance by use of injectable tetracyclines. The purported clearance of anaplasma by injectable oxytetracycline treatments in the 1970s and 1980s has been disproven by more recent studies utilizing cELISA, rtPCR and inoculation of spleenectomized cattle. Cattle that are chronically infected with Anaplasma are positive to the cELISA or real time PCR (rtPCR). Cattle that are in the incubation phase or early clinical stage are often cELISA negative, but may be rtPCR positive. This is explained by the ability of rtPCR to detect the presence of organism prior to the seroconversion that is detectable by cELISA. The cELISA remains positive in these chronically infected cattle because of the continual antigenic exposure of the chronic infection. If cattle are truly cleared of Anaplasma infection, they become negative on both rtPCR and cELISA, but it usually takes up to 90 days after the infection is cleared for the cattle to become seronegative with the cELISA.

Chronic infection must be differentiated from chronic disease. Some cattle recovering from acute infection develop a chronic nonresponsive normochromic, normocytic anemia with chronic debility and wasting (Pathology of Domestic Animals 1992) It is usually difficult to find Anaplasma bodies in these cattle. The precise reason for this chronic condition is unknown, but could be due to the organ malfunction precipitated by the profound anemia that occurs during acute disease. Acute centrolobular hepatic necrosis and renal tubular nephrosis may be precipitated by the profound anemia of acute disease.

Tetracyclines in Treatment and Prevention of Anaplasmosis: Historical Understanding and Implications
Injectable oxytetracycline has been used extensively to treat clinical disease in infected cattle and studies show that use of parenteral oxytetracycline is effective in treating clinical disease. Parenteral oxytetracycline has also been used to prevent disease in cattle during the incubation period and has shown reduced incidence of disease; however, a smaller number of cattle still developed disease following a prolonged incubation period (Lincoln 1982). Multiple studies have attempted to achieve clearance of Anaplasma with parenteral oxytetracycline. Those that showed clearance usually did so with the CF test. However, studies repeated with the more sensitive cELISA and rtPCR showed that treating with injectable oxytetracycline alone failed to achieve clearance of chronic infection which suggests that those earlier studies may have incorrectly predicted the ability of parenteral oxytetracycline to clear chronic infections. (Aubrey 2011, Rheinbold et al. 2009, Wallace et.al. 2007)

Aureomycin chlortetracycline is labeled as follows:
**Beef Cattle (under 700 lb): Control of active infection of anaplasmosis caused by Anaplasma marginale susceptible to chlortetracycline.** 350mg/hd/day.

**Beef Cattle (over 700 lb): Control of active infection of anaplasmosis caused by Anaplasma marginale susceptible to chlortetracycline.** 0.5mg/lb body weight / day.
Beef and Non-Lactating Dairy Cattle: As an aid in control of active infection of anaplasmosis caused by Anaplasma marginale susceptible to chlortetracycline when delivered in a free-choice feed. Free-choice feed must be manufactured under a feed mill license utilizing an FDA approved formulation. 0.5-2mg/lb body weight/day

Aureomycin has demonstrated effectiveness in controlling clinical anaplasmosis for decades, and is the only method approved for control of anaplasmosis in the US.

The effect of Aureomycin on Anaplasma is presumed to be related to long term exposure causing inability of the organism to replicate and propagate infection to other erythrocytes. (Kocan 2003, Rheinbold 2010). Aureomycin however has been shown to clear chronic infections when fed at 4.4mg/kg for 80 days,(Reinbold 2010) and at 1.1mg/kg for 120 days (Richey 1977). In the Rheinbold study, cattle cleared infection by rtPCR, cELISA and sub-inoculation into spleenectomized steers. These studies were not done under typical production conditions so the ability to clear anaplasmosis by Aureomycin feeding under field conditions remains to be established. Aureomycin is not labeled to clear persistent infection of anaplasmosis, and should not be promoted for that purpose; however, these studies and decades of practitioner experience have spawned the philosophical debate of whether continuous Aureomycin use in endemic areas will create naïve herds, and how Aureomycin might be applied to control disease and retain some level of endemic stability. This topic is brought up only to illustrate the divergent opinions on how Aureomycin should be applied in endemic areas.

Cleared cattle do become seronegative via cELISA and are susceptible to re-infection. The consequences of re-infection in terms of development of clinical disease are unclear. Elimination is potentially beneficial for seed stock producers sending cattle to herds in low prevalence areas and to reduce the potential for introduction of anaplasmosis into naïve herds.

Bulls require particular attention prior to and during the breeding season because of the demonstrated effects of acute disease on fertility. There may be adequate justification for year round control in bulls in endemic areas given the long recovery time to re-establish fertility in affected bulls. Due to heavier body weight, particular attention is necessary to ensure adequate intake of Aureomycin to achieve the desired results. Likewise, late term abortions due to anaplasmosis are well recognized in cows, particularly in fall calving herds where late gestation coincides with peak vector season. These seem to be time periods where there is little disagreement about the value of control.

References.


Reinbold, J. B., J. Coetzee, and R. Ganta, 2009a: Comparison of three tetracycline antibiotic treatment regimens for carrier clearance of persistent Anaplasma marginale infection derived under field conditions. Proceedings of the 42nd Annual Conference of the American Association of Bovine Practitioners (AABP), Omaha, NE


Swift Theriogenology Volume 11, Issue 4, April 1979, Pages 277–290

Diagnostic Sample Collection, Handling, Transport and Submission
Heather Walz, DVM, PhD, DACVP
Alabama Department of Agriculture & Industries
Thompson Bishop Sparks State Diagnostic Lab (TBSSDL)
Auburn, AL

Submissions to a diagnostic lab can be rewarding and can provide beneficial information that may dictate future treatments during disease outbreaks, or can help identify the cause of death and permit for management changes or removal of toxic substances. Submitting specimens to a diagnostic lab can also be frustrating, and a good relationship between practitioners and diagnostic lab staff can help alleviate some of these issues. In many cases, being able to call ahead of time will make sure all aspects of the submission are covered. It is also the best interest of pathologists for a diagnosis to be made for each case, especially a meaningful diagnosis that explains the clinical history. Generally, cases with a clear diagnosis have a faster histology turn-around time, and cases without a clear answer require additional reading and consultation with other pathologists. This document is intended to provide a basic outline for diagnostic lab submissions (with an emphasis on cattle) to the Alabama Veterinary Diagnostic Lab (AVDL) System, which includes laboratories in Auburn, Boaz, Elba, and Hanceville.

For nearly all cases, pathologists save a routine set of tissues and organs, and this set of fresh samples is frozen for future use. This can be valuable in cases where there are gross necropsy lesions that explain one death, but in subsequent days there are other deaths on the farm that appear to be caused by a different disease process and we need to look further. In these situations, we will pull tissues and organs from this storage pool and begin the testing process. The best use of client money is for formalin fixed tissues to be submitted with fresh samples as a food animal practitioner necropsy. The Alabama Veterinary Diagnostic Lab (AVDL)/Thompson Bishop Sparks State Diagnostic Lab charges $25.00 plus a $10.00 accession fee for food animal necropsies. This $35.00 fee covers all tests performed including histopathology, toxicology, bacteriology, virology, and PCR (molecular diagnostics). All of these options are not always necessary for a diagnosis but having these diagnostic tools available at a flat-rate provides both practitioners and pathologists a great opportunity to make a diagnosis, and this reduces the financial limitations that can often impair our ability to make a diagnosis.

SPECIMEN HANDLING AND TRANSPORT OF FRESH SAMPLES: All fresh tissues should be placed in leak-proof bags such as a Whirl-Pak® and double bagged. If submitting breakable items such serum tubes, wrapping the tubes in paper towels or other padded/absorbent material and enclosing within a Whirl-Pak® or Ziplock® bag is recommended. Overnight transport of fresh samples is vital for adequate tissue preservation, and overnight shipping also helps reduce issues with samples decaying on the mail carrier’s truck or at the postal facility over the weekend. External packaging should be sufficient that contents should not leak outside the container. Even with overnight transport, multiple ice packs are required to cool the specimen in an insulated container, especially during warm months. Filling most of the available space in the insulated container with ice packs is recommended during summer months, as we have seen one to two small ice packs does not sufficiently cool specimens during summer months in Alabama. Virology and PCR samples can be frozen prior to transport, but it is important that samples requiring bacterial culture remain at a temperature close to refrigeration prior and during transport. It is also beneficial to place submission forms in a separate leak-proof plastic bag (Ziplock® bag).

PATHOLOGY: Factors that limit our ability to make a diagnosis are primarily: postmortem autolysis/poor tissue preservation, freezing of tissues and organs prior to formalin fixation, and small sample sizes that may not represent the overall disease process. A 10:1 ratio of 10% formalin to tissue should be used. Formalin fixed tissues should be submitted in a wide necked container approved for use with formalin. Sealing the lid with paraffin wrap, lab or masking tape can also reduce the likelihood of spills.
**VIROLOGY and MOLECULAR DIAGNOSTICS (PCR):** It is recommended fresh tissue samples are frozen immediately. Unlike bacteriology, smaller samples are recommended (maximum of 1.0-1.5 cm diameter) because tissue maceration/digestion procedures become problematic with larger sample sizes, and this can complicate DNA/RNA extraction. These fresh samples should be aseptically collected and placed in individually labeled sterile bags (Whirl-pak®). Virology swabs should be maintained at 4°C (refrigerated) until transport on ice.

**Bovine viral diarrhea virus (BVDV):** Ear notch samples for BVDV antigen-capture (AC) Enzyme Linked Immunosorbent Assay (ELISA), or ACE, should be placed in phosphate buffered saline (PBS) in a sterile leak-proof tube and can be refrigerated for up to 72 hours. Shipping should be overnight on ice. These prefilled tubes are available from TBSSDL as a BVDV testing kit that can be shipped to producers or veterinary practitioners for a fee. If a necropsy was performed, the tissue of choice for BVDV is spleen, but lymphoid tissues such as thymus, lymph node, and ileal Peyer's patches are also useful, especially for histologic confirmation of a positive PCR. Generally, lymphoid depletion is found in persistently and transiently/acutely infected animals, and there is a dramatic collapse of Peyer’s patches due to loss of lymphocytes. In some cases, spleen, thymus, and lymph node may show less dramatic lymphoid depletion than Peyer’s patches. Other lesions found with BVDV may include cryptitis, so small intestinal and large intestinal histologic specimens can be helpful. In cases of bronchopneumonia, PCR detection of BVDV in the lung may help determine if immunosuppression from BVDV was the underlying cause for bacterial bronchopneumonia.

**Bovine herpesvirus-1 (BHV-1):** Fresh and formalin fixed lung and/or trachea are needed for a diagnosis. Histopathology on lung and trachea are beneficial to confirm a PCR positive result for BHV-1.

**Bovine respiratory syncytial virus (BRSV):** In these cases, histology is a great benefit in these cases due to prominent syncytia and intracytoplasmic inclusions that are anticipated for these cases. PCR and histology on lung samples are both important for diagnosis because viral nucleic acid is often not detected following the incubation or acute phases of infection.

**Parainfluenza-3 virus (PI-3):** PI-3 virus also causes similar intracytoplasmic inclusions as PI-3 virus, and bronchointerstitial pneumonia can closely resemble BRSV lesions. Our laboratory offers a PI-3 virus fluorescent antibody test. Fresh and formalin fixed lung are required, and PI-3 virus positive cases may not be detected following the incubation or acute phase of disease.

**Bovine coronavirus (BCV):** Fluorescent antibody testing on intestine and lung is offered at TBSSDL. BCV infects epithelial cells in the nasal turbinates, trachea, and lungs. Distal small intestine and spiral colon are recommended for enteric samples.

**Bluetongue (BT):** Fresh spleen is necessary for PCR detection, as well as serum for serologic diagnosis. These samples are most valuable in recently aborting animals. Fresh spleen is also used to detect BT infections in deer. Histologic findings are usually non-specific, unless vasculitis is detected.

**Epizootic hemorrhagic disease virus (EHDV):** Similar to BT, fresh spleen is needed for diagnosis. Serologic testing for EHDV is also possible for cattle and deer. Histologic evaluation is typically non-specific, and finding vasculitis is extremely rare. Most cases of EHDV in deer at TBSSDL are diagnosed by PCR testing.

**Mycoplasma:** Swabs, respiratory mucus or lung in a Whirl-pak® or sterile container are used with general Mycoplasma primers for screening, and species identification is performed with restriction fragment length polymorphism (RFLP).
**Mycobacterium avium paratuberculosis** (Johne's disease) in cattle: Feces should be submitted in a Whirl-pak® or sterile container for culture and confirmation by PCR. We do not currently perform PCR directly on feces. Additionally, we cannot attempt to culture sheep or goats for Johne's disease.

**Trichomonas:** Samples for *Trichomonas foetus* testing should be submitted in a TF Transit tube that should not be refrigerated or placed on an ice pack during shipment. After an incubation period in our laboratory, PCR is currently performed on Mondays, except weeks with holidays occurring on a Monday.

**SEROLOGY:**

**Anaplasma:** Serologic testing (c-ELISA) is an invaluable asset for diagnosing anaplasmosis in cattle. Cytology is also an important diagnostic tool. Cytologic examination is offered at the Auburn University College of Veterinary Medicine, and these bacteria found near the surface of the erythrocyte can be found in Diff-Quik or Giemsa stained preparations. Gross necropsy and histologic findings (most notable in the spleen and liver), and impression smears of liver, spleen, and peripheral blood can be evaluated for intraerythrocytic organisms.

**PARASITOLOGY:**

**Cryptosporidium:** Auramine O stain on feces can be performed in a variety of species.

**Fecal flotation, Direct fecal smear, Kaplan Lab McMaster's Egg Count, and McMaster’s Egg Count** are performed at TBSSDL, and 2 grams of feces are recommended for quantitative assays.

**BACTERIOLOGY:** Samples should not be frozen prior to submission. Please attempt to aseptically collect tissues, organs, and lesions, and place in sterile individually labeled plastic bags (such as Whirl-pak® bags). It is helpful for the requested test to be marked on the submission form, or please indicate what pathogen you suspect so appropriate media can be used. Tissue samples should be approximately 5 grams or larger to allow for searing of the tissue in order to reduce surface contaminants. Intestinal samples should be tied off at both ends. If submitting fluids, a red-top tube free of anticoagulant or sealed 10 ml screw-top conical vial are useful. Tissue samples are most beneficial for culture, and if a swab is going to be used, swabs yield the best results when an abscess or lesion is sampled.

**Salmonella:** Necropsy samples usually submitted for Salmonella culture include small intestine, large intestine, mesenteric lymph nodes, and liver.

**TOXICOLOGY:** Routine tissues and organs collected for toxicology include aqueous and vitreous fluid in separate red top tubes (as large of volume as possible), and quart-sized bags of fresh liver (1), kidney (1), and ruminal content (1). Serum can also be submitted for toxicology testing, but serum is not as beneficial as organs such as liver for trace mineral analysis. Serum copper testing requires for the whole blood sample to be submitted in a red-top tube, serum zinc requires a royal blue-top tube (metal-free tube), and serum selenium requires the sample to be submitted in a heparinized green-top tube. Nitrate and ammonia levels can be performed on aqueous fluid submitted in a red-top tube. A few diagnostic labs will perform ammonia levels on vitreous humor, but locally we submit ammonia levels on aqueous samples in red-top tubes to the Auburn University, Clinical Pathology Laboratory for $6.18 per sample. Total calcium and magnesium can be detected in a vitreous sample.

Table 1. Toxicology sample submissions at Thompson Bishop Sparks State Diagnostic Lab
### Recommended samples for toxicology testing

<table>
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<tr>
<th>Test</th>
<th>Sample</th>
<th>Volume/special instructions</th>
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<tbody>
<tr>
<td>Ash, bone</td>
<td>Bone-mid-femur or humerus</td>
<td>2-inch section of bone</td>
</tr>
<tr>
<td>Ash, feed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanine</td>
<td>Bait, liver, stomach contents; freeze immediately</td>
<td>100 grams needed</td>
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<tr>
<td>Feed or Hay, Visual Exam</td>
<td>Feed or hay</td>
<td></td>
</tr>
<tr>
<td>Insecticides</td>
<td>rumen content, environmental sample</td>
<td>Quart-sized leak-proof bag of rumen content</td>
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<tr>
<td>Ionophores: Panel:</td>
<td>Dry feed</td>
<td>Gallon-sized leak-proof bag</td>
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<tr>
<td>Monensin, salinomycin,</td>
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<tr>
<td>Narasin, lasalocid (by</td>
<td></td>
<td></td>
</tr>
<tr>
<td>request)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>Fresh kidney, rumen content, serum</td>
<td>Quart-sized leak-proof bag</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Liver, environmental sample</td>
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</tr>
<tr>
<td>Calcium</td>
<td>serum, bone, feed</td>
<td>serum-remove from clot and freeze</td>
</tr>
<tr>
<td>Chromium</td>
<td>liver, environmental sample</td>
<td>Quart-sized leak-proof bag</td>
</tr>
<tr>
<td>Copper</td>
<td>serum, liver</td>
<td>serum-remove from clot and freeze; quart-sized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leak-proof bag</td>
</tr>
<tr>
<td>Iron</td>
<td>serum, liver</td>
<td>serum-remove from clot and freeze</td>
</tr>
<tr>
<td>Lead</td>
<td>whole blood in EDTA (purple-top tube), fresh kidney</td>
<td>Quart-sized leak-proof bag</td>
</tr>
<tr>
<td>Potassium</td>
<td>feed, bone</td>
<td></td>
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<tr>
<td>Selenium</td>
<td>liver; heparinized whole blood (green-top tube)</td>
<td>Quart-sized leak-proof bag</td>
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<tr>
<td>Selenium</td>
<td>feed</td>
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<tr>
<td>Zinc</td>
<td>liver; whole blood in royal blue-top/trace metal free</td>
<td>serum-remove from clot and freeze; quart-sized</td>
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<td>leak-proof bag</td>
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<tr>
<td>Mycotoxins</td>
<td></td>
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<tr>
<td>Aflatoxin B1, B2, G1, G2</td>
<td>Feed</td>
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<tr>
<td>Substance</td>
<td>Sample Type</td>
<td>Storage Method</td>
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<tr>
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<tr>
<td>Ochratoxin</td>
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<tr>
<td>Fumonisin B1</td>
<td>Feed</td>
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</tr>
<tr>
<td>Deoxynivalenol</td>
<td>Feed</td>
<td>Gallon-sized leak-proof bag</td>
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<tr>
<td>Nitrates</td>
<td>Aqueous humor, serum</td>
<td>serum-remove from clot and freeze</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>feed-test based on chloride</td>
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</tr>
<tr>
<td>Strychnine</td>
<td>rumen content, bait, liver</td>
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</tr>
<tr>
<td>Urolith analysis</td>
<td>Urolith</td>
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</tr>
</tbody>
</table>
Diagnostic Test Interpretation

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These notes are intended to provide a prospective of the challenges with diagnostic test interpretation that can be encountered in a veterinary diagnostic laboratory or private veterinary practice. In an ideal situation, diagnostic test results should provide a clear answer for a simple question that the practitioner and diagnostician are seeking, which is often a yes or no answer. One of the most common situations where test results can cause interpretation problems is when a confirmatory test contradicts the initial screening test. In cases where we find a disparity between diagnostic test results, this may involve comparing PCR and serology test findings. Depending on the infectious agent, and if and when animals are vaccinated for the disease, differences between PCR findings and serology test findings can be a result of insufficient time for the animals to mount an immune response. This difference is a function of PCR detecting antigen prior to seroconversion. A positive PCR assay and negative serologic test caused by insufficient time for seroconversion can be viewed as a false negative result. Conversely, detecting vaccinal antibody serologically and using this information when seeking a yes or no answer has also been described as a false-positive result. The sensitivity and specificity of the two assays being performed is also another potential cause for differing test results, and this is especially true for PCR and antibody-based test methods. In some cases, we may find good correlation between PCR and serology test results, but this varies by the animal species and antibody test being performed.

For diagnostic assays, diagnostic labs aim to provide clients with the assay with the highest sensitivity and specificity, and test interpretation is dependent on sensitivity and specificity data. Diagnostic lab test suppliers that provide diagnostic assay test kits have abundant sensitivity and specificity data, predictive values, acceptable ranges for positive and negative controls, and other criteria necessary for test validation. Sample to positive (S/P) ratios are often calculated (equation 1) and interpreted by software provided by companies selling diagnostic lab test kits, or laboratories may manually calculate the S/P ratio if software is not available (1). These calculations can also be performed by diagnostic laboratories manually, but it is better use of a lab’s time to use software to perform the calculations and quickly provide an interpretation because the S/P ratio is dependent on the optical density readings for the positive and negative controls, which vary per kit lot and test run. Interpretation of these assays is set by the companies, and diagnostic labs are told cut-off values for positive and negative S/P ratios. Since S/P ratio interpretation varies with each diagnostic assay, the companies instruct diagnostic labs about the cut-off ranges for positive and negative results. Labs are not provided statistical data used for these S/P ratio calculations, but this information can be provided upon request. Some laboratories may validate their own assays offered by diagnostic test lab suppliers. In these cases, the test kit is usually available outside of the United States and a biologics permit is required by the USDA for this process. In these cases, the S/P ratios may play more of a role in a diagnostic lab setting. But, some laboratories only purchase USDA validated reagents and do not go through this internal validation process for kits sold outside of the U.S. Laboratories often spend the majority of their time when processing diagnostic cases running the assays then compiling all lab results for the submission. We then utilize the serologic diagnosis as a tool, not the final diagnosis unless the herd history, clinical signs, gross necropsy and histologic findings correspond with the laboratory data.

Equation 1: \[ S/P = \frac{\text{Sample Mean - Mean (negative control)}}{\text{Mean positive control - Mean (negative control)}} \]

In diagnostic veterinary medicine, some assays with historically high sensitivity and sensitivity results have been referred to as the gold standard, traditionally viewed as the most accurate test available at the time. However, for a variety of
diseases we have seen that the gold standard has changed over time, and with advances in medical technology there is not likely to be a diagnostic test that will stand the test of time. There is not a perfect diagnostic test, and there will not be a perfect diagnostic test in the future. Sensitivity is referred to as the ability of the test to detect disease in an individual for which the disease is present. The positive predictive value of this positive test result would provide us with the proportion of individuals with the disease and a corresponding positive test result. Specificity tells us the number of non-diseased individuals in a population with a negative test result. The negative predictive value should reflect if a negative test result accurately corresponds with an individual without the disease. The goal for a sensitive test is to provide the fewest false negative results for the test group with disease. Conversely, a specific test should identify the fewest positive results in a population that is disease free (2).

**Johne's disease:** Some tests do not have sensitivity and specificity values within a desirable range, such as the Johne's ELISA tests for goats, but we must rely on the clinical signs and herd history to come up with the most likely diagnosis for these cases. In some cases, submitting an animal with suspected Johne's disease for necropsy will help confirm Johne's disease when the ELISA or AGID tests and clinical history are suggestive but not confirmatory for a diagnosis. Knowing if Johne's disease is present on a farm will help with the decision-making process for additional submissions when serologic test results are positive. The sensitivity and specificity of the Johne's ELISA for cattle is less of a concern, as these numbers have improved over the past few years. However, goat Johne’s disease serologic assays still have low sensitivity rates. The difficulty with finding a definitive diagnosis with some assays has pushed the diagnostic testing realm toward more sensitive and specific assays such as PCR. We were all taught fecal culture (a 12 to 16-weeks process when cultured on solid media) is the gold standard for Johne's disease diagnosis, but for cattle it is likely direct fecal PCR for Johne's disease will be adopted as the test of choice for Johne's disease due to the faster turnaround time. Direct fecal PCR for Johne’s disease in goats has not been validated by the USDA at this time. Additionally, the Johne's ELISA for sheep has not been validated by the USDA, and additional test material is needed for test validation.

**Anaplasmosis:** Competitive ELISA (cELISA) has replaced complement fixation, the previous gold standard for diagnosis. Anaplasma cELISA, depending on the manufacturer, is used to detect *Anaplasma marginale*, *Anaplasma ovis*, and *Anaplasma centrale*, but not *Anaplasma phagocytophilum* (3). Cytologic examination of Giemsa, Wright-Giemsa, or Diff-Quik stained blood and splenic aspirates can be used for diagnosis. PCR is also a beneficial tool for detecting acute infections because acute and carrier phases of the disease affect diagnostic test results. Cases of anaplasmosis can be diagnostically challenging because we often have negative results despite testing on multiple fetal submissions, necropsy of adult cows (often only with possible icterus and enlarged spleens), and cytologic examination of blood and splenic aspirates from necropsy. Giemsa stains are often negative because carriers may have fluctuating levels of rickettsemia typically falling below the threshold needed for organism identification in blood smears, and generally $10^{2.5}$ and $10^7$ infected erythrocytes per ml have undetectable organisms in blood smears (3). Successful diagnosis requires multiple blood samples from cows in the herd, and in some cases both c-ELISA testing and PCR are indicated. c-ELISA testing targets bovine anti-major surface protein 5 (anti-MSP5) antibodies, which recognize the MSP5 protein epitope of *A. marginale* (4). The sensitivity reported by one c-ELISA manufacturer was 98% with a 100% reported sensitivity rate (3). However, published comparisons of cELISA assays with PCR has shown lower sensitivity rates (5). The clinical phase of disease can affect diagnostic lab test results; requiring interpretation of the herd history, consideration of the animal’s age, and endemic state of the herd. It can be difficult to explain recent abortion storms to clients when the herd was serologically positive for many years. A combination of diagnostic tests, necropsy, serology, and PCR are needed for a definitive diagnosis for many of these challenging cases.

**Trichomoniasis:** Primary factors affecting testing for *Trichomonas foetus* testing in enriched samples include: media storage, media expiration dates, refrigeration, sample age, sample collection, inoculation of tubes, and shipping.

A. **Media storage:** Stored uninoculated media should be kept at room temperature in the dark. TF Transit Tubes are required by most labs for PCR. These tubes are labeled by Biomed as a method to increase sensitivity of *T.*

a. To order TF Transit Tubes:

Biomed Diagnostics 1-800-964-6466
TF Transit Tube: 10 pack catalog number 12-012-001TF
Transit Tube: 50 pack catalog number 12-071-002

B. Media expiration dates and other reasons for rejection of samples: The economic impact of bull testing, interstate transport of bulls, and associated regulatory restrictions create the necessity for strict sample submission criteria. Samples submitted in expired media will be rejected. Media with altered or missing expiration dates or lot numbers will be also be rejected. Additional reasons for sample rejection include leaking InPouches™ or Transit Tubes, or refrigerated InPouches™ or TF Transit Tubes. Refrigeration for even short windows of time may kill the organism, and refrigeration increases the possibility for false negative results.

C. Sample age: Samples more than approximately 72 hours old will have a statement added to the report with the age of the sample. Samples older than approximately 4 days will be rejected. It is recommended that samples are driven to the lab, or shipped to the lab overnight by UPS or FedEx.

D. Inoculation of TF-Transit Tubes and labeling: Inoculate the media with 1-2 ml of sample and secure the cap on the tube to prevent leaking. Label the tube with the Animal ID number, tube number, date of sampling. The owner and veterinarian can also be labeled on the tube.

E. Shipping samples: Samples must reach the lab within 48 hours of inoculation. Inoculated media must remain at room temperature in the dark until the lab receives the samples. Do not refrigerate samples before shipping, and please do not ship with an ice pack. It is also not permitted to put an ice-pack on one half of the specimen container and put a divider between chilled and unchilled samples. Please include the submission form with the sample.

F. These rules for trichomonas sample submission vary by lab and state. Please check with the State Animal Health Official (SAHO) and diagnostic lab you are sending the samples to before collection, submission, and requirements for writing health certificates to ensure these requirements are met.
G. Some laboratories use PCR kits that permit pooling of samples. The PCR kit used at the State Diagnostic Lab in Auburn allows for test pools up to 5 samples. Some laboratories may determine the pool size by the prevalence of disease in the area. Pooled samples with a positive result are subsequently tested individually to determine the infection status of each animal in a positive pool.

H. Interpretation of trichomoniasis test results: Due to the number of risks involved with media and sample submission, these factors must be taken into account when interpreting test results. Generally, PCR sensitivity and specificity data are highly sensitive and specific for T. foetus DNA. Sample collection and submission can adversely alter the sensitivity rate.

BVDV:
Acute BVDV infections in immunocompetent calves and adults have historically been described as ‘acute’, ‘primary’, and ‘transient’. Currently, two types of infections are described based largely on the duration of viremia. This definition of the duration is largely associated with the clinical outcome, as transient cases are infected horizontally and develop 14-21 days of viremia, serum neutralizing antibody titers, and viral clearance. The second type of infection is persistent infection of the fetus following in utero vertical transmission of the virus from a transiently or persistently infected dam. Acute-transient infections may occur after an incubation period of 5-7 days in naïve cattle. The virus enters via the oral-nasal mucosa and initial replication occurs in the nasal mucosa and tonsils. Viremia typically begins around day 3 and peaks by day 7, but viremia has been documented to persist for up to 15 days. Viral replication is pronounced in the respiratory tract epithelium, spleen, thymus, lymph nodes, and gastrointestinal tract epithelium.

Acute-transient BVDV infections are often observed in 6 to 24 month old seronegative cattle. The age group that appears to be most susceptible is 4-6 month old calves, and this is thought to be due to the period of vulnerability caused by waning of maternally-derived BVDV specific antibodies. Maternally-derived antibodies are believed to be protective if there is not a great amount of antigenic diversity between the maternally-derived antibodies and the challenge strain of virus, and if there was adequatecolostrum quality and consumption. Consequently, failure of passive transfer can be associated with acute disease in neonatal and preweaned calves, often causing enteric disease or pneumonia. A strong correlation of transient BVDV infection with bovine respiratory disease complex and diarrheal diseases has been documented. The risk of these conditions is estimated to be doubled by transient BVDV infection. This estimate took into consideration several field investigations of BVDV incidence in animals that received treatment for respiratory disease. In a group of animals entering a feedlot, 13/29 (45%) received treatment for respiratory disease, and 8/36 (22%) of untreated calves had seroconverted. Similar evaluations have been performed for the risk of bronchopneumonia in calves that receivedcolostrum without BVDV antibody or calves receivingcolostrum from seropositive dams. Among calves exposed to BVDV at birth, 68.2% (30/44) developed moderate or severe bronchopneumonia if they received seronegativecolostrum, while 40.7% (35/86) of this group developed moderate to severe bronchopneumonia despite receivingcolostrum with BVDV antibodies. Other variables considered when evaluating the risk of other diseases following BVDV exposure, included incidence of respiratory or diarrheal disease requiring treatment in calves born into the herd when BVDV was present. Mortality rates of calves born during the period of BVDV introduction have also been evaluated. BVDV comorbidity with agents from the bovine respiratory disease (BRD) complex was associated with increased morbidity and mortality in calves. Antigen distribution of infectious bovine rhinotracheitis (IBR) was reported to be greater in calves previously exposed to BVDV. Severity of Mannheimia haemolytica infections was more often associated with increased morbidity and mortality when BVDV is present.

Bovine viral diarrhea virus is capable of infecting multiple organ systems, often concurrently. Clinical signs often observed include: oculonasal discharge, depression, anorexia, fever, oral erosions and ulcerations, diarrhea, decreased milk production in lactating cows, and increased respiratory rate. A consistent clinical finding during acute-transient
BVDV infection is decreased total leukocyte counts, primarily due to a reduction in neutrophils and lymphocytes. Virulence of the infecting BVDV strain is one of the most important factors influencing the outcome of infection, and higher virulence viruses are reported to cause increased viral loads in tissues and a faster rate of viral spread throughout the body, in contrast to viruses of lower virulence. Strain virulence has been shown to be a determinant in dissemination of virus throughout the body, and subsequently animals with greater levels of viremia demonstrate more profound clinical signs. In low virulence infections, the intestinal mucosa was documented to be a specific location in which BVDV antigen could be readily found, but infections caused by high virulence strains demonstrated antigen in endocrine tissues, the nervous system, respiratory tract, intestinal mucosa, and bone marrow. A difference in tropism for a specific lymphoid tissue has not been observed between low and high virulence strains, and all strains of BVDV are reported to show viral replication and antigen localization within the lymph nodes, Peyer’s patches, thymus, spleen, and tonsils. Several studies have examined potential reasons for differences in immune responses following low versus high virulence infections, and virulence and genotype have been noted to play a role in expression of innate immunity and pro-inflammatory and anti-inflammatory cytokines.

Subclinical infections represent the majority of BVDV infections. Mild or subclinical disease was estimated to encompass 70-90% of transient infections. This estimate has been confirmed in large dairy herds in which virus was known to be present in the herd for approximately 2.5 years. The primary impact of transient BVDV infection in cows is a decline in udder health. Reduced milk yield did not correlate with the level of BVDV antibody in bulk tank milk samples. However, there was an association of BVDV antibody with increased somatic cell counts in bulk milk tank samples. Retained placenta is also an issue for transiently infected cows delivering PI calves, as 5/12 (41.7%) dams with PI calves had retained placenta in comparison to 7/198 (3.5%) cows with retained placentas with non-PI calves.

Outbreaks of severe, peracute BVDV infections were reported in immunocompetent cattle in the 1990's. These outbreaks were distinctive due to the peracute course, high morbidity, and mortality in all age groups. Mortality rates varied with 10-20% mortality rates reported for some herds. Older cattle frequently demonstrated oral ulcers, diarrhea, fever, decreased milk production, and abortions. Based on samples collected during this period of atypical, peracute BVDV infections, diagnostic testing revealed noncytopathic BVDV to be implicated in these deaths. Nucleotide sequencing identified a novel genotype of BVDV, which was later designated as a separate species within the genus Pestivirus, BVDV-2.

A form of severe, acute BVDV infection in immunocompetent cattle is hemorrhagic syndrome. This cause of bloody diarrhea, epistaxis, hyphemia, ecchymotic hemorrhage, bleeding from injection sites or insect bites, and petechial hemorrhages is associated with thrombocytopenia, in addition to severe lymphopenia and neutropenia. Bone marrow infection by BVDV, most notably megakaryocytes, may be a primary defect leading to thrombocytopenia. Platelet defects, both quantitative and qualitative, are associated with platelet dysfunction and clinically noteworthy hemorrhage. There is some strain association, as a majority of infections are associated with ncp, BVDV-2. One report described hemorrhagic syndrome in colostrum-deprived calves with a BVDV-1b strain.

The mechanism of BVDV-induced immunosuppression has not been clearly elucidated. However, one potential cause of immunosuppression relates to the ability of the virus to cause leukopenia, most notably neutropenia and lymphopenia. Severity of leukopenia is also reported to be greater with BVDV-2 than BVDV-1. Strain dependent decreases in helper (CD4+), cytotoxic (CD8+), and gamma/delta (γ/δ) T lymphocytes, B-lymphocytes and neutrophils have been described. Speculation into the mechanism of leukopenia has involved the following theories: destruction of immune cells by BVDV, increased trafficking of immune cells into tissue sites of viral replication, and immune system removal of BVDV infected immune cells. Lymphoid depletion is a prominent feature during acute infection, primarily secondary to lymphocyte apoptosis and acute cytolysis of lymphocytes. Lymphoid tissues targeted during transient infection usually include thymus, spleen, Peyer’s patches, and lymph nodes. Previous studies have shown an association between virulence and lymphocyte depletion. Immunohistochemical staining of lymphoid organs during infection with highly
virulent BVDV strains correlated with antigen localization and lesions of lymphocyte apoptosis and depletion. A frequent clinical outcome from BVDV immunosuppression is secondary bacterial pneumonia.

Diagnostic test strategies: The two primary tests used for BVDV diagnosis are antigen-capture ELISA (ACE) testing on serum and ear notch samples, and PCR. Ear notch samples are the recommended test for detecting persistently infected cattle. PCR and virus isolation (still the gold standard) should be performed on lymphoid tissues primarily, but epithelial lined tissues such as the lungs and ileum are also valuable samples (partially due to lymphoid tissue within Peyer’s patches). It is very rare for an acute/transient infection to be detected with antigen capture ELISA testing, but this has been reported. If a positive ear-notch sample is detected, it is recommended to resample and retest in 30 days. However, some clients may only choose to use this resampling and retesting practice for valuable animals. Interpretation of BVDV test results must also include the herd history, recent introductions to the herd, and management practices. BVDV diagnostic tests can be challenging especially when working with samples with potential low viral loads such as buffy coat samples and serum, but occasionally even ear notch samples can yield confusing results when ACE findings do not match PCR results. To conclude, in a diagnostic laboratory setting ACE on ear notch samples is the best method of detecting PI cattle. PCR is also a useful, but PCR is a more expensive diagnostic tool to detect BVDV in ear notch samples, serum, buffy coats, and tissues such as spleen, thymus, lymph nodes, lung, and ileum. Any positive result should be interpreted by a veterinarian to help the owners and producers decipher what a positive result means for their herd.

References:

1. IDEXX product insert, Mycobacterium paratuberculosis Antibody Test Kit (IDEXX MAP), 06-14401-05.

BVDV references available upon request.

Infectious Bovine Keratoconjunctivitis

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Infectious bovine keratoconjunctivitis (IBK), more commonly referred to as pinkeye in cattle, is a contagious bacterial ocular infection characterized by pain, ocular discharge, corneal edema, and corneal ulcers. The causative organism is spread when noninfected animals come in contact with ocular discharges of animals infected animals. Although not a nonfatal disease, IBK can have a devastating impact on cattle production and welfare.

The highest incidence of disease is in late summer and early fall, correlating with the increase in fly populations, plant growth, pollen production, and an abundance of UV light. The condition can affect up to 80% of the herd. Calves are more susceptible than adults, as adult cattle have often developed immunity following previous infection(s). Bos taurus cattle appear to be more susceptible to pink eye than Bos indicus cattle. Bull calves tend to be affected more commonly than heifers. On average, calves affected with IBK will weigh 15-30 pounds less at weaning and often bring a reduced
price at sale depending on the severity of the condition and degree of visible ocular damage. The cattle industry has an estimated annual loss of $150 million from IBK due to production losses, lost revenue at sale, and treatment costs.

**Etiology**
The most common causative agent of IBK is a gram-negative rod, *Moraxella bovis*. The organism possesses hair-like structures on the surface of the bacterial cell called pili, which facilitate the attachment of the organism to the surface and colonization the cornea. Two specific pili are known, Q and I. The Q pili facilitate attachment to the corneal surface, while I pili facilitates maintenance of infection. It is theorized that Q pili may convert to I pili once the initial attachment has occurred, facilitating maintenance of the infection. Attachment of bacteria to the cornea and conjunctival surfaces results in conjunctivitis and keratitis.

*M. bovis* also produces hemolysins which are toxic to neutrophils recruited to combat the infection. Degenerating neutrophils release collagenases which induce the liquefaction (melting) of the cornea. Corneal inflammation associated with *M. bovis* infection frequently progresses to formation of a corneal ulcer and can potentially lead to rupture of the bulbus oculi, resulting in blindness. Strains of *M. bovis* which do not possess pili or hemolysins are non-pathogenic.

Other potential virulence factors of *M. bovis* includes phospholipases, outer membrane proteins, iron acquiring systems, and proteolytic and hydrolytic enzymes.

Clinical cases of pinkeye most commonly occur in the summer through the early fall. Environmental factors including seed awns, pollen, dust, sunlight, wind, and high ammonia can increase the risk of developing IBK by irritating the conjunctiva and disrupting the integrity of the corneal surface, facilitating the attachment of *M. bovis*. Ocular irritation also increases tear production and the associated epiphora can attract face flies which readily spread the organism.

**Transmission**
Infection of animals occurs following contact with ocular secretions containing *M. bovis* bacteria. The major vector for disease transmission is usually the face fly (*Musca autumnalis*) but inanimate objects (fomites) can also spread the infectious organism.

Face flies are attracted to ocular and nasal discharges and readily transmit the bacteria to naïve animals. Face flies efficiently spread the bacteria as they often feed on the ocular secretions of several animals in one day, facilitating rapid spread through a herd.

Asymptomatic carriers may harbor the organism for up to one year and provide a constant reservoir of infection. Young animals are more susceptible to disease as older animals typically develop surface immunity following exposure as a calf.

**Predisposing Factors**
Predisposing factors include dusty conditions, the presence of flies, physical irritants such as tall grasses and weeds, lack of pigmentation around eyes, and exposure to bright sunlight. Nutritional imbalances, such as deficiencies in protein, energy, vitamins, and minerals, can influence the disease outcomes.

**Clinical Signs**
Clinical signs can include conjunctivitis, chemosis, excessive lacrimation, corneal edema, ocular pain, blepharospasm, epiphora, photophobia, corneal ulceration, corneal rupture, and blindness. Ocular pain and visual impairment can cause reduced appetite and feed intake due to discomfort or an inability to locate feed. Minimizing the impact through preventive measures and cost-effective treatments are important measures for lessening the impact on the producers’ bottom line.
The course of the condition may vary for days to weeks with recovery taking three to five weeks. Most cases of IBK heal with minimal to no loss in vision, however, severe cases can result in corneal rupture and blindness. Prospective breeding bulls with lost vision are limited their future reproductive capacity, which can affect the financial and genetic future of the operation.

Four stages of IBK are described. Although the condition may resolve during any one of these stages, severe cases may progress through all four stages.

**Four Stages of the Disease**

**Stage 1.** Animals in this stage have excessive tearing and photophobia. Affected animals tend to seek shade and spend less time grazing. Inflammation to the eye will result in frequent blinking and injected sclera. A small centrally located ulcer often develops as the condition progresses, appearing as a small white spot. Corneal edema may result in a cloudy grayish appearance to the ocular surface. The condition can be unilateral or bilateral. Pain associated with this stage may decrease feed intake and time spent grazing, reducing daily gains.

**Stage 2.** The clinical signs in this stage are the same as stage 1 with progression of the corneal ulceration. The cloudy area in the cornea will enlarge as the disease state progresses. Portions of the iris are still visible through the anterior chamber, but vision can be impaired. Blood vessels migrating across the cornea from the limbus at a rate of approximately 1 millimeter per day aid in healing of the ulcer. This neovascularization imparts a pink or red color to the eye and led to the disease being known colloquially as “pink eye”.

**Stage 3.** Ulceration progresses to cover much of the cornea at this stage of clinical disease. Inflammation spreads to the interior of the globe and fibrin and white blood cells accumulate within the anterior chamber. The accumulation of inflammatory products results in a yellow discoloration of the globe and blocks the examiner’s view of the typically brown pigment of the iris.

**Stage 4.** The ulceration extensively covers the cornea in its entirety in Stage 4. In some cases, the iris protrudes into the ulcerated cornea and adhesions form between the iris and cornea. Impairment of internal drainage of the anterior chamber can lead to glaucoma and partial to complete blindness. In severe cases, the globe may rupture resulting in permanent blindness. Typically, enucleation is recommended for this stage of disease.

**Corneal Scarring.** After healing of the corneal epithelium, the blood vessels may remain visible for a variable period of time, along with the blue to white cloudy opacity to the cornea. The majority of cornea will eventually become transparent but there is typically a persistent white scar at the site of the initial ulceration which may impair vision slightly. Vision never returns to eyes of Stage 4 cases in which the globe ruptures.

**Diagnosis.**
Diagnosis is most commonly based on typical clinical signs and the characteristic central ulceration. In herd outbreaks, it can be beneficial to perform culture and sensitivity testing to facilitate accurate treatment options. The best samples to submit are conjunctival swabs and lacrimal secretions. Samples should be plated onto blood agar within 2 hours of collection for maximum sensitivity of detection.

Fluorescent antibody test on lacrimal secretions can sometimes be used to demonstrate the causative organism. Concurrent culture for *Mycoplasma* should be considered in cases where a herd outbreak is not responsive to treatment.

**Treatment.**
Early identification and treatment are critical to salvaging the eye. Treatments plans should always include methods to reduce transmission and bacterial shedding. If possible, animals with clinical signs should be isolated to minimize decrease transmission in the group.

Available treatments include an array of local and systemic agents. Topical products such as ointments or sprays are effective when applied multiple times per day but may be too labor intensive for the average producer. It is important to remember that many commercially available ophthalmic ointments contain medications which are prohibited for use in food animals.

In the early stages, systemic antibiotics are often effective in treating IBK and perhaps in minimizing shedding of the infectious organism. *M. bovis* is susceptible to oxytetracycline, ceftiofur, tulathromycin, and florfenicol at labeled dosages.

Although labor intensive, administration of antibiotics or antibiotic/corticosteroid mixtures by sub-conjunctival injection is widely practiced. Procaine penicillin G is the most commonly used antibiotic. Dexamethasone or other corticosteroids are often included, particularly in cases where the corneal epithelium is not disrupted.

If the ulceration has become severe, it is necessary to protect the eye from flies, UV light, tall grass, and other irritants. Application of eye patches, third eyelid flaps, or tarsorrhaphy can provide good protection with minimal discomfort.

Studies have consistently shown that although treatment will reduce healing time and decrease the discomfort in the animal, it does not prevent decreased weigh gains in affected animals.

**Prevention**

Prevention should be centered on decreasing the transmission of the bacteria, making fly control essential. A moderate fly infestation is characterized as 10-20 flies per animal during the middle of the day. Fly control can be difficult and frustrating. On most farms a single fly control system is ineffective. Implementing an integrated fly control program that addresses egg, larval, and adult life cycle stages can greatly decrease the incidence of IBK within a herd but can be time consuming for a producer.

Insecticidal ear tags (Fly tags) are an excellent product when used correctly. Newer tags provide a higher concentration of insecticide. Most tag labels require two tags per adult animal and one per calf for adequate control. Application of an insufficient number of tags can potentially lead to insecticidal resistance in the fly population, making control in future years more difficult.

If fly tags are put in too early in the year, there will be a decrease in late season effectiveness. It is important to remind producers that tags should stay in for 3-5 months and must be removed to minimize development of insecticide resistance.

Pour-on deworming products do aid in fly control, however it is important to remember that using these products multiple times throughout the year will favor in internal parasite resistance to the drug.

Dusters or rubs provide an economical approach to fly control but to have many disadvantages. Strategic placement of dusters is critical— they must be placed in areas where cattle will have to move through, need to have draped curtains to contact the face, and require maintenance.

Sprays can be effective if done several times during the fly season but are hard to apply if cattle are grazing larger pastures.
Feeding of a larvicidal or growth inhibitor products is a common practice for many producers targeting the larva form of the fly that hatch from egg that are laid in feces. These products should be mixed into rations 30 days prior to fly emergence and feed continuously until 30 days after a killing hard frost. One caveat is that face flies can travel up to 2 miles. Therefore, if the neighbors are not using these products, there may appear to be a poor response due to fly migration. Promoting dung beetles which breakdown the feces will also decrease the survival rate of the larval stage.

Commercial and autogenous vaccinations are available for producers to use. Most commercially available vaccines target the pilus antigen, however there is a great antigenic variety among these surface organelles. Experimental evidence has yielded mixed results on efficacy for prevention of clinical disease.

Biosecurity is always necessary in the control and prevention of any disease. Remember to isolate new additions or animals which have been transported and are returning to the property for a minimum of 30 days.

It is important not to forget environmental factors which can contribute to the disease state. Pasture maintenance will aid in controlling incidence of disease. Tall grasses, seedheads, and weeds can facilitate ocular irritation. Mowing of pastures in May after seedheads develop and mid-summer when the weeds have emerged as well as providing adequate space around bunks and hay feeder will reduce the risk of irritation. Mineral feeders, bunks, and hay rings should be routinely inspected for frayed or rough edges which could damage the corneal surface.

The vaccination program on the farm should include a modified live IBR vaccine. IBR infections increase the risk of *M. bovis* colonizing on the corneal surface.

In attempts to decrease the sensitivity to UV light, producers of white-faced cattle have started to select animals with pigmented eyelids.

IBK will continue to be a problem in the southeast. A well-formulates prevention and treatment program will greatly decrease the losses of animals and minimize production losses in a herd.

References:

5. Funk LD., Reccy JM, Wang C, Tait RG, O’Connor AM. Associations between infectious bovine keratoconjunctivitis at weaning and ultrasonographically measured body composition traits in yearly cattle. *JAVMA*, 2014; 244:100-106
Updates from Alabama Beef Cattle Improvement Association, Alabama Farmers Federation, Alabama Veterinary Medical Association, Auburn University Department of Animal Sciences, Auburn College of Veterinary Medicine, and Tuskegee University College of Veterinary Medicine

(No Proceedings)

NOTES
Update from the United States Department of Agriculture and Presentation of Two APHIS Approved Supplemental Training (AAST) Modules in Partial Fulfillment of USDA Accreditation
Mel Stephens, DVM
Emergency Coordinator for USDA-APHIS-VS

(No Proceedings)

NOTES
INTRODUCTION: THE VIRUS

Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen of cattle with a worldwide distribution. The economic losses associated with BVDV infection in cattle are the result of decreased weight gain, loss of milk production, reproductive wastage, and death losses. Bovine viral diarrhea virus is also well recognized for its ability to cause immunosuppression in cattle, which may result in infections by other pathogens. Bovine viral diarrhea virus is the prototypic member of the genus *Pestivirus*, which also includes two other viruses of veterinary importance: classical swine fever virus and border disease virus. Isolates of BVDV can be classified *in vitro* as cytopathic or noncytopathic, and this classification of the virus is referred to as the biotype. The noncytopathic biotype predominates in the cattle population. There is also significant genetic and antigenic heterogeneity among BVDV isolates. BVDV has been divided into two different species based upon their genotype: BVDV1 and BVDV2. BVDV2 infections have been associated with severe peracute disease, neutropenia, and a "hemorrhagic syndrome," which is characterized by severe thrombocytopenia, and death.

Bovine viral diarrhea virus isolates may be subdivided based upon differences in the nucleotide sequence, and these specific differences in the viral nucleic acid sequences are the basis for genotyping. The classification of genotype is independent of biotype, as there are cytopathic and noncytopathic BVDV1 and BVDV2 isolates. Several techniques are available for identifying the different genotypes of BVDV. Most genotyping procedures involve the use of molecular biology techniques that take advantage of sequence differences in specific genome segments, or monoclonal antibody profiles. Reverse transcription and polymerase chain reaction of the 5'UTR has been the most widely used, but reverse transcription and polymerase chain reaction of other regions of the BVDV genome are proving acceptable. Two distinct genotypes of BVDV exist, and the BVDV1 and BVDV2 may be further divided into subgenotypes based upon analysis of the 5' UTR sequence. Analysis and comparison of nucleic acid sequences between BVDV1 and BVDV2 genotypes have revealed greater than 30% dissimilarity. This dissimilarity is concentrated in the regions of the viral genome encoding for E2 and the 5' UTR. The E2 glycoprotein is the target for neutralizing antibody production by the humoral immune system during infection. Questions have been raised as to the ability of vaccines containing BVDV1 to induce protection to BVDV2 infection. An antigenically divergent BVDV2 may escape the neutralizing humoral immune response to the E2 glycoprotein elicited by vaccination with a BVDV1-containing vaccine. This has been demonstrated when a BVDV2 isolate was recovered from a PI calf born to a healthy cow that had been vaccinated with an inactivated vaccine containing BVDV1.

The origin of type BVDV2 is unknown, but it is likely that BVDV2 has existed prior to its association with outbreaks of severe peracute disease and the hemorrhagic syndrome. An analysis of BVDV isolates from 1981 was performed, and several BVDV2 isolates were identified. In addition, the recovery of a BVDV2 isolate was just as likely in the 1981-1985 period as in the 1991-1994 period during which outbreaks of severe peracute BVDV were occurring. Also, phylogenetic survey of the 5'UTR genomic sequences of BVDV1 and BVDV2 isolates has revealed a similar level of sequence variation within each genotype. This finding suggests that these two genotypes have been evolving for a similar time span. An explanation for the emergence of BVDV2 and its association with severe peracute disease and the hemorrhagic syndrome cannot be provided; however, most Canadian herds involved in outbreaks of severe peracute BVDV infections had not been vaccinated against BVDV, or the vaccination protocols were incomplete. Use of vaccines containing BVDV1 provides at least partial protection from BVDV2, but the protections appears to be of limited duration. For example, a modified-live vaccine containing BVDV1 provided a disease-sparing effect following challenge with a virulent isolate of BVDV2, whereas unvaccinates developed severe BVDV infection.
BVDV PREVALENCE AND HOST RANGE
Cattle are considered the natural host for BVDV, and BVDV is distributed in cattle populations throughout the world as indicated by serologic surveys. Surveys in North America have indicated individual-animal seropositive rates between 40% to 90%, while herd-level seroprevalence varies from 28 to 53% depending on geographic region. The prevalence of PI cattle is much lower and is generally believed to be less than 1% of all cattle. PI cattle may cluster within certain groups of cattle, thus elevating the prevalence within populations.

Bovine viral diarrhea virus does not possess strict host specificity. Classically, pestivirus isolates have been assigned to BVDV1, BVDV2, classical swine fever virus, or border disease virus according to the species from which they were isolated, with most BVDV, classical swine fever virus, and border disease virus isolates being recovered from cattle, pigs, and sheep, respectively. Evidence of BVDV infection, as demonstrated by the identification of antibodies, exists in seven of the ten families within the mammalian order Artiodactyla. Over 50 animal species within the families Antilocapridae, Bovidae, Camelidae, Cervidae, Giraffidae, Suidae, and Tragulidae have been reported susceptible to BVDV infection. In addition to cattle, species that have been reported susceptible to BVDV infection include pigs, sheep, goats, bison, captive and wild cervids, and Old World and New World camels, with recent accounts of BVDV infections in alpacas and wild cervids in North America receiving much attention. Clustering of pestivirus strains among three host groups (domestic ruminants, camels, deer) has been proposed; however the implications for transmission between these clusters are unknown. Identification of heterologous PI hosts may have important implications for the epidemiology of BVDV, most importantly as these non-bovid PI animals may serve as reservoirs for BVDV.

CLINICAL MANIFESTATIONS OF BVDV: TRANSIENT INFECTIONS
Much focus in BVDV control has focused on the PI carrier, and rightly so; however, transient infections with BVDV pose significant economic hardship to cattle producers. Additionally, transiently infected cattle may also transmit virus into naïve cattle populations. The terms, transient and acute and primary, are often used interchangeably to describe the infection of BVDV in an immunocompetent host. With respect to acute infections (infection in immunocompetent cattle), multiple clinical forms of BVDV exist, including subclinical, clinical (BVD), BVDV-induced immunosuppression, severe BVD, a hemorrhagic syndrome, and venereal infections. Peracute BVD and the hemorrhagic syndrome are two clinical forms of BVDV infection in cattle that have been associated with the BVDV-2 genotype. Although the outbreaks of severe peracute BVD and the hemorrhagic syndrome have been associated with BVDV-2 isolates, viral virulence does not equate with genotype. Infections with BVDV-2 have also resulted in subclinical to mild disease under natural and experimental conditions. Like BVDV-1, isolates of BVDV-2 can be involved in the entire spectrum of clinical manifestations ranging from subclinical to severe clinical disease.

Bovine Viral Diarrhea (BVD):
The majority of BVDV infections in immunocompetent and seronegative cattle are subclinical. If observed closely, cattle undergoing a subclinical infection may develop pyrexia, a mild leukopenia, and a decrease in milk production. The source of BVDV is usually a PI animal, and exposed cattle develop BVDV-specific neutralizing antibody. Bovine viral diarrhea (BVD) is the term used to describe the clinical form of BVDV infection in immunocompetent cattle. Clinical signs of BVD include diarrhea, depression, ocuionalal discharge, anorexia, decreased milk production, oral ulcerations, pyrexia, and mild leukopenia.

BVDV-induced Immunosuppression:
Although the majority of BVDV infections in immunocompetent cattle are transient and self-limiting, it is apparent that when infection occurs in the presence of other micro-organisms, BVDV can contribute to a disease that becomes clinically evident. Numerous studies, under both natural and experimental conditions, have demonstrated the relationship between BVDV and other infectious agents, suggesting that BVDV has the ability to induce
BVDV affects the immune system in a number of different ways leading to an increased susceptibility to other infectious agents. Impairment of lymphocyte and neutrophil function and decreases in the number of circulating and tissue immune cells have been identified in BVDV-infected cattle. These abnormalities, combined with environmental and/or management stressors, contributes to immunosuppression and concurrent infection with other pathogens.

Hematologic abnormalities may be observed in cattle undergoing an acute, or primary BVDV infection, and thrombocytopenia and leukopenia are the most frequently reported. A transient leukopenia occurs in most cattle undergoing a primary BVDV infection. Varying degrees of white blood cell count depressions have been reported. The time frame for depressions in the white blood cell count to be observed is between 3-12 days after infection. In an experimental BVDV infection model using the BVDV-2 isolate 890, infected calves develop a severe leukopenia that begins several days after inoculation. Different studies have reported differences in the white blood cell differential following experimental challenge. In immunocompetent calves experimentally infected with BVDV, the main decreases in the total leukocyte count have been in the numbers of neutrophils and lymphocytes. Neutropenia has been observed as the major hematologic abnormality for some isolates, while lymphopenia has been observed as the major abnormality using other BVDV isolates. Other studies have documented reductions in both lymphocytes and neutrophils. The mechanism of leukopenia is unknown; however, BVDV has a propensity to replicate in immune system cells. A result of this replicative cycle is the destruction of some of these cells and impairment of surviving cells. During acute infection, lymphoid depletion is observed in the thymus, spleen, lymph nodes, and gut associated lymphoid tissues (Peyer’s patches). In addition, viral antigen can be found in the gut associated lymphoid tissues and the myeloid progenitor cells within the bone marrow of experimentally infected calves, and this finding may partially explain the lymphopenia and neutropenia observed during primary BVDV infection. The consequence of infection in these critical immune system cells may result in the inability to clear bacterial infections of the gastrointestinal and respiratory tract.

Specific changes in immune system function occur during primary BVDV infection. The immune cells that have been reported to be affected by BVDV in immunocompetent cattle include lymphocytes, neutrophils, and monocytes and macrophages. Multiple in vitro and in vivo studies have been performed. Experimental in vitro studies have demonstrated that BVDV can infect lymphocytes and macrophages and affect their function. From experimentation, it appears both B- and T-cell function are affected during BVDV infection. Dysfunction in both B- and T-cells has been demonstrated by poor culture response to phytohemagglutinin, pokeweed mitogen, and concanavalin A stimulation. Infection of peripheral monocytes in vitro caused a significant decrease in the chemotactic responses to lymphokine. Immunohistochemical and flow-cytometric studies have demonstrated that BVDV infects and replicates in antigen-presenting cells. Infection of monocytes with BVDV has been shown to stimulate the synthesis of cytokines that may be responsible for the reduced ability to stimulate T-cell responses to specific antigens and mitogens. A decrease in the secretion of TNFα from LP5- or Salmonella-stimulated bone marrow-derived macrophages that had been infected with BVDV has been reported.

Increased susceptibility to other secondary infections is a consequence of BVDV-induced immunosuppression. Some pathogens may induce disease alone, but in the presence of BVDV, disease is enhanced. Substantial data exist demonstrating that BVDV infection is important in multiple-etiologic diseases.

**BVDV-2 and Severe, Peracute BVD:**
Prior to 1993, it was believed that the majority of BVDV infections in immunocompetent, adult cattle resulted in subclinical or mild disease. In 1993-1994, an atypical form of BVDV infection was recognized in Canada and the United States. The disease had a peracute course, caused high morbidity, and resulted in substantial numbers of deaths in all
age groups. Interestingly, the new form of BVDV was able to cause severe disease and death in immunocompetent cattle. Some adult cattle died within 40 hours of onset of clinical signs. In Quebec, mortality due to BVDV in veal operations was estimated at 25% of 143,000 calves for 1993. The outbreak in Ontario involved 150 dairy, 600 beef, and 100 veal herds with mortality of up to 50% in some herds. Analysis of BVDV isolates from outbreaks in Canada and the United States has confirmed that they were of the BVDV-2 genotype. Clinical manifestations of severe, peracute BVD include diarrhea, pyrexia, decreased milk production, and oral ulcerations in some cases. In addition, concurrent diseases such as pneumonia and abortion were frequently reported in herds experiencing outbreaks. Prior to these described BVDV-2 outbreaks, there was a tendency to depreciate the importance of BVDV infection in immunocompetent cattle. The reports of these severe BVDV infections clearly demonstrate that some isolates of BVDV can cause severe life-threatening disease in immunocompetent cattle.

**BVDV-2 and the Hemorrhagic Syndrome:**

Bovine viral diarrhea virus infection in cattle has been associated with a hemorrhagic syndrome characterized by thrombocytopenia. The hemorrhagic syndrome appears to be associated with noncytopathic BVDV isolates, and thus far, BVDV-2 has been associated with the majority of hemorrhagic syndrome cases. Thrombocytopenia in association with BVDV infection was first reported in New York. These first descriptions occurred in calves and adult cattle naturally infected with BVDV. In a retrospective case study, 15 animals ranging in age from 1 to 8 years were diagnosed with BVDV and thrombocytopenia. In these 15 naturally infected animals, the platelet counts ranged from 2,000 platelets/µl to 33,000 platelets/µl. Bloody diarrhea was the most common clinical sign associated with the thrombocytopenic BVDV infections in these 15 cattle. Other clinical signs included epistaxis, petechial hemorrhages, ecchymotic hemorrhages, and bleeding from injection sites or insect bites. Cattle with platelet counts less than 25,000 platelets/µl had the most severe signs of bleeding. Thrombocytopenic BVDV infections were experimentally reproduced shortly after the original descriptions in naturally infected cattle appeared. With respect to the platelet count following experimental inoculation, a decline in the platelet count was observed to occur as early as 3 days after inoculation, with maximum platelet count depression occurring between days 9 and 17 following infection. Clinical signs of hemorrhage have been observed in all reports of experimental BVDV infections resulting in thrombocytopenia. The extent and location of hemorrhages in experimentally infected calves are similar to the reports describing BVDV infection in naturally infected calves. Hemorrhages have been observed during physical examination of infected calves on the oral mucosa, ventral surface of the tongue, eye (hyphema), sclera and conjunctiva of the eye, and subcutis, especially in areas surrounding the carpus and hock. Prolonged bleeding from venipuncture sites was a common finding in BVDV-infected calves. Epistaxis was also observed, but less frequently than other antemortem hemorrhages. The exact mechanism of BVDV-induced thrombocytopenia is unknown; however, bone marrow infection with BVDV may be involved in the pathogenesis. Bovine viral diarrhea virus infects megakaryocytes as evidenced by the presence of BVDV antigen in bone marrow megakaryocytes revealed by immunofluorescent antibody staining and immunohistochemical antibody staining, suggesting that megakaryocyte infection may be important in the etiology of BVDV-induced thrombocytopenia.

**Venereal Infections with BVDV:**

Venereal infections have also been identified following BVDV infection in nonimmune, immunocompetent cattle. Decreased conception rates as a result of fertilization failure have been reported in association with BVDV infection in nonimmune, immunocompetent cattle. The ability of BVDV to cause repeat breeding and result in early embryonic death has been controversial. An early infection study demonstrated that conception rates were not different between cattle infected at the time of insemination when compared to uninfected controls. Many studies performed since then have demonstrated the contrary. Infection of cattle prior to insemination results in impaired conception rates. Part of this impairment in conception is the result of ovarian infection and dysfunction as a result of BVDV viremia. Viral antigen and ovaritis have been described in acutely infected cattle with BVDV. Conception and pregnancy rates are lower if the animals are viremic at the time of insemination. Further field studies have supported this theory that BVDV is involved in early embryonic death and repeat breeding syndrome. Retrospective determination of serologic status
has indicated that cows that seroconvert during the early gestation period have significantly lower conception rates than cows that were immune (conception rate for seroconverting cows: 22%; for immune cows: 79%).

Bovine viral diarrhea virus infection in seronegative, immunocompetent bulls often results in infertility and poor semen quality. In addition, semen quality may remain inferior for as long as 76 days following acute infection with BVDV. While BVDV can be isolated from semen of some acutely infected bulls after viremia subsides, the ability to isolate virus from semen ceases when serum antibodies are detectable. Virus in semen collected from an acutely infected bull prior to seroconversion (12 days post-inoculation) infected 5% of inseminated heifers. Subsequently, horizontal transmission of virus from these infected heifers to pregnant animals resulted in the production of PI fetuses.

In 1998, a unique, localized, persistent infection with BVDV was identified in the testes of a seropositive, nonviremic bull at an artificial insemination center. Despite absence of viremia, the bull continuously shed infectious BVDV in semen throughout his life. Since this report, persistent, localized testicular infections with BVDV have been experimentally reproduced in post-pubertal bulls. After experimental acute infection of these bulls, virus persisted within testicular tissue of some bulls for at least 7 months.

CONTROLLING BVDV IN CATTLE HERDS

Methods to control BVDV infection within beef cattle herds involve 3 major, yet basic principles: 1) identification and elimination of PI carrier animals, 2) enhancing immunity through the vaccination of susceptible animals, and 3) strict biosecurity measures to prevent introduction of PI carrier animals.

Identification and elimination of PI carriers:

Cattle that are PI with BVDV continuously shed large amounts of virus and serve as the major mechanism to spread BVDV within and among cattle populations. Clinical signs of persistent infection may include poor performance and poor growth rates, increased morbidity in the form of respiratory and gastrointestinal disease, and mucosal disease. It is very important to note that not all PI cattle perish as young calves, and some survive well into adulthood.

The individual animal prevalence of PI carriers in the cattle population is small, and has typically been observed in the range of 0.5 to 3.0%. Some PI’s will survive to adulthood, but in general, PI cattle have poor survivorship, with approximately 50% perishing in the first year of life. In terms of herd prevalence, data are variable. In randomly selected beef herd, 4% (3 of 76 herds) contained PI cattle.

Persistently infected cattle can be identified by a number of diagnostic tests. Lately, the ear notch skin biopsy immunohistochemistry test or the ear notch skin biopsy antigen capture ELISA test have been most useful for identifying PI carriers. The advantages of these tests are that they only identify PI carriers, and they can be used on any aged animal, as colostral antibody consumption does not interfere with the interpretation of the test. In individual animals, the definitive test for diagnosing BVDV infection is virus isolation in cell culture, but this test is largely becoming replaced by PCR in diagnostic laboratories. As long as live virus exists in sample for virus isolation, this method is very reliable. However, results of virus isolation could vary depending on the sample type. When serum samples are utilized for virus isolation, neutralizing antibodies from dam’s colostrum in calves less than 3 months of age could interfere with the assay. In addition to virus-infected tissues, the ideal sample for virus isolation is mononuclear cells from whole blood. Whole blood virus isolation is not affected by neutralizing antibodies for virus isolation, because the cells are washed. It is suitable for acute infected animals and calves under 3 months of age when the serum virus isolation is not reliable. Some ncp isolates grow slowly in certain cells. In some cases, immunofluorescent or immunoperoxidase assay may not detect virus antigen or may show false positive results, depending on types of antibodies used and background(Deregt and Prins, 1998). Because of these factors, another diagnostic test by RT-PCR may be necessary to confirm virus infection. The RT-PCR is very sensitive and can detect presence of virus genome, regardless of dead or live virus. The combination of virus isolation and molecular technique may decrease the chance of false negative and positive results, and help confirming presence of virus.
Herd screening for PI carriers is most applicable for herds that have a confirmed BVDV problem. It is problematic to screen the herd if new introductions of cattle are taking place, as this is a common reintroduction of the virus into the herd. In addition, if biosecurity procedures are not in place to prevent reintroduction of the virus, the herd screening may fail. In herd basis BVDV diagnosis, cost, sensitivity and time should be considered because of large numbers of samples. Antigen capture ELISA on ear notches or pooled PCR using serum or ear notch samples can be used to provide an economical way to screen herds for PI animals. The ag-ELISA should not be used to detect acute infection, and PI calves less than 3 months of age, because of neutralizing antibodies in serum. The IHC is another method for herd screening, because of sensitivity, rapidity and ease. It is appropriate for PI animal screening, not for acute infections. Pooled RT-PCR test can be also used for herd screening purpose. Pooling of whole blood, serum or milk samples is an economical way to screen herds. This strategy can be used on the whole herds including calves with colostrum. The pooling of 640 milk samples can be detected by RT-PCR if one PI animal’s milk sample is included. However, the life span of a PI animal should be considered. Most PI animals may die before they reach the lactating herd. The 100 fold-diluted sample of single viremic serum with \(10^{0.12}\text{TCID}_{50}/\text{ml}\) can be detected by RT-PCR assay, and single PI animal is detectable in pools of 200-250 negative samples. When identifying PI cattle in large herds with 80 pooled serum samples, more than 3% prevalence increased the least cost and diminished the competitive benefit of pooled testing.

As ongoing herd surveillance, VN and sentinel animal antibody surveillance may be used. The VN is the only test routinely used to detect and quantify antibodies specific for BVDV. This test is used to demonstrate seroconversion by testing acute and convalescent sera. Therefore, it should be used only in unvaccinated and greater than 3 month old animals. Using sentinel animal is an innovative method for monitoring virus circulation within a group of animals. The sentinel should be not be a PI and should remain unvaccinated within the herd. If BVDV is introduced into the herd, seroconversion in the sentinel may be used to demonstrate BVDV infection.

**Vaccination:**
Nothing seems to be more on the minds of producers than what vaccine is the best to control BVDV in their cattle operations. Choices are many. More than 150 federally licensed vaccines are available for BVDV in the United States. The majority of these vaccines were licensed prior to a complete understanding of the pathogenesis of BVDV infections. In addition, the majority of these vaccines have not been tested to determine if they are efficacious in preventing transplacental infection with BVDV. Prevention of transplacental infection is the ideal measure of vaccine efficacy. In general, the currently available vaccines provide adequate protection against clinical disease. Studies describing protection against fetal infection using BVDV1 and BVDV2 combination vaccines have been performed for some vaccines and are likely ongoing for many others. Designing a vaccine program is critical in helping to control BVDV associated losses and giving producers a sense of security. Dr. Ed Dubovi, Cornell University, has said it best: “In general, vaccines do not fail; vaccination programs fail.” Timing of vaccination is a critical issue. Maximizing immunity during the early periods of gestation is most likely to reduce BVDV-associated reproductive losses. This is achieved through the use of prebreeding vaccination and boosting. Vaccination programs aimed at preventing reproductive losses may have different timing than vaccination programs aimed at preventing losses associated with clinical disease, such as pneumonia.

**BVDV vaccine efficacy:**
When discussing BVDV vaccination efficacy, it is first important to discuss reasonable expectations following vaccination and to remember that disease and infection are not synonymous terms. Although vaccines are an important component to BVDV prevention and control, they are not 100% efficacious, meaning that no vaccine will prevent all infections from occurring. Reasons for lack of efficacy of vaccination against BVDV are many, and include factors related to the administration of the vaccine and factors related to the ability of the host to respond to the vaccine.
The requirements for vaccine licensure in the United States were first described within the Code of Federal Regulations (CFR) 113.311 for ‘Bovine virus diarrhea vaccine” (MLV) and 113.215 “Bovine virus diarrhea vaccine, Killed virus.” These licensing documents describe the requirements for immunogenicity. To summarize from the CFR113.311 guidelines for modified-live BVDV viral vaccines (http://edocket.access.gpo.gov/cfr_2003/9cfr113.311.htm), immunogenicity was determined by testing for neutralizing antibodies in 20 vaccinated calves as compared to 5 unvaccinated calves. Efficacy was determined by challenging the calves with virulent BVDV two to four weeks after vaccination. A BVDV vaccine is considered immunogenic if 19 of the 20 vaccinated develop an antibody response (>1:8 titer), with efficacy being defined as vaccinates not developing leukopenia where 4/5 unvaccinates did. These early licensing requirements did not address efficacy related to reproductive disease or reproduction-related label claims. The vaccine claims for protection of the fetus against BVDV were described in the Center for Veterinary Biologics Public Notice 02-19 (http://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_02_19.pdf). The label claims for BVDV reproductive effects are divided into claims for fetal protection and claims for abortion (maternal and/or fetal causes). Here, the label claims are type-specific, i.e. BVDV 1 or BVDV 2 protection. Supporting data for the label claim is performed according to Veterinary Services Memorandum 800.202. Three categories for label claims are: 1) aids in the prevention of abortion, 2) aids in the prevention of persistently infected calves, and 3) aids in the prevention of fetal infection or aids in the prevention of fetal infection including persistently infected calves. Most clinical trials evaluating vaccine efficacy are performed with the goal of achieving efficacy claims for the prevention of persistently infected calves. These studies are characterized by vaccination prior to breeding, then challenging the pregnant cattle between days 75-90 of gestation, and finally testing the fetuses on or after 150 days of gestation. Virus isolation procedures are performed on fetal tissues, and those fetuses from which BVDV is isolated are considered to be persistently infected.

Protection from clinical disease:
Initially, the effectiveness of BVDV vaccination was focused on limiting clinical disease due to BVDV infection. Numerous studies have demonstrated the ability of vaccination to protect against overt clinical disease associated with BVDV, and many trials have investigated the ability of commercially available BVDV vaccines in protecting against the high virulent BVDV 2 strains isolated from outbreaks of severe peracute BVD in the late 80’s and early 90’s. Experimental and field data indicate vaccination with either inactivated or modified-live BVDV vaccines are effective at reducing or obviating clinical disease. In addition, modified-live viral vaccines containing BVDV 1 strains are effective at limiting or preventing clinical disease when vaccinated animals are subsequently challenged with a virulent BVDV 2 strain. Protection from clinical disease is important for stocker/backgrounder and feedlot operations, and immunity to BVDV has been demonstrated to be protective against bovine respiratory disease complex. Preconditioning cattle by vaccinating cattle against BVDV prior to an expected exposure (commingling and shipping) reduces the effects of exposure of cattle to BVDV.

Prevention of fetal infection:
For the reproductive herd, vaccination against BVDV should protect against viremia to prevent dissemination of virus throughout the host, including preventing infection of the reproductive tract and fetus. In the past decade, the focus for vaccine efficacy has shifted from protection against clinical disease to protection against fetal infection. Published studies indicate the protection against fetal infections following BVDV vaccination varies, with influences by use of inactivated or modified-live vaccine, the timing of challenge, and the degree of homology between vaccine and challenge strains. In general, most experimental studies indicate significant, although incomplete, protection against fetal infection using modified-live viral vaccines, and partial protection using inactivated viral vaccines. The genotype of the challenge strain is important, and fetal protection is superior when animals are challenged with strains from the same genotype. An important observation regarding all of the fetal protection studies so far is that although protection may not be 100%, the level of protection is superior to that observed when proper vaccination is not utilized as evidenced by higher rates of PI animals in unvaccinated cattle. Recently, cattle PI with BVDV have been used for the challenge exposure of vaccinated and control cattle in BVDV vaccine efficacy studies evaluating fetal
protection. (Ellsworth et al., 2006; Grooms et al., 2007; Leyh et al., 2011; Rodning et al., 2010) These types of studies provide a more natural and rigorous challenge method.

Within the United States cattle population, there are three major subtypes, BVDV1a, BVDV1b, and BVDV2a, with the BVDV1b subtype predominating from diagnostic laboratory submissions and PI prevalence studies, accounting for 78% of bovine persistent infections in one North American Study. (Fulton et al., 2006) Because of the finding of higher isolation rates for BVDV 1b strains and the fact that commercial vaccines for BVDV contain BVDV 1a and/or BVDV 2a strains, logic would dictate that challenge experiments should be performed using cattle PI with BVDV 1b strains. Two recent clinical trials have evaluated commercial modified-live viral vaccines in their efficacy in preventing fetal infections. Like previous studies, these studies have demonstrated that modified-live BVDV vaccines provide significant (85-96%), although incomplete, protection against fetal infection.

**Vaccination strategies:**
Designing a vaccine program is critical in helping to control BVDV associated losses and giving producers a sense of security. In general, vaccines do not fail; vaccination programs fail. Timing of vaccination is a critical issue. Providing young calves with immunity can help reduce disease and death associated with BVDV infections. Timing of vaccination has been performed to co-incide with the decay of colostral antibodies, which may occur as early as a few weeks to as long as 8 months of age. Recent research has demonstrated that vaccination of young calves that possess colostral antibodies can result in an immune response that provides protection against clinical BVD later on in life.

Vaccination programs aimed at preventing reproductive losses may have different timing than vaccination programs aimed at preventing losses associated with clinical disease, such as pneumonia in weaned calves. Maximizing immunity during the early periods of gestation is most likely to reduce BVDV-associated reproductive losses. This is achieved through the use of prebreeding vaccination and boosting. The use of a modified-live viral vaccine at 1 month prior to breeding has been recommended to the point of being indisputable. However, vaccinating or using booster vaccines in the early lactation period of dairy cows (15-45 days in milk) can be an immunological challenge for cows due to negative energy balance. Delaying vaccination until after this period may provide better immunity.

**Summary:**
Nothing seems to generate more opinions regarding BVDV than what vaccine is the best to control BVDV, and in fact, choices and opinions are many. Even though controversy exists, most everyone believes that when vaccines are given correctly, at appropriate times, to healthy cattle, they are better than not vaccinating at all. In general, currently available vaccines provide adequate protection against clinical disease. Prevention of infection in pregnant animals is the ideal measure of vaccine efficacy.
Biosecurity:
Strict biosecurity measures to prevent further introduction of PI carriers into herds is considered an additional preventive measure. Preventing contact between susceptible cattle and cattle infected with BVDV is a key component for biosecurity. The biosecurity plan that is developed may be quite different for a seasonal-calving beef herd and a continuous calving dairy herd. In the latter example, susceptible pregnancies are present at any one time in the herd.

Biosecurity measures include isolation and testing of new additions to the herd. The most common method by which BVDV is introduced to a herd is through the addition of cattle. Acutely infected animals or PI carriers can transmit the virus into new susceptible cattle. Newly purchased animals should be quarantined and screened for the presence of BVDV. Quarantine for a minimum of 3 weeks should minimize the potential for acutely infected individuals to spread the virus into the resident herd. For PI carriers, testing prior to entry into the resident herd is imperative. For the purchase of pregnant cattle, the fetus should be tested as soon as possible after parturition.

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NOTES
Using Pregnancy Analytics App to Diagnose Reproductive Inefficiency

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Introduction

Good reproductive efficiency is critical for economic sustainability of beef cow-calf herds. Two standard measurements of reproductive success for beef cowherds are the percentage of cows exposed to bulls that are identified as pregnant at a mid-gestation evaluation, and the percentage of pregnant cows that give birth to a live calf. In addition to these standard performance assessments, converting fetal age data to a pregnancy distribution that displays pregnancy percentages by 21-day periods can provide enhanced information to assist in the diagnostic work-up for sub-optimal reproductive efficiency and to guide the design of intervention strategies. The value of fetal age data can be amplified by further segregating reproductive distributions by animal age and/or other management groups when evaluating a herd with reproductive or production shortfalls.

Beef cow reproduction is limited by two key factors, the first being a relatively long period of infertility following calving and the second being that only 60% to 70% of successful matings between a fertile bull and fertile cow will result in a viable pregnancy at the time pregnancy status is determined at mid-pregnancy. We know that approximately 30% to 40% of fertile matings result in either failure of fertilization or death of the early embryo, but in most situations, the mated, but non-pregnant cow will express heat and ovulate a fertile egg about 21 days after her last heat and will have another 60% to 70% probability of conceiving and maintaining a pregnancy. Fertile cows that have three opportunities to be bred by a fertile bull (each with a 65% probability of a successful pregnancy) will have a 96% probability of being pregnant at the time of a preg-check about one-half way through pregnancy.

If nearly all the cows in a herd calved early enough so that they have resumed fertile cycles by the 21st day of the next breeding season, and the bulls are fertile and able to successfully mate, then the ideal pregnancy pattern would have about 60% to 65% pregnant in the first 21 days of the breeding season, 85% to 90% pregnant by the 42nd day of breeding, and about 95% pregnant after 63 days of breeding (Figure 1).

Figure 1: Pregnancy distribution goal for a 63-day breeding season
BCI Pregnancy Analytics App: Gathering Pregnancy Data Chute-Side

The BCI Pregnancy Analytics App is being used by veterinarians to enhance monitoring and evaluating cowherd breeding season success. Veterinarians know that being able to visualize the percentage of a cowherd that becomes pregnant each 21-days of the breeding season can provide important information to identify the contributing causes for situations when a lower than desired percentage of the herd becomes pregnant, or to identify areas for improved reproductive efficiency. Until now, collecting and evaluating that information while at the chute during preg-checking has been difficult. Data entry for the BCI Pregnancy Analytics App is even easier than using a paper-and-pen method and has the benefit of data analysis that is automatically available immediately after the last cow is palpated.

The only data required by the Pregnancy Analytics App are the dates for the start and end of the breeding season and an estimate of the fetal age for each cow’s pregnancy. Additional information such as cow id, cow age, body condition score, and breed (or other descriptor) can be added to enhance the value of the preg-check information. After preg-check data is entered, projected calving dates are generated and graphs are created to display the distribution of the upcoming calving season. These pregnancy patterns can help identify the most likely contributing factors when investigating herds with lower than desired percent pregnant.1,2

Veterinarians can be fairly precise estimating fetal age early in gestation but the ability to estimate fetal age accurately decreases as gestation progresses.3 Therefore, to confidently place cows/heifers within fetal age groups, pregnancy diagnosis should occur no more than 120 days after the start of the breeding season. This opportunity to place females within fairly tight “stages” or 21-day periods is a great advantage for producers and their veterinarians when evaluating the nutritional and reproductive status of the herd’s recent past and in planning to optimize the upcoming nutritional and marketing options for the herd. While it is true that recording calving dates also allows producers to generate calving distributions, by analyzing the pregnancy distribution soon after the end of the breeding season, information is generated 6 to 7 months earlier than calving data.

For a 63-day breeding season, the ideal distribution should resemble Figure 1. Producers should strive for nutritional and management systems that allow at least 60% of the exposed females to become pregnant in the first 21 days of the breeding season.4 The majority of the remaining females should become pregnant in the second 21-day period. Moreover, 5% or less of the herd should be non-pregnant at the end of the breeding period.

Another way to evaluate pregnancy distribution data is to determine the percent of the available (non-pregnant) cattle that become pregnant each 21-day period of the breeding season. Recognize that as the breeding season advances and cattle that become pregnant are no longer available to get pregnant again, the percent of the herd that becomes pregnant each 21 days is not the same as the percent of available (non-pregnant) cattle that become pregnant each 21 days. To display this important measure of reproductive success using the Pregnancy Analytics App, click on “% Pregnancy Success”. A table will then be displayed that reports the percent of the non-pregnant cows at the start of each 21 days that became pregnant within that 21 days.
Looking at the percent of the herd that became pregnant each 21 days does not immediately inform the veterinarian about how fertility is changing over the breeding season. Examining the pregnancy distribution displayed in Figure 2 provides evidence that 25% of the non-pregnant cows became pregnant in the first 21 days. But it is not as clear that in the second 21 days, of the cows that weren’t pregnant already, 40% became pregnant; (which is 30% of the herd), and in the third 21 days, of the cows that weren’t pregnant, 65% became pregnant (which is 30% of the herd), and finally, that in the 4th 21 days, 65% of the available (non-pregnant) cows became pregnant.

Based on expected pregnancy success when both cow and bull fertility is optimum, the “% Pregnancy Success” goal should be between 60%-70% for every 21-day period of the breeding season. Using the herd represented in Figure 2, by the third 21 days there is no problem with fertility in the cows or bulls. The % Pregnancy Success values clearly indicate that the reproductive problems in this herd occurred during the first two cycles and in the last two cycles of the breeding season, fertility was optimal.

**BCI Pregnancy Analytics App: Interpreting the Charts and Tables**

At the time of pregnancy diagnosis, veterinarians can estimate fetal age and evaluate the palpable or ultrasonographic characteristics of non-pregnant reproductive tracts (Figure 3). If low pregnancy percentage is due to failure to conceive due to cows not resuming fertile cycles post-calving or bulls failing to deliver fertile semen to the cow reproductive tract, reproductive tract examination should reveal the characteristics of a non-pregnant uterus with no indication of previous pregnancy or uterine pathology. Timing of pregnancy diagnosis relative to reasons for low pregnancy percentage due to non-infectious (e.g. stress) or infectious (e.g. Trichomoniasis) causes of early gestation pregnancy loss reveals that the loss occurs a few weeks to a few months prior to examination and may or may-not be associated with still-detectable uterine involution or pathology. Because infectious agent or toxin causes of fetal loss often occur in late gestation just prior to or following examination for pregnancy status, examination of non-pregnant reproductive tracts should reveal characteristics of involution or uterine pathology.
Once all the data collected at the time of pregnancy diagnosis is organized for analysis, an in-depth and efficient evaluation of the herd reproductive success can be conducted. The reasons for low pregnancy percentage during any 21-day period of the breeding season can be placed into one of three categories: 1) an inadequate percentage of females were having fertile estrous cycles, 2) the bulls were not able to deliver adequate amounts of fertile semen to the female’s reproductive tract, or 3) infectious or non-infectious agents prevented or ended pregnancy. The charts and graphs produced by the Pregnancy Analytics App along with the physical examination findings of the reproductive tract and cow body condition at the time of pregnancy diagnosis can guide history questions, further physical examination, herd record evaluation, and diagnostic laboratory testing to assist the veterinarian’s evaluation of the possible rule-outs as likely or unlikely causes of the undesired pregnancy distribution.

1) **Inadequate percentage of females were cycling by the 21st day of breeding**

Although Figure 1 depicts an ideal herd, many times the evaluation of herd preg-check data reveals a much different pregnancy distribution. Figure 4 illustrates a very common distribution. In this situation, the percentage of cows open at the end the breeding season would not necessarily alert the veterinarian to a problem if the breeding season lasts long enough, and an evaluation of the distribution is needed to begin a diagnostic work-up.
The distribution and the Percent Pregnancy Success in Figure 4, allows the veterinarian to conclude confidently that females did not become pregnant early in the breeding season. The cause of poor fertility early in the breeding season that improves over time can be due to either female or male problems. Additional information, such as the number of bulls in the pasture that passed a Breeding Soundness Examination, the Body Condition Score of the herd at the start of the breeding season, and the date during the previous calving season when one-half the calves had been born would be helpful for narrowing the rule-outs.

One common reason a herd has a pregnancy distribution like that depicted in Figure 4 is that a similar pregnancy distribution the previous year resulted in many cows calving in the 3rd or later 21-day period of the calving season. Because the average bovine pregnancy lasts 283 days, one can calculate that a cow/heifer must rebreed within 82 days after calving in order to maintain a yearly (365-day) calving interval. The typical amount of time from calving to the resumption of fertile cycles (postpartum period) for 90% of a herd’s mature cows is 60 to 80 days (Figure 5). For first-calf heifers, the number of days post-calving for 90% to resume fertile cycles is closer to 100 to 120 days. If the breeding season begins on the same date as the previous year (and the breeding season lasts 63 days) the breeding season will commence 63 to 82 days postpartum and end 123 to 142 days past calving for cows calving in the first 21-day period of the previous calving season.

Therefore, all early-calving cows (including first-calf heifers) are expected to have the opportunity to cycle and be bred several times during the breeding season. Cows calving in the second 21-day period will be to 43 to 62 days postpartum at the start of the breeding season and 103 to 122 days past calving at the end of the breeding season. Once again, this timing should allow the mature cows to resume cycles and have the opportunity to be bred several times during the breeding season. First-calf heifers should also resume cycling early enough in the breeding season to have one or two opportunities to be bred. In contrast, for those cows that calve in the fourth 21-day period, calving has just finished as the breeding season begins and for those in the fifth 21-day period, the breeding season has begun prior to the time they calve. Limited (or non-existent) time from calving until the start of the breeding season essentially eliminates the potential for nursing cows, and even more so first-calf heifers, to rebreed early in the breeding season (if at all).
Without implementing culling, nutrition, and heifer development changes in herds with flat pregnancy distributions similar to the herd in Figure 4, it is very difficult to influence the percentage of the herd pregnant in the first 21-days of the breeding season. Reasons that a herd with a previously ideal calving distribution can deteriorate to a less-than-ideal situation includes females too thin at calving, poor postpartum cowherd nutrition, subfertile bulls, or infectious or non-infectious pregnancy loss.5,7,8,9

2) **Bulls did not deliver adequate amounts of fertile semen to the female reproductive tract**

If reproductive performance is initially adequate - indicating that conception occurred and pregnancy was maintained early in the breeding season, the veterinarian can assume that fertile bulls were turned out with fertile, cycling cows, the herd was free of pregnancy wasting disease, and the postpartum period and energy reserves (as indicated by BCS) were adequate. A sharp decline in Percent Pregnancy Success during the breeding season should cause the veterinarian to investigate whether bulls developed testicular or musculoskeletal problems that prevented the production or delivery of fertile semen and whether herd replacements brought in after the start of the breeding season could have introduced a venereal disease.

Figure 6 illustrates a problem that is seen fairly frequently in herds with one bull for each breeding pasture. Although multiple-bull breeding pastures are more resilient to breeding failure due to bulls being unable to successfully mate cows compared to single-bull pastures, because of potential problems arising from injuries due to bull-on-bull fighting, social dominance by subfertile bulls, and isolation of groups of cows in an extensive breeding pasture without one or more bulls present, multiple-bull pastures can also have poor reproductive efficiency due to bull problems and can have reproductive distributions similar to Figure 6.

The breeding season in this example (Figure 6) starts out with a high percentage of cows cycling, good cowherd fertility and good bull fertility. Because 55% of the herd becomes pregnant in the first 21-day period, the veterinarian can be confident that the pre-breeding feeding/supplementation program offered adequate nutrients for a fairly high level of reproductive performance. It is also evident that the bull(s) was able to cover the breeding pasture, find the cows displaying estrus, and successfully breed the cycling cows.

The dramatic decrease in pregnancy percentage during the second 21-day period of the breeding season is strong evidence for bull infertility. The cause can be testicular or musculoskeletal insult.10 The incremental increase in the percentage of available (open) cows bred in each of the following 21-day periods in Figure 4 indicates that bull fertility is gradually returning.

| % Pregnancy Success | 1st 21-d | 2nd 21-d | 3rd 21-d | 4th 21-d | 1st 21-d
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<td>55%</td>
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In a situation where the veterinarian evaluated overall pregnancy percentage but not the pregnancy distribution for the herd depicted by Figure 6, the percentage of open cows would indicate herd fertility problems, but the cause of the high open percentage would not be evident. With limited information, one could guess that nutrition or cow fertility were to blame. And, as with this example, a bull may pass a breeding soundness examination both before and after being placed in the breeding pasture without revealing that a fertility problem existed during the breeding season. By categorizing and displaying the information gained at pregnancy diagnosis the cause of the problem becomes more obvious.

A breeding soundness examination (including a through physical examination) at the time the breeding season problem is discovered may supply information about penile, testicular, foot and leg or other musculoskeletal problems during the breeding season. However, lack of identifiable pathology following the breeding season does not rule-out a physical (locomotion, mounting, intromission) or semen quality problem several weeks to months earlier.

3) Infectious or non-infectious agents prevented or ended pregnancy

In situations when early pregnancy loss leads to negative effects on the reproductive distribution, the problem occurred after the breeding season started and before the time of pregnancy diagnosis. In addition to the effect of pregnancy loss on the reproductive distribution, in some situations, non-pregnant cows may exhibit palpable evidence of an involuting uterus at the time of mid-gestation pregnancy diagnosis.

Non-infectious pregnancy loss very early in gestation (before maternal recognition of pregnancy) due to environmental or nutritional stress placed on the cow or fetus should not result in uterine pathology and would not be expected to have negative carry-over effects in the next 21-day period of the breeding season. In contrast, non-infectious pregnancy loss after maternal recognition of pregnancy (around day-13 after estrus) will result in a delayed return to normal fertility until after the embryo is resorbed or expelled and the hypothalamic-pituitary axis has resumed normal estrous cycle activity – which may be later than the 21-day period following the initial conception. Early, non-infectious pregnancy loss that occurs before pregnancy is detectable by palpation or ultrasonography is unlikely to be differentiated from failure to conceive.

Infectious pregnancy loss may result from fertilization failure or very early embryonic death so that palpation or ultrasonographic examination is indistinguishable from failure to conceive or early non-infectious pregnancy loss. However, because many common causes of infectious pregnancy loss in North America have peak incidence after the pregnancy could have been diagnosed, it is expected that evidence of previous pregnancy will remain for several weeks after fetal loss in some of the affected cows. The length of time that pregnancy loss would be evident is influenced by the stage of gestation at the time of pregnancy loss and whether or not uterine pathology accompanied the pregnancy loss.

Infection with the protozoa *Trichomonas foetus* (Trich), which is transmitted during mating, is an important cause of early gestational loss in North America because it is diagnosed in many cattle-dense areas and because it can cause a high percentage of exposed cows to lose their pregnancies. The pregnancy distribution of a herd infected with Trich will vary depending on what the distribution would have been without infection and the timing of Trich introduction into the herd.
If Trich entered the herd prior to the start of the breeding season so that a high percentage bulls are already infected, the cows will become pregnant at a time similar to last year’s breeding season, but infected cows are likely to lose their pregnancies approximately 15 to 80 days into gestation, at which time the embryo or fetus dies and is resorbed or aborted. A period of female infertility is expected to last for another two to six months as a result of infection. The magnitude of loss is expected to approach 30% to 50% of exposed cows. If Trich entered the herd during the breeding season or few bulls were infected at the start of the breeding season but the number of infected bulls increased as the breeding season progressed, then the reproductive distribution is greatly influenced by what the distribution would have been without Trich exposure, and the speed at which additional bulls become infected.

Other causes of early gestational loss (e.g. *Campylobacter fetus ss venerialis*, Bluetongue virus, *Leptospira borgpetersenii* serovar hardjo type hardjobovis, bovine viral diarrhea virus) will have a similar effect on the reproductive distribution but the magnitude of pregnancy loss is not expected to be as high as with Trich. Infectious and toxic causes of pregnancy loss commonly expressed in mid- to late-gestation include: Bovine Herpes virus 1 (Infectious Bovine Rhinotracheitis – IBR), bovine viral diarrhea virus (BVDv), *Neospora caninum*, *Leptospira* sp., pine-needle toxicosis, and others. Pregnancy losses in mid- to late-gestation are likely to occur after the time of pregnancy diagnosis and the effect is not limited to one period of the reproductive distribution. If pregnancy losses have occurred by the time that pregnancy status is determined, evidence of that loss is likely to be found upon palpation of the non-pregnant uterus of some affected cows.

**Second-level analysis of gestational age data**

To capture more information from fetal aging, the distribution of breeding dates can be analyzed not only by 21-day intervals, but also by category within those 21-day intervals. The herd depicted by Figure 7 has a pregnancy percentage of 94.5%, which meets the overall herd goal for a 63-day breeding season. In addition, 61.8% of the herd became pregnant during the first 21 days of the breeding season – which exceeds the 60% cut-off associated with good cow and bull fertility at the start of the breeding season. From these observations, one could conclude that the herd has normal fertility and that there are no nutritional and reproductive management problems.

![Figure 7. Herd with a good pregnancy distribution that has a hidden problem](image)

Looking at *Percent Pregnancy Success*, during the first 21 days of the breeding season 62% of the available cows become pregnant. In the second 21 days, 55.6% of the non-pregnant cows become pregnant; which is 21% of the herd. In the third 21 days, 68% of the available cows become pregnant; which is 11.5% of the herd. These measures of reproductive success also indicate that overall herd fertility is good, but there is an indication that fertility may be sub-optimal during the second 21-days and closer examination of the information is warranted.

If the data collected at pregnancy diagnosis for the herd depicted in Figure 7 is further analyzed by breaking it into age categories for each 21-day period (Figure 8), the pregnancy distribution for the first-calf heifers indicates that management for this herd is not satisfactory. The important diagnostic information is that while the mature cows performed very well throughout the breeding
season, the Percent Pregnancy Success of the first-calf heifers is good during the first 21-days of the breeding season but dramatically decreases during the second 21-day period before returning to 70% for the final 21 days of breeding.

The fact that the first-calf heifers performed well the first 21 days of the breeding season is important to recognize because my bias when first-calf heifers perform worse than mature cows is that the deficit is because it took them longer to begin fertile cycles after calving and therefore, they performed poorly early in the breeding season. But the information provided by the Pregnancy Analytics App for this herd indicates that the first-calf heifers did not experience a delayed return to estrus. The problem was the second 21 days. Without the second-level analysis, I would probably assume that the heifers were too thin when they calved or that the producer should move the heifers’ breeding season so that they could calve earlier than the mature cows. Because of the information provided by second-level analysis of data collected at preg-check, I am able to identify “which” cattle were not pregnant, and “when” during the breeding season fertility was reduced; and for this example, I am most interested in investigating bull issues confined to the second 21-day of the breeding season.

Having this type of analytics available immediately after pulling the palpation sleeve off isn’t diagnostic by itself; but as I am talking to the producer, I can confine my history questions to the first-calf heifers during the second 21 days of the breeding season (which for this herd would have been the last two weeks of June through the first week of July). If I use any diagnostic testing, I will focus my testing on the bulls in the first-calf heifer breeding pasture.

Summary
Information gathered at the time of pregnancy diagnosis is very valuable to both veterinarians and beef producers, particularly if fetal age is estimated within 21-day periods. Any pregnancy test that does not include an estimate of fetal age will not provide information about when a cow became pregnant or when during the breeding season which categories of cows did not become pregnant – it can only identify that a particular cow is not pregnant. Despite the importance of reproductive performance to cowherd profitability and sustainability, without an efficient and convenient method to collect and analyze preg-check data, the value is difficult to capture.

Nutrition, genetics, animal husbandry, male and female reproductive soundness, and health all influence the distribution of pregnancy within a herd. By combining uterine palpation or ultrasonographic imaging to determine pregnancy status with analysis and graphing of the information, veterinarians can identify strengths and weaknesses within the management system of a herd. The Pregnancy Analytics App allows the knowledge and skill of the veterinarian to be augmented by: efficient digital data entry and immediate creation of
commonly used herd reproductive assessments in order to enhance communication between the veterinarian and producer.

References

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