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The Armed Forces Institute of Pathology
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CASE I – 25078 (AFIP 2936460)

Signalment: One-year-old, male, Basset Hound, dog.

History: The dog was admitted at the Universidade Federal de Minas Gerais (UFMG) Veterinary Hospital with a history of lameness of the left hind limb for the past 4 months, and progressive weight loss. Radiological changes included a periosteal proliferative reaction in the metaphysis of the left femur suggestive of either an inflammatory or a neoplastic lesion (in spite of the age of the dog). Samples were obtained by fine needle aspiration for cytological exam, and a serum sample was processed for serological diagnosis of leishmaniasis.

Gross Pathology: Soon after euthanasia, fragments of the proximal metaphysis of the left femur were submitted for histopathology and a necropsy was not performed.

Laboratory Results: The serological tests for leishmaniasis yielded the following results: Indirect immunofluorescence: reactive 1/160; Complement fixation: reactive 1/160; ELISA: positive

Non-neoplastic mesenchymal cells and macrophages containing intra-cytoplasmic amastigotes were observed by microscopic examination of fine needle aspirates from the bone lesion. Immunohistochemical staining was strongly positive for *Leishmania* sp..

Contributor's Morphologic Diagnoses: 1. Bone, periosteum: Periostitis, histio-plasmacytic with periosteal proliferation and immature bone formation, osteoblastic

hyperplasia and hypertrophy, intense osteoclasia, and innumerous intra-cytoplasmic amastigotes in macrophages, chronic, focal, moderate.
2. Bone marrow: Fibrosis, chronic, multifocal; and accumulation of macrophages containing intra-cytoplasmic amastigotes, diffuse, moderate.

Etiologic diagnosis: Protozoal periostitis

Etiology: *Leishmania chagasi*

Contributor's Comment: Visceral leishmaniasis (VL) is a disease caused by protozoa from the genus *Leishmania*, particularly *L. donovani*, *L. infantum*, and *L.* chagasi.[5] VL is a significant public health concern in several Brazilian States, including the State of Minas Gerais. The dog is considered the most important reservoir for human VL, particularly in urban areas.[1,2,3]

The clinical manifestation of canine VL is usually chronic, and associated with cachexia, cutaneous lesions, hepatomegaly, splenomegaly, and lymphadenopathy.[5] Osteo-articular involvement in cases of VL has been described in dogs,[4,6,8] and is usually due to either the inflammatory response to the parasite or accumulation of immune complexes in the joints.[6]

Although VL is widespread in Brazil, periosteal proliferation with the intensity and localization described here is extremely uncommon. As in several other areas in Brazil, VL is considered an emerging disease in the State of Minas Gerais. In spite of the large number of dogs with VL presented to the UFMG Veterinary Hospital, this was the first case in which the primary complaint was a skeletal condition due to periostitis associated with *Leishmania* sp.

Joint lesions occur in approximately 37.5% of VL cases. These changes are often associated with reluctance to walk, arthralgia, and periosteal proliferation in the periphery of the joint.[7] Interestingly, in this case the dog had no clinical signs of VL such as lymphadenopathy, which is one of the most frequently observed clinical signs.[5]

The diagnostic approach employed in this case allowed us to establish the etiology of the process to the level of classification as *Leishmania* sp.. However, considering the geographic distribution of the donovani complex Leishmania species, it can be assumed that the agent involved in this case was *Leishmania chagasi*, which is the agent of VL in the New World.

Considering the significance of the dog as a major reservoir for human VL, particularly in urban areas, it is important for clinicians to keep unusual clinical manifestations of VL including skeletal changes in their list of differentials.

AFIP Diagnosis: Bone, proximal metaphysis of left femur (per contributor): Osteomyelitis and periostitis, plasmacytic and histiocytic, multifocal, moderate, with reactive bone formation, periosteal fibroplasia, and myriad intrahistiocytic amastigotes, etiology consistent with *Leishmania* sp., Basset Hound, canine.

Conference Comment: Leishmaniasis is a zoonotic disease caused by many pathogenic species of the genus *Leishmania*. Histologically, the amastigotes are 2-4 μm, round to oval, with clear cytoplasm and a kinetoplast perpendicular to the nucleus. The kinetoplast is a specialized mitochondrion.[9] Organisms are usually located in the cytoplasm of macrophages, but have also been reported in neutrophils, eosinophils, endothelial cells and fibroblasts.[6] Clinical pathology findings in cases of leishmaniasis include hypergammaglobulinemia, hypoalbuminemia, nonregenerative anemia, thrombocytopenia, uremia and proteinuria.[7]

Differential diagnoses include *Histoplasma capsulatum, Sporothrix schenckii, Trypanosoma cruzi,* and *Toxoplasma gondii. H. capsulatum* and *S. schenckii* can be differentiated by special stains for fungal organisms, such as GMS or PAS. Unlike *Leishmania* sp., *T. cruzi* is located primarily within muscle and its kinetoplast is parallel to the nucleus.

As the contributor stated, periosteal proliferation to this extent is an unusual finding with leishmaniasis. More common causes of periosteal proliferation in dogs include hypertrophic osteopathy, *Hepatozoon americanum* infection, craniomandibular osteopathy, osteosarcoma and osteomyelitis.[11]

Although endemic throughout much of the world, there are only rare reports of leishmaniasis in dogs in the southern and midwestern United States. In 1999, *L. infantum* was diagnosed in an outbreak of foxhounds in the northeastern US. Beagles and Bassett hounds housed in the same kennel and with a similar travel history as the foxhounds were seronegative. The cause of the increased susceptibility of foxhounds to leishmaniasis is unknown.[10] The clinical disease depends largely on whether the animal mounts a predominantly Th1 or Th2 response to the parasite. The development of a Th1 immune response is important in the control of leishmanial infections. Th1 cells secrete interferon-gamma, which activates macrophages to kill the parasites. Whereas a predominantly Th2 response results in the release of IL-4, IL-10 and IL-13 which inhibit the activation of macrophages thereby preventing the killing of leishmanial organisms, and stimulate immunoglobulin production which may result in immune complex deposition.[7,9]

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CASE II – 03-03451 (AFIP 2948752)

Signalment: 4 month-old Arabian foal.

History: This foal had a fever for 2 days and a 4 day history of dyspnea, coughing, and mild subcutaneous edema of the head and neck. Euthanasia was elected due to a poor prognosis. No clinical signs appeared in other foals or the adult horses of the same farm.

Gross Pathology: The cranioventral pulmonary areas (approximately 60-70% of total lung surface area) were bilaterally consolidated, failed to collapse, and had a corrugated surface. On cut section, there were random multiple and variably-sized (0.2 to 2 cm), multifocal to coalescing abscesses that contained casseated white necrotic material.

Laboratory Results: High numbers of *Rhodococcus equi* were grown on culture and seen on smears.

Contributor's Morphologic Diagnosis: Lung: Subacute Bronchopneumonia, multifocal to coalescing, suppurative and histiocytic with intralesional intrahistiocytic rods consistent with *Rhodococcus equi*.

Contributor's Comment: *Rhodococcus equi* (*R. equi*) is a Gram positive facultative intracellular pathogen that causes significant respiratory disease with an occasional enteric form in foals less than 6 months of age.[1] Histology is characterized by chronic suppurative/pyogranulomatous bronchopneumonia and ulcerative enteritis. Although rare, infections are also recorded in other mammals including goats. Transmission is primarily by inhalation and rarely by ingestion and may be facilitated by poor dusty conditions. *R. equi* is characterized by the presence of virulent and avirulent strains. Despite the fact that most environmental isolates are avirulent, the isolates from diseased foals are always virulent.[2] The virulent isolates are characterized by the presence of an 85 or 90 kb virulence-associated plasmid (Vap).[3] Virulent strains can survive within the macrophages likely due to the products of Vap genes.[4]

AFIP Diagnosis: Lung: Bronchopneumonia, pyogranulomatous, multifocal, severe, with myriad intrahistiocytic coccobacilli, Arabian foal, equine.

Conference Comment: *R. equi* causes pyogranulomatous pneumonia with abscessation, lymphadenitis, ulcerative enterocolitis, and less commonly, osteomyelitis in foals. *R. equi* has been reported to cause lymphadenitis in swine, sheep, cattle, llamas and cats. Disseminated infections are reported in goats, primarily causing hepatic and pulmonary abscesses. Unlike foals, avirulent strains of *R. equi* may cause disease in goats.[5] Although all foals are susceptible to *R. equi*, those with compromised immune systems from failure of passive transfer or

combined immunodeficiency (CID), are particularly vulnerable to diseases such as those caused by *R. equi*, *Pneumocystis carinii* and equine adenovirus.

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CASE III – 0303046 (AFIP 2937764)

Signalment: Approximately 6 month-old female laboratory Beagle (*Canis familiaris*) (9 kg).

History: Five day duration of sudden onset muscle rigidity especially noticeable in the neck and shoulder. Muscle mass appeared to be increased.

Gross Pathology: Most proximal muscles in each limb were pale and firmer than normal, especially in the thoracic limbs and neck. Fresh samples of affected muscles 20x10x5mm would stand out straight horizontally when held by one end (compared to samples from normal dogs, which hang down vertically).

Laboratory Results: Elevated CPK (762 U/l compared to lab normal of 242 U/l), normal liver enzymes, total protein, WBC normal (with no eosinophilia), RBC parameters normal.

Contributor's Morphologic Diagnosis: Myopathy, characterized by degeneration, necrosis, and regeneration, with minimal to mild histiocytic inflammation.

Contributor's Comment: The proximal muscles of the thoracic limbs were most severely affected, but pelvic limb muscles, diaphragm, temporalis and pharyngeal muscles were affected to a lesser extent. Section provided is from the rhomboideus or serratus ventralis muscle, both of which were markedly affected. Microscopically, most muscles examined, including several muscle groups that appeared grossly normal (e.g. diaphragm and esophagus), were characterized by differing degrees of myopathy (degeneration and regeneration). The myopathy was characterized by nuclei located centrally instead of peripherally (minimal in mildly affected muscles), wide variation in cross-sectional diameter, and in more extensively affected muscles by multifocal cytoplasmic hypereosinophilia (usually in central sarcoplasm), granularity, and loss of cross-striations. Granularity of sarcoplasm was due to abnormal organization of myofibrils (myofibril disarray). Occasional fibers were necrotic. Inflammation (predominantly histiocytic, with occasional neutrophils) was present in the most severely affected muscles, but was not a prominent feature (i.e. was considered a response to necrosis). Rarely, ring fibers were noted. Regeneration was present and characterized by rowing of nuclei, which were plump, euchromatic, and had prominent nucleoli; associated cytoplasm was usually slightly basophilic.

Based upon the intense rigidity of the muscles of this dog clinically and immediately post mortem, and on the microscopic findings, the myopathy in this dog is consistent with a diagnosis of "myotonic dystrophy". Many myotonic dystrophies are inherited (e.g. in Chow Chows, Miniature Schnauzers, Staffordshire terriers, myotonic goats, and humans), although myotonia can also occur following administration of cholesterol lowering agents, corticosteroids, and rarely in cases of hypothyroidism.

<u>Myotonic Dystrophies:</u>
Myotonic dystrophy is the most common form of muscular dystrophy in humans, with an estimated incidence of 1 in 8,000. The two types of heritable myotonic dystrophies in people are designated as type 1 (DM1, Steinert's disease) and type 2 (DM2, proximal myotonic myopathy or PROMM). Both are dominantly inherited, multiorgan diseases.

Muscle pathology in both DM1 and DM2 includes central nuclei (sometimes in chains), angular/atrophic fibers, hypertrophic fibers (hence wide variation in fiber diameter), necrotic fibers, fibrosis, and deposition of adipose tissue.[1] DM2 is known as proximal myotonic myopathy because muscle symptoms (pain, stiffness, myotonia, and weakness) characteristically involve proximal limb muscles.

Both DM1 and DM2 also cause a variety of extramuscular effects in a proportion of patients. These include cardiac conduction abnormalities, cataracts, diabetes, testicular failure, and hypogammaglobulinemia. DM1 also results in mental retardation and skeletal abnormalities in the congenital form.[1]

DM1 is due to DNA $(CTG)_n$ repeats that cause a "gain-of-function" at the RNA level, wherein $(CUG)_n$ RNA transcripts accumulate, resulting in aberrant splicing of chloride channel pre-mRNA, loss of CIC-1 (chloride channel) protein from the membrane surface, and therefore reduced membrane conductance to chloride.[3] DM2 results from $(CCTG)_n$ repeats having similar effects to DM1.[1] Reduced membrane conductance results in membrane hyperexcitability, with subsequent degeneration, necrosis, and attempts at regeneration.

Naturally-occurring animal models of the human disease include the myotonic goat and various dog breeds in which myotonia is inherited. An autosomal dominant mutation in the goat CIC-1 gene results in reduced channel conductance and hyperexcitability. Similarly, in miniature Schnauzer dogs, a missense mutation in CIC-1 has been identified causing recessive myotonia congenita by a similar mechanism.[4] These models have assisted in understanding the electrophysiology and function of the chloride channels, whereas transgenic mouse models of the human disease were used to elucidate the RNA splicing regulation abnormalities and "gain-of-function" mechanism of the $(CTG)_n$ and $(CCTG)_n$ repeats observed in humans.[2,5]

AFIP Diagnosis: Skeletal muscle: Myocyte degeneration and necrosis, multifocal, moderate, with regeneration, variation in fiber size, satellite cell proliferation, and endomysial fibrosis, Beagle, canine.

Conference Comment: Muscular dystrophies (MD) are a heterogeneous group of inherited disorders that cause progressive muscle weakness and wasting. In humans, MDs are divided into several groups, the most common of which are X-linked MD, autosomal MD, and myotonic dystrophy. The MDs all have similar histological lesions of muscle degeneration, necrosis and regeneration. Clinical correlation, genetic testing and electromyography are often used for definitive diagnosis of a specific MD.[6]

Myotonia refers to a sustained involuntary contraction of a group of muscles. Humans affected with myotonic dystrophy describe "stiffness" and an inability to release their grip after a handshake. The contributor has provided an excellent overview of the pathogenesis of myotonic dystrophy. Alterations in the CIC-1 (chloride channel) protein results in reduced chloride conductance, membrane

hyperexcitability, and ultimately muscle degeneration. There are several animal models of myotonic dystrophy. Mutations in the CIC-1 (chloride channel) protein have been documented in the myotonic (or "fainting") goat and the miniature Schnauzer.[4] Myotonic dystrophy has also been reported in the Chow Chow and the Staffordshire Terrier. Gross lesions are variable, primarily depending on the stage of the disease, and range from muscular atrophy to hypertrophy. In this case, the clinical and gross findings of muscle rigidity correlate with the diagnosis of myotonic dystrophy.

The best known example of X-linked MD in humans is Duchenne MD. The cellular defect occurs in the gene encoding for the dystrophin protein. Dystrophin connects the intracellular contractile apparatus and the extracellular connective tissue matrix. Animal models include the *xmd* dog, *mdx* mouse and cats. In most species, X-linked MD causes muscular atrophy. However, affected cats develop muscular hypertrophy and the condition is known as "Hypertrophic feline muscular dystrophy". The muscles of the neck, tongue, diaphragm and pectoral girdle are most commonly affected.[7]

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CASE IV – 201602-1 (AFIP 2956376)

Signalment: 4 week-old female Simmental cow (*Bos bovis*).

History: This calf had watery and mucoid diarrhea over a long period of time. The calf was presented with dehydration and acidosis. It had lesions between the digits and on the muzzle.

Gross Pathology: At necropsy, especially in the esophagus, rumen and small intestine there were multiple linear erosions. In the small intestine, Peyer's patches were moderately depleted. Multiple erosions in the interdigital clefts and on the lower lip were seen.

Laboratory Results: Serology for Bovine Viral Diarrhea virus: antigen positive / antibody negative.

Contributor's Morphologic Diagnosis: Rumen: Rumenitis, erosive, multifocal, subacute, mild to moderate with single cell necrosis and hydropic degeneration of epithelial cells, bovine.

Contributor's Comment: Multifocally, mostly the basal layer of the epithelium and occasionally the upper layers show hydropic degeneration (swollen cells characterized by intracellular edema and clear pale eosinophilic vacuoles within the cytoplasm) and there are scattered apoptotic cells (characterized by hypereosiniphilia and condensed or karyorrhectic nucleus). There is some accumulation of lymphocytes surrounding these degenerated epithelial cells (satellitosis). In a focal extensive area, on the epithelium, there is a mild hyperkeratotic, orthokeratotic sometimes parakeratotic, layer with mild serocellular crusts and there are scattered small pustules (not on all slides). The epithelium is mildly eroded and mildly and irregularly hyperplastic.

Etiology: Bovine pestivirus

The Bovine Viral Diarrhea virus belongs to the family Flaviviridae, genus Pestivirus. It is a small, enveloped, positive strand RNA virus. The Bovine Viral Diarrhea virus (BVDV) is found in cattle; but can also infect sheep, goats and pigs and has been isolated in many wild and captive African species including the rhinoceros, giraffe

and eland. There are two biotypes--cytopathic (CP) and noncytopathic (NCP), each with two serovars.

For the pathogenesis, the virus is shed in fluids (saliva, blood, oculonasal discharge, urine, feces, semen, uterine secretions, amniotic fluid, fetal tissue and blood). The primary replication takes places in the tonsils and oropharyngeal lymphoid tissues. The virus enters circulating monocytes. It is transported to lymphoid tissues and the subepithelial connective tissues of the dermis and the GI tract and spreads locally to the overlying epithelial cells. The outcome depends on viral strain and virulence, immune status of the host, whether or not the animal is pregnant, and the stage of pregnancy. Infection with some NCP-BVDV may produce a thrombocytopenia and hemorrhage. The virus can also inhibit macrophage chemotaxis and neutrophil function (animals may have concurrent pneumonia or mastitis).

Acute Bovine Virus Diarrhea:

Most BVDV infections in immunocompetent, nonpregnant cattle are subclinical and of the NCP type. Clinical disease is usually seen in seronegative, immunocompetent cattle from 6-months to 2-years old.

Transplacental infections can occur with CP strains:

Between 50-100 days: fetal death, abortion, mummification

Between 100-150 days: congenital defects (microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, microphthalmia, thymic aplasia, hypotrichosis, alopecia, brachygnathism, growth retardation, pulmonary hypoplasia)

Transplacental infections can also occur with NCP strains:

Before 100-125 days: immunotolerance and persistent infection (bovine fetuses become immunocompetent at 150-200 days)

After 150-200 days: calves may be born with neutralizing antibodies and may be unthrifty, slow growers or show no clinical signs

Persistent Infection:

Several factors have influenced the persistence of BVDV in cattle. Non-lytic infections produced by non-cytopathic BVDV strains, and the ability to evade the host immune response, are the primary mechanisms of persistence. In addition, some man-made factors have provided opportunities for BVDV to persist in cattle populations. Others mechanisms unique to BVDV probably result from its adaptation to cattle as a primary host.

The ability to induce fetal persistent infections is a unique aspect of BVDV pathogenesis. An additional requirement of this mechanism is a non-lytic infection with BVDV, which does not adversely affect fetal development and maturation.

This unique phenomenon is the primary mechanism whereby BVDV is maintained in cattle populations providing for direct and indirect transmission. Although persistently infected (PI) animals may represent approximately one percent of the cattle population, they shed virus and initiate further virus replication and genetic variation. Therefore, control and prevention programs must focus on prevention of persistent infections and identification and removal of PI animals. Breaking the cycle of exposure of pregnant animals in the first 125 to 150 days of gestation is the key to preventing persistent infections.

As an RNA virus, BVDV generates mutations that precipitate antigenic changes. Changes in the E2 glycoprotein are the primary sites of variation in neutralizing epitopes. Recently, the phylogenetic classification of BVDV isolates as type Ia, Ib, and II has emphasized the significance of genetic variation. Infection of immunocompetent animals with BVDV stimulates cross-reactive antibody and provides protection from disease due to infection with diverse strains. Due to the ease with which BVDV crosses the placenta, the fetus may remain susceptible to infection although the pregnant dam is protected by cross-reactive antibody. Variations in BVDV have led to vaccine failures against fetal infection due to differences between vaccine virus and field virus. However, continued genetic and antigenic variation is responsible for the circulation of BVDV in susceptible cattle and the development of persistent fetal infections in susceptible pregnant animals. The move toward multivalent vaccines is in response to the recognition of the importance of genetic variation of BVDV.

Recently, Voges et al. reported a chronic BVDV infection in the testicles of a bull that was previously acutely infected with the virus. The bull was not viremic and possessed high levels of anti-BVDV antibody while shedding approximately $10^3 CCID_{50}$ of virus/ml of semen. Currently, studies are being conducted to determine the prevalence and potential of chronic persistent infections that may follow acute BVDV infections. The establishment of chronic infections would provide an additional mechanism for BVDV to persist in cattle populations.

Recognition of the pathogenic mechanism of immunotolerant fetal persistent infections was an important step in the evolution of BVDV control and prevention. The identification and removal of PI animals is an important component of current prevention and control methods. Due to the prevalence of PI animals and their shedding of BVDV they represent a high risk and are justifiable targets of control methods. However, it is clear that BVDV has many mechanisms at its disposal to ensure that it can persist and be maintained in cattle populations. When this is considered, the slow progress in preventing and controlling BVDV infections is understandable. In addition, this aspect will be an important consideration as increased emphasis is placed on the eradication of BVDV from cattle.

Mucosal disease (MD) develops when immunotolerant cattle (infected with a NCP strain in utero) are infected with a CP strain, or it may occur with introduction of an exogenous CP virus or a mutation of the endogenous NCP virus that becomes CP. Cattle with MD can infect other animals in the herd. The greater the genetic homogeneity between the CP and NCP strains, the shorter the clinical course. Less similar viruses produce a disease with a more protracted clinical course. Case fatality rates approach 100%.

Typical gross findings are:

With BVD: Erosions or shallow ulcerations of the oral cavity

With early MD: Erosions in oral and nasal mucosa, esophagus (linear), rumenal papillae, abomasum, omasum, cecum, and colon; ulcers at the interdigital cleft, vulva and testis; blunting of the oral papillae; Peyer's patches swollen, necrohemorrhagic +/- diphtheritic membrane

With chronic MD: Alopecia and hyperkeratosis (especially on the neck), chronic erosive lesions in the mouth and skin at mucocutaneous junctions, and around hooves and horns

Typical histological findings are:

Severe, acute inflammation in intestinal mucosa, especially overlying Peyer's patches, destruction of underlying crypts, stromal collapse, lymphocytolysis

Hyaline degeneration or fibrinoid necrosis of blood vessels; vasculitis in multiple organs accompanied by a mild-to-moderate mononuclear cell infiltrate in the vessel walls and perivascular tissues

Mesenteric lymph nodes and spleen: lymphocytolysis and lymphoid depletion

Erosions in the skin similar to those in the mucosa

Differential diagnoses are:

Pestiviral thrombocytopenia: similar clinical signs, diarrhea less pronounced, with profound thrombocytopenia

Rinderpest (Paramyxoviridae-Morbillivirus): intranuclear/intracytoplasmic inclusion bodies, syncytia

Malignant catarrhal fever (Herpesviridae - gammaherpesvirus): similar gross findings plus conjunctivitis and corneal edema; lymphoblastic and lymphocytic necrotizing vasculitis

Infectious bovine rhinotracheitis (Herpesviridae - alphaherpesvirus): Similar gross findings; epithelial necrosis, intranuclear inclusions.

Diseases with oral lesions only: foot and mouth disease (Picornaviridae - Aphthovirus); vesicular stomatitis (Rhabdoviridae-Vesiculovirus); bluetongue (Reoviridae-Orbivirus); bovine papular stomatitis (Poxviridae-Parapoxvirus); and necrotic stomatitis or oral necrobacillosis (Fusobacterium necrophorum)

Diseases with diarrhea only: salmonellosis (*Salmonella dublin* and *S. typhimurium*), winter dysentery ("coronavirus"), paratuberculosis (*Mycobacterium avium paratuberculosis*), and intestinal parasitism

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AFIP Diagnosis: Rumen: Rumenitis, erosive, subacute, multifocal, moderate, with epithelial degeneration and necrosis, Simmental, bovine.

Conference Comment: The contributor has provided an excellent overview of Bovine Viral Diarrhea virus (BVBV). BVDV is a common cause of erosive and ulcerative lesions on epithelial and mucosal surfaces. Histopathology and additional laboratory testing can be used to differentiate BVDV from the list of differential diagnoses that the contributor provided. Although there is some variation among slides, the majority of them have the erosions described by the contributor. Additional pestiviral diseases include Border Disease in sheep and Classical Swine Fever.

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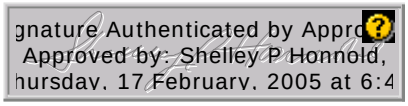

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