CASE I – 02-3629 (AFIP 2885737)

**Signalment:** Three year-old, female, Barbados-Moreno X, ovine.

**History:** Three of forty sheep in a dry lot developed black, hard, encrusted skin of the ear tips, lips, and nose. The lesion involved only non-pigmented skin. The lesions developed three days after the sheep were fed a moldy, pelleted complete horse feed. The sheep were fed oat hay in addition to the pellets. Inspection of the baled hay revealed some mild discoloration of the edges of the bale but no obvious mold or extraneous weeds.

**Gross Pathology:** All three of the sheep had black, hard, encrusted skin of the lips, nose, eyelids and ear tips except where the skin was pigmented or covered by an ear tag (Fig. 1-5). Heavily wooled parts of the body and feet were not affected. The livers were diffusely pale.

**Laboratory Results:** Elevated GGT (201 IU/L) and bilirubin levels (Total bilirubin = 5.0 mg/dl) were present in serum of one ewe collected before euthanasia. Aerobic cultures of the lung, liver, and spleen yielded no significant isolates. The moldy, pelleted horse feed was negative for aflatoxin B1, aflatoxin G1, sterigmatocystin, zearalenone, zearalonal, ochratoxin A, citrinin, diacetoxyscirpenol, neosolaniol, nivalenol, deoxynivalenol, fusarenone-X, T-2, and HT-2.

**Contributor’s Morphologic Diagnosis:** Cholangiohepatitis, subacute, diffuse, severe, with intraluminal crystals, bile ducts, and occasional hepatocyte necrosis. Resulting in: Secondary photosensitization with multifocal cutaneous necrosis of skin of ear tips, lips, nose, and eyelids.
Contributor’s Comment: In the liver there is diffuse periportal fibrosis and dilation of bile ducts. Basophilic, wispy, acicular crystals are present in bile duct lumina. Sometimes, these are surrounded by multinucleated giant cells. The portal connective tissue contains infiltrates of mononuclear cells, principally lymphocytes and plasma cells. There are increased bile duct profiles. Small numbers of necrotic hepatocytes, often surrounded by neutrophils, are present in lobules. A few hepatocytes contain crystalline material. There is mild, diffuse hepatocellular vacuolation.

The lesions are consistent with “crystal-associated cholangiohepatitis”. This is a well-recognized syndrome of photosensitization secondary to liver injury associated with the formation of crystalline material in the bile ducts. The disease is associated with the consumption of certain plants including Kleingrass (Panicum coloratum), Agave lechequilla, Tribulus terrestris, Narthecium sp., Nolena texana and Brachiaria decumbens. There is also a report of photosensitivity and liver disease with crystal formation in goats consuming young green oats (Avena sativa) infected with the fungus Drechslera companulate.

The puncture vine (Tribulus terrestris), in combination with the mycotoxin, sporodesmin produced by Pithomyces chartarum, causes the disease known as geeldikop and is a major cause of secondary photosensitization in South Africa. Mycotoxins have not been associated with the other plants listed. Steroidal saponins derived from the plants are suspected to be the source of the crystals but this has not been substantiated.

The moldy, pelleted horse feed was temporally associated with the outbreak of disease in this flock but we were not able to identify a mycotoxin in the feed. None of the plants known to be associated with this syndrome were present in the environment except for the oat hay. The owner could not recall exactly when the bales were purchased relative to the first clinical signs. It is interesting to note the case report of goats developing hepatogenous photosensitization and crystal-associated hepatopathy following grazing of mold infected green oats. The hay was not grossly moldy but fungal cultures were not performed.

AFIP Diagnoses:
1. Liver: Fibrosis, portal, diffuse, moderate, with mild lymphoplasmacytic cholangiohepatitis, biliary hyperplasia, and intrabiliary crystals, Barbados-Moreno X, ovine.
2. Liver: Hepatitis, necrotizing, neutrophilic, random, multifocal, mild.

Conference Comment: Conference attendees agree that the portal distribution of the lesions is consistent with the pathogenesis of toxin concentration within the
biliary system. The cause of the randomly distributed neutrophilic hepatitis is not evident. It is not thought to be associated with the intoxication.

There are three types of photosensitization, based on the source of the inducing agent, that produce gross lesions similar to those seen in this case. Gross lesions are comparable in all three types of photosensitization. Photosensitization results from the activation of photodynamic substances within the skin by ultraviolet light. This results in free radical formation, either directly or through activation of xanthine oxidase, which causes the tissue damage.4

Type I, or primary photosensitization, results from ingestion of a plant with photodynamic properties, such as Hypericum perforatum (St. John’s wort), Fagopyrum sp. (buckwheat), Ammi majus (bishop’s weed), or the anthelmintic, phenothiazine. Type II photosensitization is caused by a defect in porphyrin metabolism, such as congenital porphyria and congenital protoporphyria in cattle. In both cases, enzyme deficiencies lead to the accumulation of photodynamic pigments. In congenital porphyria, cattle are deficient in the enzyme uroporphyrinogen III cosynthetase. In addition to photodermatitis, affected cattle have discolored teeth and bones, anemia, and porphuria. Congenital protoporphyria occurs in Limousin cattle due to a deficiency of ferrochelatase, and causes photodermatitis. Finally, Type III is called hepatogenous photosensitization and is secondary to any hepatic injury that interferes with bile excretion. Phylloerythrin, a breakdown product of chlorophyll, is normally excreted in the bile. Accumulation of phylloerythrin causes photosensitization if the animal is exposed to the appropriate wavelength of solar radiation that activates the photodynamic agent.4 The contributor noted several plants associated with hepatogenous photosensitization.

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References:

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CASE II - 03M0512 (AFIP 2888770)

Signalment: 12 month, male, mixed breed, bovine, *Bos taurus*.

History: This animal had a brief history of drainage from the right ear and swelling of the right side of the head. Loss of sight in the left eye was suspected. Euthanasia with captive bolt was performed.

Gross Pathology: In the calvaria, a 2.5 - 3 cm diameter tan, firm, multilobular mass was present extending from the right vestibulocochlear nerve medially into the brain on the right side. Lungs were adhered to the diaphragm and thoracic cavity.

Laboratory Results: Immunohistochemistry demonstrated immunoreactivity for *Mycoplasma bovis* in and around the caseous and purulent foci (Fig. 1).

Contributor’s Morphologic Diagnoses: Ear/brain: chronic polypoid pyogranulomatous and plasmacytic otitis media, interna, and meningoencephalitis, associated with *Mycoplasma bovis* infection.

Contributor’s Comment: Speculation in the present case is that an inflammatory polyp extended from the middle ear along the vestibulocochlear nerve with invasion of meninges and parenchyma of medulla oblongata and cerebellum. Not all sections contain cerebellum. The cause of the inflammation was likely chronic infection with *Mycoplasma bovis*. Immunohistochemistry, as in this case, has been used successfully to detect the organism in lung abscesses in calves with fatal pneumonias that were culture positive for the organism. Otitis media caused by *Mycoplasma bovis* has been described in preweaned dairy calves. Clinical findings included ear droop, epiphora, head tilt, and recumbency. Tympanic bullae often had fibrinosuppurative to caseous exudate with fibrous thickening of the tympanic mucosa and mononuclear cell infiltrates. Other causes of otitis media in calves include *Haemophilus somnus*, *Pasteurella multocida*, *Streptococcus* spp., *Actinomyces* spp., and *Railletia auris* (an ear mite).

Cases of otitis media in preweaned calves were likely associated with cases of subclinical *M. bovis* mastitis, given isolation of *M. bovis* from the bulk tank of the herd of origin. *M. bovis* is an important bovine pathogen causing respiratory disease and arthritis in addition to mastitis, reproductive disease, and otitis media. In Europe it is reported to be responsible for 1/4 to 1/3 of calf pneumonias. Its prevalence is likely underestimated with bacteria such as *Mannheimia haemolytica*,

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Pasteurella multocida, and Haemophilus somnus being more commonly and easily isolated.\textsuperscript{3} In one study, feedlot cattle with chronic respiratory disease and/or arthritis had high rates of \textit{M. bovis} identification from lungs and joints by immunohistochemistry, often with bovine virus diarrhea virus (BVDV) infection also identified. \textit{M. bovis} from lungs or joints and along with BVDV were the most common pathogens persisting in tissues of animals failing to respond to antibiotic therapy.\textsuperscript{2}

Besides \textit{M. bovis}, other mollicutes isolated from cattle include \textit{Mycoplasma mycoides} subsp. \textit{mycoides} (the cause of contagious bovine pleuropneumonia), \textit{Mycoplasma dispar}, \textit{Ureaplasma diversum}, \textit{Mycoplasma bovirhinis}, and \textit{Mycoplasma canis}.\textsuperscript{3} Because of the lack of a cell wall, these organisms are inherently refractory to several groups of antibiotics and some strains are becoming resistant to other antibiotics, making control difficult. As of early 2003, no vaccine was available.\textsuperscript{3}

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\textbf{AFIP Diagnosis:} Brain: Meningoencephalitis, pyogranulomatous, multifocal, moderate, with hemorrhage and neuronal and white matter necrosis, mixed breed, bovine.

\textbf{Conference Comment:} The lesions are most severe in the sections containing cerebellum; however, some slides contain only sections of less severely affected brainstem.

The contributor reviews \textit{M. bovis} infection and its implication as a predisposing factor in infection with other bacterial and viral agents. This stresses the importance of considering mycoplasma as a primary pathogen, especially in respiratory disease, as it is frequently masked by other common and more easily cultured secondary bacterial agents.\textsuperscript{3}

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\textbf{References:}
2. Haines DM, Martin KM, Clark EG, Jim GK, Janzen ED: The immunohistochemical detection of \textit{Mycoplasma bovis} and bovine viral diarrhea

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CASE III – 03-2702 (AFIP 2888614)

Signalment: 3 month old, intact female pit bull dog, *Canis familiaris*, canine.

History: This dog was euthanized by the County Animal Shelter due to neurologic signs that included incoordination, spasms, tremors, and abnormal use of its legs. The dog was also reported to have diarrhea and ocular mucous discharge.

Gross Pathology: Tan fecal material soiled the perirectal skin. Lung lobes were distended and had prominent rib impressions. Multifocal pinpoint white foci were present in all lobes. The right main-stem bronchus contained red, tenacious mucous. The thymus was markedly atrophied. Intestinal contents were watery and brown to clear.

Laboratory Results: Fecal parasite examination revealed high numbers of *Isospora* sp. oocysts. Rabies IFA of sections of brain were negative. Immunohistochemistry for canine distemper virus was positive on sections of spleen (performed by Washington Animal Disease Diagnostic Lab).

Contributor’s Morphologic Diagnoses: Spleen: Marked, diffuse, lymphoid atrophy and multifocal follicular lympholysis, with intranuclear inclusions.

Contributor’s Comment: There is severe depletion of lymphocytes in the splenic cords and periarterial lymphoid sheaths. The underlying reticular stroma, dendritic cells, and ellipsoids are exposed. Pale foci in the mantle and marginal zones of the follicles contain lymphocytes and mononuclear cells with nuclear pyknosis and karyorrhexis. In these regions, dendritic cells have swollen nuclei with marginalized chromatin and central eosinophilic viral inclusions. Additional microscopic lesions (not submitted) include bronchointerstitial pneumonia with syncytial cells containing intracytoplasmic and intranuclear inclusions, and mild nephritis localized to the papillae with myriad eosinophilic intracytoplasmic inclusions within the transitional epithelium.
Canine distemper virus is a negative-strand RNA *Morbillivirus* of the family *Paramyxoviridae*. Morbilliviruses cause measles, rinderpest, peste-des-petits ruminants, equine morbillivirus infection, and phocine, dolphin, and porpoise distemper. Canine distemper virus is most commonly transmitted via respiratory aerosols; transplacental infection may also occur. Following respiratory exposure, the virus replicates within macrophages and disseminates to lymph nodes, the spleen, and to other organs\(^1\).

Severe lymphopenia and immunosuppression are hallmarks of the disease and thymic atrophy is one of the most consistent gross lesions. Increased lymphocyte apoptosis has been demonstrated in lymph nodes and the thymus and may be the cause of immunosuppression.\(^2\) CD4+ T cells have also been shown to be preferentially depleted.\(^3\) This spleen nicely demonstrates the severity of lymphocyte depletion, along with the etiologic agent. Differential diagnosis includes canine herpesvirus and canine adenovirus 1 infection. The spectrum of lesions and positive immunohistochemistry results confirm canine distemper virus as the etiology.

Distemper virus infection of the CNS causes both grey and white matter lesions. It has been compared to toxic and metabolic myelinopathies and multiple sclerosis. The mechanism of demyelination has been the subject of investigation. Demyelination in the CNS is associated with down-regulation of myelin gene transcription and degeneration of oligodendrocytes. Interestingly, viral particles are much more abundant in microglia, astrocytes, ependyma, and neurons than oligodendrocytes.\(^4\) In contrast to lymphoid depletion, demyelination does not appear to be result of oligodendroglial necrosis or apoptosis.\(^5\) The presence of viral nucleic acid but not intact virions within oligodendrocytes suggests that defective infectious particles may be a cause of the demyelination. Other theories, including immune-mediated, antibody-dependent mechanisms, cytokine-mediated, and astrocyte-dependent processes have also been proposed.\(^4\)

**AFIP Diagnosis:** Spleen: Lymphoid necrosis and depletion, diffuse, marked, with reticuloendothelial eosinophilic intranuclear inclusion bodies, pit bull, canine.

**Conference Comment:** The contributor gives a concise review of this important infectious disease. Canine distemper virus (CDV) has a wide host range and affects multiple species in the families *Canidae* (dingo, fox, coyote, wolf, jackal), *Ailuridae* (pandas), *Mustelidae* (ferret, mink, skunk, badger, weasel), *Procyonidae* (raccoon, coati), *Ursidae* (bear), and *Felidae* (large cats - lions are especially susceptible). Similar disease syndromes occur in marine mammals caused by
phocine distemper virus (pinnipeds), and viruses of the cetacean morbillivirus group (dolphins and porpoises).  

Secondary infections due to the immunosuppressive effects of CDV are especially important. Toxoplasmosis, bordetellosis, and canine adenovirus 2 are common sequelae to CDV infection.

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References:

CASE IV - 02-4408 (AFIP 2888675)

Signalment:  Twenty day-old Holstein calf.

History:  Calf submitted with a history of respiratory signs and diarrhea.
**Gross Pathology:** The calf had a small thymus and small erosions or ulcerations of the oesophagus and abomasum. Intestinal content was liquid and yellowish. Atelectatic lesions were present in cranioventral regions of the lungs.

**Laboratory Results:** BVD virus was detected in the colon with the immunofluorescence technique. Search for bovine coronavirus and rotavirus with the FA technique was negative. Bacterial cultures were negative.

**Contributor’s Morphologic Diagnosis:**
Mucosal colitis with crypt necrosis

**Contributor’s Comment:** In this colon, there is a mild and multifocal exfoliation of the superficial epithelium. Many crypts dilated and filled with mucus and necrotic cells are denuded or lined by flat, cuboidal or low columnar enterocytes. There is a multifocal infiltration of the lamina propria by lymphocytes and plasma cells. The extensive necrosis of the crypts of Lieberkühn noted in this colon is compatible with a BVD virus infection destroying their epithelial lining. The bovine coronavirus causing a similar colitis was not demonstrated in the present case. This case emphasizes the fact that BVD virus should always be considered a potential cause of diarrhea in young calves.

**AFIP Diagnosis:** Colon: Colitis, neutrophilic, acute, diffuse, mild, with crypt abscesses, crypt epithelial cell necrosis, and epithelial regeneration.

**Conference Comment:** Bovine pestivirus consists of two biotypes, cytopathic (CP) and noncytopathic (NCP), based on their effects in cell culture. Infection with NCP biotype occurs primarily in immunocompetent, non-pregnant cattle and often produces subclinical infection. Affected animals may develop acute disease with fever, lethargy, diarrhea, and mild oral erosions. Whether the animals develop clinical signs or not, they are considered reservoirs of infection and are lifelong shedders. (Clarification: Postnatal BVDV infection of immunocompetent, non-pregnant cattle is usually subclinical and the cattle clear the virus, usually in 2-3 weeks. They definitely do NOT become lifelong shedders. Even when they are shedding maximally, they are not very efficient transmitters, as several studies have shown. Only persistently infected (infected at 1-4 months of gestation) cattle are lifelong shedders.) Some NCP BVDV strains have been associated with abortion, and some with thrombocytopenia and hemorrhagic disease in calves and, rarely, adult cattle; however, only NCP BVDV cause persistent infection.

If a fetus is infected in utero with NCP biotype during the first four months of gestation (before the immune system fully develops), the calf may be aborted, born
weak, or may exhibit congenital defects such as cerebellar hypoplasia. If the calf survives, it is considered immune tolerant because its immune system does not recognize the NCP virus as being foreign. This calf is now a persistently infected carrier.\textsuperscript{1,2}

If the persistently infected animal is then infected with a CP strain (possibly by mutation from an NCP strain), the animal becomes superinfected and develops fatal mucosal disease. Both the CP and NCP biotypes are antigenically similar, so the immune system fails to recognize the CP strain as foreign and does not protect the animal. Mucosal disease is characterized by erosions in the oronasal, esophageal, and gastrointestinal mucosa, blunting of buccal mucosal papillae, and necrosis of Peyer's patches, often with a diphtheritic membrane. The animal often has severe diarrhea with dehydration, and may die quickly. If the animal survives the acute disease, chronic mucosal disease develops, characterized by healing ulcers in the oral cavity, erosive and ulcerative dermatitis of the pastern, and laminitis.\textsuperscript{1,2}

Although not present in this case, additional classic histological lesions of BVD infection include Peyer's patch necrosis, lysis of gut-associated lymphoid tissue, and herniation of the crypts of Lieberkühn into the submucosal space previously occupied by Peyer's patches.\textsuperscript{1,2}

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References:
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