VACCINES FOR BOVINE RESPIRATORY DISEASE
(3 CE Hours)

Learning objectives

- Understand the overall concepts of the body’s defense mechanism.
- Identify the initial diagnostic overview.
- Understand the epidemiological background and considerations.
- Explain how the body defends itself against infectious diseases.
- Explain how to diagnose and monitor the progress of infectious diseases.
- List the contraindications and differential diagnostic considerations.
- List vaccine considerations.

Introduction

The cattle and beef industry is a major economic factor in the United States:

<table>
<thead>
<tr>
<th>ITEM</th>
<th>2002</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retail equivalent value of U.S. beef industry (billion $)</td>
<td>60</td>
<td>76</td>
<td>73</td>
</tr>
<tr>
<td>Total United States beef consumption (billion $)</td>
<td>27.9</td>
<td>27.3</td>
<td>26.9</td>
</tr>
<tr>
<td>Value of U.S. cattle and calf production (billion $)</td>
<td>27.1</td>
<td>35.6</td>
<td>31.8</td>
</tr>
<tr>
<td>U.S. beef production (commercial carcass weight, billion pounds):</td>
<td>27.09</td>
<td>26.56</td>
<td>26.07</td>
</tr>
<tr>
<td>U.S. beef exports (billion $):</td>
<td>2.629</td>
<td>2.972</td>
<td>2.828</td>
</tr>
<tr>
<td>U.S. commercial slaughter (number of cattle, millions):</td>
<td>35.735</td>
<td>34.4</td>
<td>33.3</td>
</tr>
<tr>
<td>Cattle inventory (number of cattle, millions):</td>
<td>96.1</td>
<td>94.5</td>
<td>93.7</td>
</tr>
</tbody>
</table>

Milk and milk products represent the second most important production value for the livestock industry. Dairy products include fluid milks, cheese, yogurt, butter, dried milk, condensed milk and whey products. In 2007, the United States had 9 million head of dairy cows producing 185 billion pounds of milk and some dairy herds are as large as 15,000 head or more. The importance and intensity of the cattle industry, both dairy and beef, obviously is considerable.

To maintaining the level of production and hopefully improve productivity, the animals must remain healthy and be raised and managed without unnecessary stress throughout their lives. Aside from appropriate breeding and management practices, the prevention of infectious diseases must be a priority.

Such practices must include circumspect care in transferring animals between herds, veterinary and serological examination and if necessary, quarantine, good nutrition, and the control of external and internal parasites. Good management practices tend to provide stronger and healthier cattle, better able to resist and respond to the stresses of unexpected diseases. Still, vaccination to specific infectious disease-producing pathogens likely to occur in a particular region is essential.

The bovine respiratory disease complex is the most common and most costly beef cattle disease in the United States. Also called shipping fever, there are many factors involved in its occurrence: stress (weaning, marketing, transportation, mixing cattle from various sources), weather and the environment, and nutrition set the stage for parasitic, viral and bacterial infections.

A study in 2006 suggests that the economic loss of the bovine respiratory disease complex, including treatment and lower yields, was $13.90 per animal, not including the handling and labor costs associated with it – a considerable cost to the livestock economy in the United States when correlated to a total count of 93.7 million head of cattle in 2009. An estimate published in 2000 suggests a loss of 640 million dollars annually in the United States caused by this disease.

Signs start to appear about 14 days after the stress event. Initial symptoms include fever; discharge from eyes, nose and muzzle; cough; and rapid and noisy open-mouth breathing. The animal appears distressed with droopy ears, extended head, bowed back, stands alone and isolated, and is off feed.

Involved in the bovine respiratory disease complex are a multitude of pathogens: viruses such as bovine herpesvirus-1 (causing infectious bovine rhinotracheitis, red nose, infectious pustular vulvovaginitis ), bovine virus diarrhea virus (bovine virus diarrhea, mucosal disease), parainfluenza-3 virus (mild, undistinguished fever), bovine respiratory syncytial virus (lower tract respiratory disease) among others, and bacterial pneumonia (Manheimia hemolytica, Pasteurella multocida, Hemophilus somnus, Ureaplasma diversum etc).

These bacteria are found in the environment normally associated with healthy cattle. With some of the viruses often presenting only a minor illness in the infected calf, bacteria will find entry through damaged epithelial surfaces in the lung alveoli and bronchioli and the respiratory tract and exacerbate the disease leading to considerable losses in production yields and, quite often, to death of the diseased animal.

Infectious viral diseases

**Bovine Herpesvirus-1**

Bovine Herpesvirus-1 is the causative agent for infectious bovine rhinotracheitis (IBR). It belongs to the family of Herpesviridae and is found worldwide causing disease in cattle. It affects the upper respiratory tract and is present in almost all herds, usually causing disease in the non-immune animals and those of lowered levels of immunity. Periods of stress will tend to precipitate and enhance the disease and accelerate virus shedding.

In combination with bacterial infections, it is implicated in the shipping fever syndrome. It is highly contagious and produces disease of the respiratory tract, breathing difficulties, cough, excessive salivation and nasal discharge, enteritis, generalized systemic disease, mucopurulent eye infection and conjunctivitis, encephalomyelitis, mastitis, genital infections and abortions. There usually is fever, depression and anorexia.

The virus is spread by aerosol and bodily secretions from the eyes, nasopharynx and by sexual contact through the reproductive system as well as vertically through the placenta. Abortion and stillbirth occurs within three months post infection, usually in the second half of pregnancy. The disease may also appear as infectious pustular vulvovaginitis (IPV), which is sexually transmitted and strictly localized to the genital area and does not produce respiratory symptoms. In the latter case, there is pronounced switching of the tail, a reddened swollen vulva with small pustules, developing to erosions and ulcers, heavy thick brownish-white discharge and frequent painful urination. The bull’s penis and prepuce may be inflamed and show pustules and discharge as well (infectious balanoposthitis). Lesions are not always visible, and the infection can be passed on by artificial insemination.

The disease has an incubation period of one to three days post-contact. Other than symptomatic treatment, antibiotics may be advised to prevent secondary infections.

Confirmation of the diagnosis is possible through virus isolation from nasal or genital swabs or washings, mucous membranes of the respiratory tract and from tonsil, lung and bronchial lymph nodes. In aborted fetuses the virus can be isolated from the fetal organs, placenta and afterbirths. Additional proof of diagnosis is the presence of specific antibody and a rise in titer in paired samples.

Even after recovery, the animal will continue to shed the virus. The virus remains lying latent in endings of the sciatic and trigeminal nerve. In the newborn calf, the virus produces, as a rule, enteritis and death. The disease is not usually fatal, but when complicated by secondary bacterial pneumonia, it may lead to death. In the young, the infection is much more severe, causing generalized systemic disease, fever, diarrhea, respiratory distress, discharge from all orifices, loss of coordination, convulsions and death.

In the absence of bacterial complications, lesions remain restricted to the local mucosae appearing as petechiae, ecchymotic hemorrhages, focal necrosis frequently coalescing to plaques. Serous and serofibrinous exudates, often containing blood, can be found in paranasal sinuses, larynx, pharynx and trachea. The regional lymph nodes are swollen and, often, hemorrhagic. As long as it stays uncomplicated by bacterial co-infection, the disease lasts only about five to 10 days.
### Table 1 – Direct fluorescent assay (dFA) to locate and identify AG

<table>
<thead>
<tr>
<th>Step</th>
<th>Material</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specimen</td>
<td>Fix on microscopic slide or flat-bottomed microtiter plate.</td>
</tr>
<tr>
<td>2</td>
<td>Diluent containing BSA*</td>
<td>Rinse repeatedly; BSA to block uncovered plastic area.</td>
</tr>
<tr>
<td>3</td>
<td>Fluorochrome-labelled pathogen-specific AB</td>
<td>Add to infected matter and incubate for required time and temperature.</td>
</tr>
<tr>
<td>4</td>
<td>Test diluent</td>
<td>Rinse repeatedly: remove unattached antibody.</td>
</tr>
<tr>
<td>5</td>
<td>UV microscope</td>
<td>Read; along with positive and negative control samples.</td>
</tr>
</tbody>
</table>

* BSA= bovine serum albumin; the specific antibody will attach to, identify and light up the specific antigen.

### Table 2 – Indirect fluorescent assay (iFA) to locate and identify AG

<table>
<thead>
<tr>
<th>Step</th>
<th>Material</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specimen</td>
<td>Fix on microscopic slide or flat-bottomed microtiter plate.</td>
</tr>
<tr>
<td>2</td>
<td>Diluent containing BSA</td>
<td>Rinse repeatedly; add BSA to block uncovered plastic areas.</td>
</tr>
<tr>
<td>3</td>
<td>Pathogen-specific AB</td>
<td>Add to infected matter, incubate required time and temperature.</td>
</tr>
<tr>
<td>4</td>
<td>Test diluent</td>
<td>Rinse repeatedly: remove unattached antibody.</td>
</tr>
<tr>
<td>5</td>
<td>Fluorochrome-conjugated AB against pathogen-specific IgG</td>
<td>Add and incubate for required time and temperature.</td>
</tr>
<tr>
<td>6</td>
<td>Test diluent</td>
<td>Rinse: remove unattached fluorescein labeled AB.</td>
</tr>
<tr>
<td>7</td>
<td>UV microscope</td>
<td>Read; along with positive and negative control samples.</td>
</tr>
</tbody>
</table>

Test will light up the specific antibody that identifies the test antigen.

### Table 3 – Western Blot to locate and identify antigen

<table>
<thead>
<tr>
<th>Step</th>
<th>Material</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus or antigen</td>
<td>May require lysis of cells with RIPA buffer, NP-40, Triton X-100.</td>
</tr>
<tr>
<td>2</td>
<td>SDS-PAGE</td>
<td>Sort by size/molecular weight.</td>
</tr>
<tr>
<td>3</td>
<td>Copper Stain (0.3 M CuCl2)</td>
<td>To visualize fractions and control SDS-PAGE process.</td>
</tr>
<tr>
<td>4</td>
<td>0.1-0.25 M Tris/0.25 M EDTA pH 8.0</td>
<td>To destain; add 1X Tris-glycine buffer + methanol 20% for transfer.</td>
</tr>
<tr>
<td>5</td>
<td>SDS-PAGE fractions</td>
<td>Apply electrical field to transfer onto nitrocellulose blotting paper.</td>
</tr>
<tr>
<td>6</td>
<td>Ponceau red (0.2% in TBST)</td>
<td>Visualize fractions and control effective transfer, destain with TBST.</td>
</tr>
<tr>
<td>7</td>
<td>THE BLOT: Blocking</td>
<td>Rinse with TBST+ non-fat milk protein or BSA to block sticky surface.</td>
</tr>
<tr>
<td>8</td>
<td>Specific primary antibody in TBST</td>
<td>Add, incubate overnight at 4°C (may keep blocking agent in TBST or not).</td>
</tr>
<tr>
<td>9</td>
<td>TBST</td>
<td>Rinse repeatedly 5 min or more each time to remove unattached AB.</td>
</tr>
<tr>
<td>10</td>
<td>Enzyme linked secondary antibody</td>
<td>Add, incubate, shake at r° for 1-2 hours (no blocking agent in TBST).</td>
</tr>
<tr>
<td>11</td>
<td>TBS-T</td>
<td>Rinse to remove unattached secondary antibody.</td>
</tr>
<tr>
<td>12</td>
<td>Add luminal substrate, ECL, ECL+</td>
<td>Read enhanced chemiluminescence, photograph (CCD Camera), scan.</td>
</tr>
</tbody>
</table>

Sample material is separated by molecule size with SDS-PAGE, (sodium dodecyl sulfate polyacrylamide gel electrophoresis), separation products transferred to nitrocellulose blotting paper (the blot) and identified by specific antibody: TBS-T (tris buffered saline +0.1% Tween20); horseradish peroxidase-conjugated antibody against primary antigen-specific antibody; ECL+: enhanced chemiluminescence.

### Diagnosis

The determinant diagnosis rests on virus isolation and identification in primary bovine fetal testes cells. Other cell cultures of bovine origin have been used, secondary lung or kidney cells or the Madin–Darby bovine kidney cell line (MDBK).

In the absence of a cytopathic effect, a blind subpassage may be necessary. Cytopathic effects develop within less than five days and can be identified by enzyme-linked or fluorescent isothiocyanate-conjugated antibody against BHV-1. Immunohistochemistry also lends itself to the identification of virus or antigen in biopsy material.

The direct immunofluorescent assay is presented in Table 1 (to the left). The test specimen to be identified is fixed on a microscopic slide via section, imprint or smear and flooded with specific antibody to the virus or antigen. This type of antibody is available commercially in fluorescein-conjugated form, which allows location and identification of the antigen to which it has become attached under fluorescence microscopy. The indirect fluorescent assay follows the same principle: the antigen is tagged by specific antibody produced in a given animal species (rabbit, goat, guinea pig etc) which is then located and identified by fluorescein-labeled antibody to the particular rabbit, goat or guinea pig IgG. Indirect fluorescent or enzyme-tagged antibody assays are usually more efficient and economical because only one conjugate is required for many different antigens.

Enzyme-linked antibody can be used in similar fashion: horseradish peroxidase is conjugated with the specific antibody directly or with the antibody directed against the gamma-globulin of the donor animal employed for the production of the specific antibody. The presence of the enzyme-labeled antibody attached to the virus or antigen in the test specimen is made visible and measurable by the naked eye or spectrophotometrically. The enzyme will convert colorless chromogenic substrates, such as TMB (3,3’5,5’-tetramethyl-benzidine), DAB (3,3’-Diaminobenzidine) or ABTS (2,2’-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid) and convert them to colored molecules. It can also act on chemoluminescent substrates in Western Blotting kits producing enhanced chemiluminescence providing considerably greater, 10- to 100-fold, sensitivity of detection.

Of course, the polymerase chain reaction can be used to amplify and identify the virus presence. This test is based on matching short, virus specific DNA sections, which are commercially available, with matching sections from the test specimen and amplifying it many fold for identification.

Antibody assays will substantiate the results of virus isolation. Virus-neutralizing antibody can be measured in cell cultures where a minimal CPE producing dose will be prevented from producing cytopathic effects by the antibody present in the test serum dilutions. There are different procedures to perform enzyme-linked immunosorbent assays (ELISA) for antibody.
Table 4 – Polymerase chain reaction (PCR) to confirm genetic identity

<table>
<thead>
<tr>
<th>Process step</th>
<th>Materials</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling</td>
<td>(1) Target or template DNA: in stool, CSF, infected tissue, their exudates, urine</td>
<td>Is found by specific primers, identifying key for a specific gene section of a specific pathogen, seeking out and attaching only to the matching DNA region for assembly and polymerisation</td>
</tr>
<tr>
<td>Initialization</td>
<td>(2) Opposing primers (3', 5') (3) Taq DNA polymerase (4) dNTP's and (5) buffer (6) Divalent cations (Mg²⁺) (7) Monovalent cations (K) (8) Thermal cycler</td>
<td>Target/template DNA, dNTPs (in excess), primers (excess), Taq DNA polymerase, buffer + Mg²⁺ K; DNA thermal cycler; Combine reagents in test tube (0.2 to 0.5 ml size) 10μl - 200μl working volume; Place in thermal cycler, heat to 94º C to 96º C for 5 min (1-9 min)</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94º C to 98º C for 20 to 30 seconds: melt complementary hydrogen bonds between double strands of DNA, separate into two single stranded complementary molecules</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>50º C to 65º C for 20 to 40 seconds, Anneale single-stranded primer to complementary single-stranded DNA target; temperature/ time critical to limit non-specific annealing (background noise); polymerase cum primer-template hybrid will commence DNA synthesis</td>
<td></td>
</tr>
<tr>
<td>Elongation</td>
<td>72º C DNA polymerase copies new DNA strand from template; will polymerize 1,000 bases per minute</td>
<td></td>
</tr>
<tr>
<td>Repeat 1-2-3</td>
<td>Recycle steps 1, 2, 3 30 to 40 times, each time re-duplication of product;</td>
<td></td>
</tr>
<tr>
<td>Final elongation</td>
<td>70º C to 74º C for 5 to 15 minutes after last cycle to allow full extension of single-stranded DNA molecules</td>
<td></td>
</tr>
<tr>
<td>Hold till use</td>
<td>4º C to 15º C</td>
<td></td>
</tr>
<tr>
<td>AGEP</td>
<td>Agarose gel electrophoresis to separate PCR products by size; read using ethidium bromide or cybre green stain</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 – Indirect enzyme-linked immuno-sorbent assay (iELISA) to detect and measure AB

<table>
<thead>
<tr>
<th>Step</th>
<th>Material</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific antigen</td>
<td>Coat bottoms of polystyrene microtiter plate with known specific antigen</td>
</tr>
<tr>
<td>2</td>
<td>Incubator</td>
<td>Allow attachment to surface (4º C several hours, overnight)</td>
</tr>
<tr>
<td>3</td>
<td>Diluent with BSA</td>
<td>Rinse, remove unattached antigen; BSA blocks uncovered plastic</td>
</tr>
<tr>
<td>4</td>
<td>Test AB sample, dilutions</td>
<td>Add to AG coated surface</td>
</tr>
<tr>
<td>5</td>
<td>Water bath</td>
<td>37º C for 2 hrs</td>
</tr>
<tr>
<td>6</td>
<td>Diluent</td>
<td>Rinse repeatedly to remove unattached test AB sample</td>
</tr>
<tr>
<td>7</td>
<td>Enzyme labeled AB to test AB</td>
<td>Add, allow to react according to instructions (37º C for 1 hr)</td>
</tr>
<tr>
<td>8</td>
<td>Diluents</td>
<td>Rinse repeatedly to remove unattached enzyme labeled AB</td>
</tr>
<tr>
<td>9</td>
<td>Chromogenic substrate</td>
<td>Add, incubate for limited time, stop enzyme action, read signals, optical density, in spectrophotometer</td>
</tr>
</tbody>
</table>

Kits for the various ELISA procedures are available commercially. Only one of those procedures, the indirect ELISA, will be shown in the table here included.

**Vaccines**

Modified live virus vaccines as well as inactivated virus vaccines are available and provide protection against the disease and significantly reduce spread of the virus within the vaccinated herd. The basics of vaccine production and quality control procedures here expounded at some detail will, more or less, apply to most vaccines.

There are essentially four aspects to vaccine production:

1. The maintenance and ongoing quality control of master and production seed virus.
2. The maintenance and ongoing quality control of the vaccine virus production substrate.
3. The production process itself and quality control of all its steps and components.
4. The end product and its quality control.

**Master and production seed:** Much time and effort, months if not years, has been expended to attenuate viruses and confirm safety and efficacy in the laboratory as well as in the field under actual use. The vaccines produced from them must be safe, potent and reproducible and must be so from batch to batch to batch for years. Origin, passage and storage history must be on record and continue to be recorded with every vial that is being removed for production.

All tests on the new production seed must meet identical lot requirements. The attenuation process that led to a given master seed may have been (a) numerous passes in cell culture under strict control of virus titer, virulence, potency and sterility; or (b) growing and passing the virus at lower than normal body temperature to select a seed virus by low-temperature mutation that will not grow at the body’s fever temperature; or by (c) genetic engineering, i.e. adding or removing parts of the viral genome and modifying the antigenic properties of the virus. The seed virus for an inactivated vaccine, except for the attenuation process, must be subject to the same quality control processes of sterility and reproducibility of product.

**Vaccine production substrate:** Continuous cell cultures utilized for vaccine production are
subject to the same prerequisite as the virus master seed: Origin, passage and storage history must be on record and continue to be on record with every vial that is removed. Absence of extraneous agents and tumorigenicity are sine qua non. There is a maximum limit of 20 passages away from the master seed cell lot for use in vaccine production.

Vaccine production itself consists of inoculating optimum amounts of virus into monolayer or suspension cell cultures and harvesting the culture at the time of highest virus titer. This requires an understanding of the growth process for the particular virus being produced and the ongoing monitoring of virus yields and sterility. In the process of logarithmic growth, even minor deviations from procedure, fluctuations of incubator temperature, glassware washing process, lot to lot variations of cell culture plasticware and medium components may lead to subadequate lots and production losses.

The final product and its quality control concern safety and sterility, potency and long-term stability as the main concerns. Stabilizer and freeze-drying processes must be rigidly controlled. It may affect the immediate product titer or lead to losses upon storage. Stability and storage are of lesser concern for an inactivated vaccine.

At least two blind passages of an inactivated vaccine are necessary to exclude the possibility of surviving virus. Inactivation procedures include heat, formaldehyde, betapropiolactone. The vaccine must deliver relatively large doses of antigen to be effective and requires at least one booster dose, followed by yearly re-boosting. Adjuvants are used to potentiate, accelerate, prolong and enhance the immune response to the antigenic components of an inactivated vaccine. There are mineral compounds (aluminium phosphate, aluminium hydroxide or alum, calcium phosphate) frequently used in human vaccines as adjuvants. These adjuvants slow the release of the vaccine antigen and extend the time of its interaction with the cells responsible for the body’s immune response. The more recently developed synthetic, highly purified subunit vaccines elicit only a limited immune response and require adjuvants for effectiveness.

Other adjuvants in use include oil emulsions (possible problem: emulsion stability); saponin derivatives (Quil A, QS-21); squalene, a precursor for steroids (cholesterol, hormones, vitamin D), widely used in Europe and Canada as adjuvant for inactivated influenza vaccines, along with vitamin E and polysorbate 80 as emulsifier; bacterial breakdown products; and virosomes (presently approved for influenza and hepatitis vaccines). Virosomes are empty influenza virus shells carrying the desired antigen and, at the same time, facilitating attachment and fusion into the body’s immune cells.

To guarantee consistency and reproducibility of production standard, operating procedures are a must: Use standard record keeping, batch production and quality control records, initialed, dated and witnessed whenever possible. According to the U.S. Department of Agriculture’s Code of Federal Regulation, the following tests must be performed for a final product batch: absence of bacteria, fungi, mycoplasma, and extraneous viruses.

In addition, the injection of a twofold dose of the inactivated vaccine must not produce adverse reactions in young bovine Herpesvirus-1 seronegative calves. For the live virus vaccine, a 10-fold dose must be given to bovine Herpesvirus-1 seronegative calves and not produce adverse reactions, such as significant local or systemic reactions, fetal infection or abortion and must not revert to virulence after five serial passages.

Potency of the live virus vaccine is determined by virus titration confirming a virus vaccine titer between the minimum release titer and one-tenth of the highest safe dose. Potency of the inactivated vaccine, usually combined with an adjuvant to enhance the immune response, is confirmed by proof of its efficacy in calves through vaccination challenge: Ten 2- to 3-month-old seronegative calves are vaccinated and challenged three weeks later intranasally with a virulent strain of bovine Herpesvirus-1. While non-vaccinated control calves will exhibit the typical clinical picture, those vaccinated show no or only very mild indications of an effect.

Virus titer isolated from the nasopharyngeal mucus of vaccinated calves should be less than 1 percent of those from non-vaccinated control animals, and virus excretion should disappear at least three days before that of the controls. There should be no continued attenuated virus shedding after vaccination, and the vaccine-induced immunity should last for at least one year.

To verify long-term stability of the live virus vaccine, titration must be carried out three months past the shelf life date and a passing virus titer confirmed. Moisture content, concentration of preservatives and pH must be confirmed.

The modified live virus vaccine is given either intranasally, into the muscle or subcutaneously. The intranasally administered vaccine may produce immunity within days of dosage and is considered useful even in the event of a threatening outbreak. It is also safer to use for pregnant cattle. The intramuscular administration may cause abortions.

Breeding animals should be vaccinated at 6 to 8 months of age before breeding and then every year. Feedlot calves should be immunized two to three weeks before addition to the feedlot.

Vaccines with inactivated virus must be shown to be of the required consistency (viscosity, stability of emulsion, concentration of preservative). The inactivated, adjuvant-enforced vaccine is injected intramuscularly or under the skin, and at least two doses are required.

Although vaccination will protect vaccinated herds from the disease and concomitant losses in productivity, it will interfere with the eradication efforts necessary to completely eliminate the disease. Vaccinated cattle will have antibody which is indistinguishable from the one produced by the virulent virus strain, suggesting that those animals might be shedding the active disease-producing virus. A control and eradication program obviously becomes impossible when the vaccine-induced antibody might be hiding virus-shedding animals. It also would make it impossible to move, transport or sell immune animals.

Thus, glycoprotein E (gE)-deleted marker vaccines were developed that allow the distinction between vaccine-induced antibody and disease-induced immunity and permit the identification of likely virus shedders for elimination. Both attenuated live virus and inactivated vaccines with the lacking glycoprotein E marker have been produced and found effective.

A glycoprotein E (gE)-deleted inactivated recombinant virus vaccine is licensed for use in the European Union. These marker vaccines and specific antibody test systems identifying antibody produced by them (IgE-ELISA) are the basis for today’s test-and-removal programs in the European Union and elsewhere. All new animals should be held in quarantine, tested for the presence of antibody and if not excluded, receive the marker vaccine. Any animal considered for move or sale should receive the marker vaccine to exclude suspicion of virulent virus shedding.

**Bovine virus diarrhea**

Bovine virus diarrhea (BVD) belongs to the family of *Flaviviridae*, serocomplex pestivirus. This virus family also includes the classical swine fever virus (hog cholera) and border disease virus (hairy shaker disease in lambs). It is an icosahedral, single stranded RNA virus with a glycoprotein envelope, 40-60nm in size. It is very common and occurs worldwide. It is present in almost all herds of cattle everywhere and infects other ruminants (deer, llama).

The main route of infection is the oronasal one. The virus remains infective for hours, if not days, in fecal matter, nasal and other body secretions. The disease it causes ranges from very mild – in fact, 70 to 90 percent of infected cattle may show no symptoms at all – to severe: fever, depression and decreased feed intake, disease of the respiratory tract (tachypnea, nasal discharge), the gastrointestinal tract (ulceration of muzzle and gum, diarrhea) and the reproductive system (fetal death, abortion, stillbirths, congenital defects). Often it will affect the immune mechanism of the body, leading to immunosuppression and enhanced susceptibility to complicating bacterial and viral infections.

There are two types of bovine virus diarrhea virus: Type I, which is cytopathogenic, i.e., it destroys its host cell in cell cultures, and Type II, which is not cytopathogenic and does not kill the infected cell. However, both will produce the disease and both are antigenically related.

The disease syndrome is usually more severe in cattle infected by the Type II virus. Aside from a low-grade fever and decreased feed...
intake, there may be bleeding from the eyes and bloody diarrhea, thrombocytopenia, generalized hemorrhagic disease and frequently, death. At necropsy, visible indications of the disease are bleeding into the large muscles and organs, and clotted blood in the intestinal tract.

While most acutely infected cattle will shed the virus temporarily and recover, some animals may become persistently infected and will continue shedding the virus (carrier animals). Persistently infected animals (BVD-PI) are the primary source of virus spread. It takes only an hour of contact with a persistently infected carrier to infect a susceptible animal. Prevention and control of this disease depends on recognizing those carrier animals and removing them from the herd. The BVD-PI Ear Notch Testing Program has been designed for this purpose.

The test consists of a rapid screen of pools of 36 ear notch samples by rt-PCR followed by individual testing with AC-ELISA if the pool was positive. Other tests to identify the virus or antigen include fluorescent antibody staining (FA) or immunohistochemistry (IHC) of the tissue sample (section, smear, imprint). These tests detect the virus but do not distinguish between an acute, transiently infected animal or a persistent shedder. A second sample, taken three to four weeks later, is necessary to differentiate between transient and persistent infection. In very young calves, the presence of passive, maternal antibody may interfere with virus detection.

Positive animals should be quarantined until determined to be persistently infected. If persistently infected, they should be euthanized or put to slaughter or be sent to a feedlot for BVD-positive cattle. The dam that produced a persistently infected calf should be retested to confirm that she is not persistently infected. If she is not persistently infected, she should be considered immune for life. Only about one in 10 dams having produced a persistently infected calf are likely to be persistently infected themselves and should be culled.

Calves born to infected dams will develop immune-tolerance and remain persistently infected and continue to shed the virus for life. Usually a fetus that has been infected during the first five months of gestation may become immunotolerant and survive as persistent shedder of the virus. On the other hand, it may die or develop severe birth defects (brain, eye abnormalities, cerebellar hypoplasia, skeletal deformities etc.). Fetuses infected during the latter half of gestation may develop antibody and survive.

The virus can be found in mucus, saliva, feces, urine, semen as well as stillbirths and abortion products. It can exist in biting insects (mosquitoes and ticks) and fetal calf serum. Transmission is also possible via contaminated moving trucks, other cattle holding equipment and veterinary carelessness.

Developing solid herd immunity should be the main thrust of prevention. Inactivated as well as live attenuated viruses are available. All new arrivals should receive the modified live virus vaccine and be held in isolation for at least three weeks before entering the herd or feedlot. Breeder cattle should receive the vaccine at 6 months of age and again before breeding. Presence of antibody in the mother will protect the fetus and provide it with maternal protection even after birth. Colostrum usually contains high levels of antibody and will continue protecting the newborn (passive immunity) as long as the calf is allowed to suckle.

The serology of the calf will indicate seropositivity, and the presence of antibody may interfere with active immunization. Vaccination should not be performed before 1 week of age, and re-vaccination should be considered to cover the calves in which the initial vaccination may not have been successful. As passive antibody levels wane, the chance of active immunization will increase.

There are four or five Type II BVD virus vaccines and a multitude of Type I BVD virus vaccines (about 140 or so). Although the vaccines provide some cross protection, alternating use of Type I and Type II vaccines would provide better coverage. The modified live virus vaccines provide better cross protection than the inactivated vaccines.

Proper herd management must include identification and removal of persistently infected, virus-shedding animals, the understanding that residual pre-natal antibody and colostral antibody may interfere with the active immunization process and that stress-producing events should be kept to a minimum.

Animals appearing sick should be removed from their cohorts to a holding pen for sick animals until cause of the disease has been established. Contact with outside cattle, other ruminants, including deer, and equipment from possibly infected areas (markets, abattoirs) should be made impossible. Bulls used for natural or artificial insemination must be proven negative before use.

The presence of the infectious agent may not be noticeable in the absence of overt disease, especially because most infected cattle show no or only minor symptoms. This makes serological testing an important aspect of efficient herd management. Carrier animals that had been infected during the early stage of gestation and have become immunotolerant will not have detectable antibody but will still be carrying and shedding the virus. Super infection with a more virulent strain of bovine virus diarrhea can lead to mucosal disease, which is generally fatal.

Symptoms include high fever, therapy-resistant severe watery, bloody diarrhea of green discoloration and fetid smell containing strands of fibrin, ulceration of oronasal mucosae, mucopurulent discharge, coronary band ulcerations, cough, emaciation and a rough feces-contaminated hair coat. Acute mucosal disease will often lead to death within less than 10 days.

The chronic type of mucosal disease is debilitating and may take months before death. According to the Academy of Veterinary Consultants, the following control measures should be instituted:

- Diagnose and remove BVD-PI cattle.
- Vaccinate to reduce rate of BVD infection.
- Prevent introduction of BVD-PI animals.
  - Maintain a closed herd.
  - Do not purchase replacement cattle from auctions.
  - Buy only certified BVD-free livestock.
  - Quarantine new arrivals and show stock for 30 days and test for BVD-PI.
  - Test purchased pregnant cows and their calves at birth.
  - Use only pretested and certified BVD-PI-free bulls.
- Keep detailed records (origin, history, reproduction, health).
- Investigate gestational problems and aborted or sick newborns.

### Diagnosis

Laboratory diagnosis includes tests for virus isolation and identification (CPE in cell culture and its neutralization, rt-PCR) and antigen detection (immunohistochemistry, antigen-capture ELISA) as well as tests for antibody detection and measurement (virus neutralization, ELISA for IgG, IgM). Virus isolates detected in the indicator cell cultures must be identified to confirm their identity. A battery of specific antibody to a variety of likely viruses is mixed with the virus isolate, allowed to incubate and then inoculated into the indicator cell cultures. The antibody neutralizing cytopathic effects will identify the virus.

With minor modification, the plaque reduction neutralization test is applicable to this procedure. White blood cell counts are helpful in detecting leukopenia and thrombocytopenia.
and loud breathing. Severity of the disease discharge from muzzle and eyes, tachypnea The clinical picture includes fever, cough, serous from infected cattle. to animal via aerosolized droplet distribution seems to increase in fall. Transmission is animal most widespread respiratory diseases. Incidence Serological surveys suggest that it is one of the contagious and found in many countries. It is considered an initiator for the development about 150 nm in diameter. It causes only minor a pneumovirus, belonging to the family Paramyxoviridae. It is spherical and enveloped and Paramyxoviridae. is a pneumovirus, belonging to the family Paramyxoviridae. It is a single-stranded, enveloped RNA virus that produces typically a syncytial-cell-like cytopathic effect in susceptible cell cultures. It infects cattle, sheep and goats and other ruminants. It exists worldwide in most cattle populations and usually involves the young. The presence of maternal antibody does not prevent the disease but it often serves to mitigate its severity.

Parainfluenza Virus – 3
Parainfluenza Virus – 3 (PI-3) is a single-stranded RNA virus, belonging to the family Paramyxoviridae. It is spherical and enveloped and about 150 nm in diameter. It causes only minor affection in the upper respiratory tract, if any at all, except when it is associated with complicating secondary viral or bacterial infections.

It is considered an initiator for the development of secondary bacterial pneumonia and is highly contagious and found in many countries. Serological surveys suggest that it is one of the most widespread respiratory diseases. Incidence seems to increase in fall. Transmission is animal to animal via aerosolized droplet distribution from infected cattle.

The clinical picture includes fever, cough, serous discharge from muzzle and eyes, tachypnea and loud breathing. Severity of the disease picture is greatly enhanced when combined with other viral (BVD, IBR) or bacterial diseases. When superimposed by bacterial infection, bronchopneumonia is the most likely source of death. Uncomplicated, there may be a minor pneumonia with bronchiolitis and congested alveoli and hemorrhage with low or no mortality. Both modified live virus and inactivated virus vaccines are available. They are usually combined with the IBR vaccine. The presence of antibody is detected in serum and nasal secretions by hemagglutination inhibition, virus neutralization and complement fixation. The complement fixation test seems to be slightly less sensitive.

**Bovine respiratory syncytial virus**
Bovine respiratory syncytial virus (BRSV) is a pneumovirus, belonging to the family Paramyxoviridae. It is a single-stranded, enveloped RNA virus that produces typically a syncytial-cell-like cytopathic effect in susceptible cell cultures. It infects cattle, sheep and goats and other ruminants. It exists worldwide in most cattle populations and usually involves the young. The presence of maternal antibody does not prevent the disease but it often serves to mitigate its severity.

In seronegative calves, it produces a severe lower tract respiratory disease and predisposes the affected animal to complicating secondary infections. With a relatively high morbidity rate, its mortality remains below 20 percent. Fever is high (102 to 108 degrees F), the animals seem depressed, are off feed, show tachypnea, cough, discharge from eyes, nose and muzzle. As the disease progresses there will be open-mouthed breathing, respiratory distress and, occasionally, subcutaneous emphysema.

Although nothing much can be done against the viral aspects of the disease, treatment with antibiotics and sulfonamide is recommended to control bacterial complications. Pathologically, there is diffuse interstitial pneumonia, emphysema and edema and, frequently, bacterial bronchopneumonia. There are syncytial cells in the bronchial epithelium, inclusion bodies in the cytoplasm, edema and the development of hyaline membrane.

**Diagnosis**
Diagnosis depends on virus isolation, which is not always possible because of the fleeting presence of the virus. The detection of the viral

### Table 7 – Hemagglutination inhibition test (HI) to detect and measure antibody

<table>
<thead>
<tr>
<th>Step</th>
<th>Material</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serum sample</td>
<td>Dilute in 0.01 M PBS in test tube or microtiter plate by two-fold</td>
</tr>
<tr>
<td>2</td>
<td>Hemagglutinin (4 – 8 HA Units)</td>
<td>Add to serum dilution, mix and incubate</td>
</tr>
<tr>
<td>3</td>
<td>Red blood cells (0.4 %)</td>
<td>Add to test tubes, shake and incubate</td>
</tr>
<tr>
<td>4</td>
<td>White background</td>
<td>Read hemagglutination pattern</td>
</tr>
</tbody>
</table>

Equipment: test tubes, micro titer plates (round bottom); Specimen: serum sample from the field to confirm disease, study epidemiology and prevailing immunity, vaccine field trials; Nota bene: All test sera to be used in HA tests must be pre-adsorbed with packed RBCs to remove non specific agglutinins

### Table 8 – Modified Live Virus Vaccines

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>PRODUCT</th>
<th>PRODUCER</th>
<th>BOOST</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV</td>
<td>BOVISHELD™ BRSV (2ml/im)</td>
<td>Pfizer Animal Health</td>
<td>3 weeks + yearly</td>
<td>OK for pregnant cows.</td>
</tr>
<tr>
<td>BRSV VAC® (2ml/im)</td>
<td>Bayer</td>
<td>3 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Monovalent bovine respiratory syncytial virus vaccine (modified live virus vaccine)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBR-PI3</td>
<td>BOVISHELD™ IBR-PI3 (2ml/im or sc)</td>
<td>Pfizer</td>
<td>Yearly</td>
<td>Not for pregnant cows or calves nursing pregnant cows.</td>
</tr>
<tr>
<td>TSV-2® Intranasal (1ml per nostril)</td>
<td>Pfister Animal Health</td>
<td>Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bi-valent infectious bovine rhinotracheitis virus combined with Parainfluenza-3 virus (modified live virus vaccine)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd-Vac™-2 (2ml/im or sc)</td>
<td>Upjohn/Pharmacia</td>
<td>Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBR-PI3 48™ (2ml/im)</td>
<td>Boehringer</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tri-valent: bovine respiratory syncytial virus + infectious bovine rhinotracheitis virus + Parainfluenza-3 virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brsv ibrv pi-3</td>
<td>BOVISHELD™ IBR-PI3-BRSV (2ml/im)</td>
<td>Pfizer Animal Health</td>
<td>2-4 weeks, BRSV only</td>
<td>Not for pregnant cows or calves nursing pregnant cows.</td>
</tr>
<tr>
<td>BRSV VAC®-3 (2ml/im)</td>
<td>Bayer</td>
<td>2-4 weeks, BRSV Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tri-valent: bovine viral diarrhea virus + infectious bovine rhinotracheitis virus + Parainfluenza-3 virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bdrv ibrv pi-3</td>
<td>BarVac™3 (2ml/im)</td>
<td>Boehringer</td>
<td>Yes</td>
<td>Not for pregnant cows or calves nursing pregnant cows.</td>
</tr>
<tr>
<td>Herd-Vac™3 (2ml/im or sc)</td>
<td>Pfizer Animal Health</td>
<td>Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyramid™MLV-3 (2ml/im or sc)</td>
<td>Ayerst</td>
<td>Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quatro-valent: bovine respiratory syncytial + bovine viral diarrhea + infectious bovine rhinotracheitis + Parainfluenza-3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRSV bdrv ibrv pi-3</td>
<td>BOVISHELD™4 (2ml/im)</td>
<td>Pfizer Animal Health</td>
<td>2-4 weeks, BRSV only; Yearly</td>
<td>Not for pregnant cows or calves nursing pregnant cows.</td>
</tr>
<tr>
<td>BRSV-VAC®4 (2ml/im)</td>
<td>Bayer</td>
<td>2-4 weeks, BRSV only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyramid™MLV-4 (2ml/im or sc)</td>
<td>Ayerst</td>
<td>Yearly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NB:</strong> calves vaccinated within the first six months of life must be re-vaccinated at time of weaning.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
antigen by immunohistochemistry, enzyme-linked or fluorescent antibody staining is more reliable. Antibody levels rise early on during the course of infection and results on paired serum samples maybe deceiving. The presence of maternal antibody in young calves may not allow the development of an active antibody response.

Aside from these general management practices, comprehensive vaccination programs must be a priority. Modified live vaccines and inactivated vaccines are available and will reduce losses associated with the disease. Most vaccines today are combination vaccine to reduce cost and effort. There are many kinds of vaccines: killed virus vaccines, often inactivated by different means and containing different antigens. Ordinarily only of harmless, commensal nature, the presence of Pasteurella multocida, Mannheimia hemolytica, and Haemophilus somnus, combined with stressful events like travel, auctions, the cattle market, infection with other pathogens and the viruses here described, may lead to serious disease and death. All three cause acute bronchopneumonia. With Mannheimia hemolytica, there is usually a fulminant disease with severe congestion and hemorrhages, red discoloration, fibrino-necrotic pneumonia and fibrinous pleuritis, thromboses, and focal lung necroses. Pasteurella multocida shows a similar picture, but very much less severe. Haemophilus somnus causes a purulent bronchopneumonia often associated with extensive pleuritis. It can cause acute septicemia and dissemination of the infection throughout the body. It is known to have caused infectious thromboembolic meningo-encephalitis, also called brain fever in feedlot cattle. Its symptoms are high fever, lameness, discoordination, posterior paresis, unwillingness to move, circling, unnatural holding of head, abnormal eye movements, lateral recumbency, opisthotonus (spasm of the muscles causing backward arching of the head, neck, and spine), and death.

In cattle exhibiting early developing symptoms, the mortality rate is greater than 90 percent. Accordingly, many of the above vaccines have been combined with bacterial components. Bacterial vaccines incorporate bacterins, serotype-specific bacterial surface antigens, bacterial growth products, supernatants, surface extracts, breakdown products and inactivated whole bacteria. Continuous cultures of bacterial populations for the production of immunizing antigens are grown in large volume bioreactors under strictly controlled culture conditions (temperature, pH, agitation, aeration). Depending on culture conditions, the bacterial suspension may have to be concentrated by centrifugation, precipitation with aluminum hydroxide or filtration to provide a sufficiently antigenic product.
Commencement of treatment immediately,

Early detection of a diseased individual

Avoiding dust and mud when feeding.

To prevent bacterial complications. Add the

Inactivation of the bacterial culture product can

be achieved by the addition of formaldehyde,

merthiolate (thimerosal), β-propiolactone

or binary ethylenimine. To reduce the risk

of accidental contamination, chemical preservatives,

fungistatic agents and antibiotics may be added to

the vaccine.

Bacterins (inactivated whole bacteria) and

bacterial extract vaccines are given two to four

to six weeks apart, with an annual booster dose

or prior to stress situations such as weaning

and shipping.

The complexity of the bovine respiratory disease

class, the various possible triggers and

or prior to stress situations such as weaning

The choice of which of the many available

to use is up to the advising veterinarian

and his consideration of animal history and

risk assessment. It seems that the low-risk

group and the one with no exposure to likely

infectious contacts should be vaccinated first

and are the ones to be released first from their

holding drylots. The high-risk group with high

likelihood of prior exposure should be held

under observation, and care should be taken to

keep them isolated and not to allow them to pass

infections from one animal to the other.

Modified live virus vaccines seem to produce a

quicker response, often within days. Although

passive maternal antibody may interfere, it is

to vaccinate and then revaccinate later

to make certain of the effectiveness of the vaccine.

References and recommended reading


VACCINES FOR BOVINE RESPIRATORY DISEASE
Final Examination Questions

Choose True or False for questions 1-10 then complete your test online at www.elitecme.com.

1. Bovine respiratory disease complex, also known as shipping fever, is the most common and most costly beef cattle disease in the United States
   False

2. Manheimia hemolytica, pasteurella multocida, hemophilus somnus and ureaplasma diversum are found in the environment and are normally associated with healthy cattle.
   True

3. Bovine Herpesvirus-1 has an incubation period of three to five days post-contact. Other than symptomatic treatment, antibiotics may be advised to prevent secondary infections.
   False

4. After full recovery from Bovine Herpesvirus-1, the animal will no longer shed the virus.
   False

5. Cytopathic effects develop after six days and can be identified by enzyme-linked or fluorescent isothiocyanate-conjugated antibody against BHV-1.
   True

6. For the live virus vaccine, a twofold dose must be given to bovine Herpesvirus-1 seronegative calves and not produce adverse reactions, such as significant local or systemic reactions, fetal infection or abortion.
   False

7. Breeding animals should be vaccinated at 6 to 8 months of age before breeding and then every year. Feedlot calves should be immunized two to three weeks before addition to the feedlot.
   True

8. About one in 10 dams having produces a persistently infected calf are likely to be persistently infected themselves and should be culled.
   True

9. Visual examination of a bull prior to collecting semen for artificial insemination can confirm the bull’s health and absence of infectious disease.
   True

10. New arrivals should be evaluated and have their temperature taken as soon as they arrive. If any animals in the shipment have an elevated temperature, or show evidence of disease, all animals from the shipment should be held in a “sick-pen” for further observation.
    True