The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
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WEDNESDAY SLIDE CONFERENCE 2009-2010

Conference 10

16 December 2009

Conference Moderator:

Dr. Fabio Del Piero, DVM, PhD, Diplomate ACVP

CASE I: S105/07 (AFIP 3067177).

Signalment: Adult female Friesian milk sheep (*Ovis ammon aries*).

History: While being kept for educational purposes during 2004 at the University of Veterinary Medicine Hannover, Clinic for Pigs, Small Ruminants, Forensic Medicine and Ambulatory Service, the sheep tested positive for infection with maedi-visna virus (MVV) and came in 2005 for further diagnostic reasons to the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health. The animal eventually developed in 2007 severe dyspnea and coughing, and became recumbent. It was then euthanized and submitted for necropsy.

Gross Pathology: The lungs showed multifocal, subpleural and peribronchiolar nodular lesions up to 2 mm in diameter. These foci were grey and slightly elevated (**fig. 1-1**). Within the right cranial lobe there was a 3 x 2 x 2 cm sharply demarcated area of necrotizing and suppurative bronchopneumonia. The caudal mediastinal lymph nodes were enlarged and showed caseous necrosis and abscess formation.

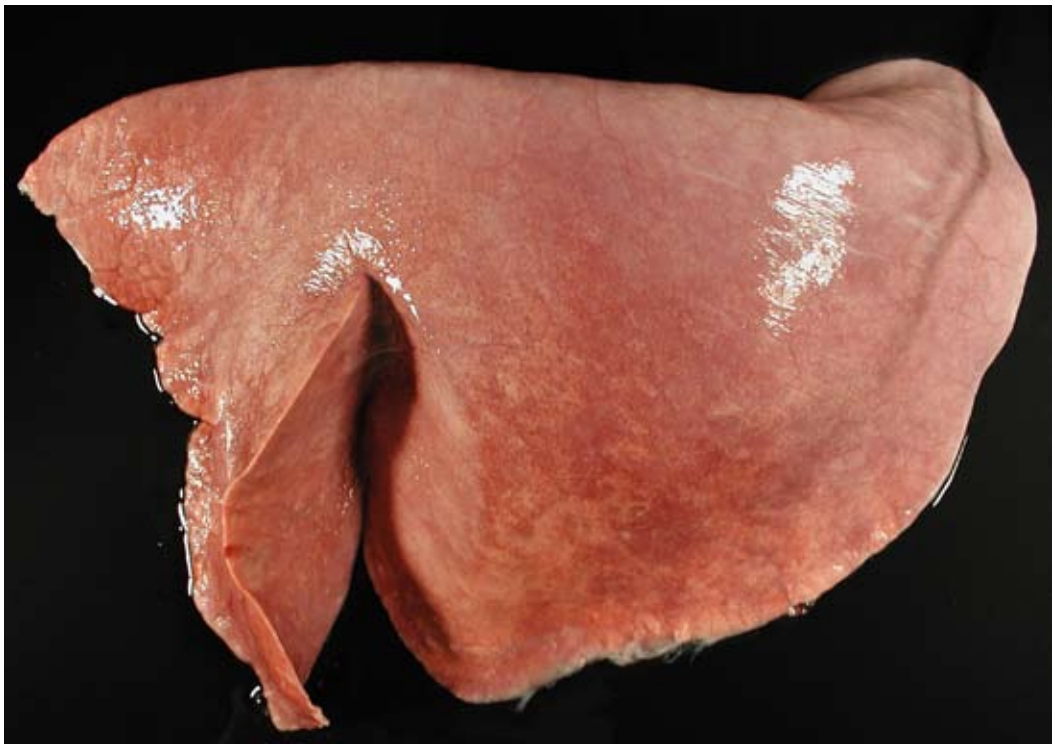
Laboratory Results: Serological testing in 2004 by competitive ELISA revealed antibodies against MVV (more specific data not available). Bacteriological culture of lymph nodes revealed heavy growth of *Corynebacterium pseudotuberculosis* admixed with *E. coli*.

Histopathologic Description: Lung: Multifocally, there are large, up to 1 mm in diameter, aggregates and sheaths of numerous lymphocytes and fewer macrophages in a follicular pattern (BALT hyperplasia) within the parenchyma or subpleural tissue, preferentially adjacent to bronchi, bronchioles and blood vessels (**fig. 1-2**). Diffusely, alveolar septa are slightly thickened by few infiltrating lymphocytes and macrophages and rare neutrophils. Around terminal bronchioles there are prominent bundles of smooth muscle cells (hypertrophy) (**fig. 1-3**). Occasionally, in the alveolar lumina there are few detached pneumocytes.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, bronchointerstitial, lymphocytic and histiocytic, multifocal, moderate, chronic with smooth muscle hypertrophy; etiology consistent with maedi-visna virus infection.

Contributor's Comment: Maedi is a persistent, chronic and progressive viral disease in sheep and goats

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.



1-1. Lung, sheep. There are multifocal, raised, grey nodular subpleural and peribronchiolar lesions up to 2 mm in diameter. Photograph courtesy of Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald, Germany, jens.teifke@fli.bund.de.

that often results in lifelong infection. Cases have been documented in numerous countries worldwide, particularly continental Europe, the United Kingdom, Canada, the United States, Peru, Kenya, South Africa, Israel, India, Myanmar, and the southern regions of the former U.S.S.R. The word, "maedi," is of Icelandic origin meaning, "shortness of breath." However, as a disease found globally, it has been called by a variety of names—Graaf-Reinet disease in South Africa, Zwoegerziekte in the Netherlands, La bouhite in France, and Montana, Marsh's, or Ovine Progressive Pneumonia (OPP) as well as Lymphoid Interstitial Pneumonia (LIP) in the United States.[4] Maedi sometimes couples with visna, another slowly progressive disease causing meningoencephalitis in sheep and goats. "Visna" is the Icelandic word for "wasting," which aptly characterizes a major clinical sign of the disease.

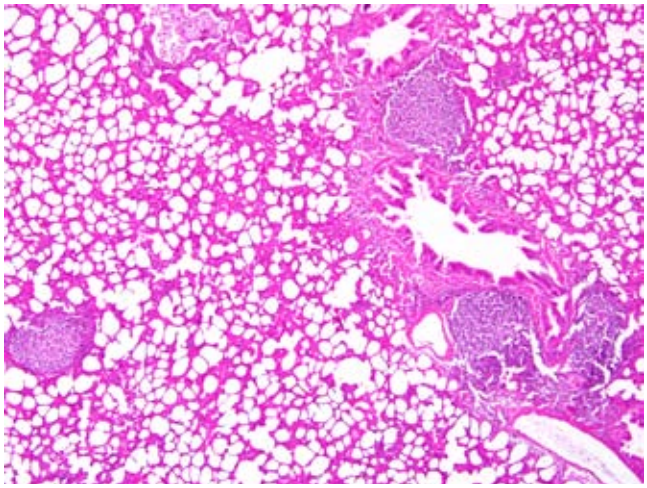
Maedi and visna are caused by the ovine maedi-visna virus complex (MVV). It is important to note, however, that the two diseases result from different strains of the virus.[6] MVV is a non-oncogenic retrovirus from the genus *Lentivirus* in the *Retroviridae* family. Retroviruses are spherical, enveloped virus particles with single-stranded, diploid, positive-sense RNA genomes. They possess a reverse transcriptase allowing them to integrate their genomes into the host cell DNA as a provirus. MVV is a small ruminant lentivirus (SRLV) exhibiting antigenic similarities to caprine arthritis encephalitis virus (CAEV). It can be subdivided into 5 groups (A-D) and further subtypes. MVV subtypes A1 and A2 seem to exclusively affect sheep, while A5, A7, B1, C, and D specifically affect goats. Subtypes A3, A4, A6 and B2 affect both sheep and goats.[5]

MVV is transmitted both horizontally and vertically. It can also be spread between species by direct contact. The primary mode of transmission has been via ingestion of colostrum or milk by the newborn.[5] Interestingly, there has been a higher risk of MVV infection when a newborn animal is bottle-fed colostrum from a seropositive ewe than when the newborn naturally suckles the same ewe.[3] Respiratory secretions constitute a secondary mode of transmission to ingestion of colostrum.[5]

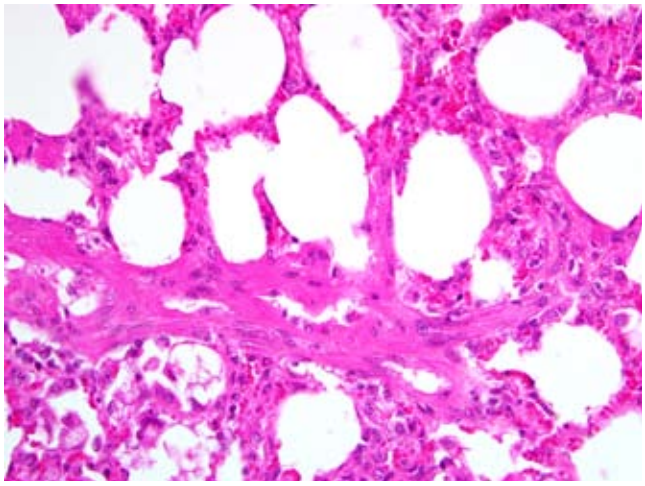
Many sheep are seropositive for MVV, but few show clinical disease. The incubation period for the virus complex tends to be two to three years, possibly extending to eight.[6] Consequently, clinical signs will generally not appear in animals before they are two years old with the highest concentration of disease cases from five to ten years of age.[2] The primary manifestations of maedi are as follows: 1) a slow and progressively worsening dyspnea and 2) weight loss resulting in undernourishment, despite a normal appetite. With the appearance of clinical signs, maedi causes nearly 100% fatality.

On gross pathologic examination of maedi-diseased animals, lungs are heavier than normal and appear expanded. Rib impressions may also be apparent. The lungs will typically be mottled and gray. The cut surface is commonly dry and exudate cannot be extruded. Regional lymph nodes—mediastinal and tracheobronchial—are often enlarged.[4]

The general pathogenesis of MVV is not fully understood. Viral RNA and proviral DNA can be detected in a broad spectrum of cells, including dendritic cells, lymphocytes,



1-2. Lung, sheep. Multifocally, there is prominent nodular hyperplasia of peribronchiolar and perivascular lymphoid tissue and a diffuse cellular infiltrate within the interstitium. (HE 40X)



1-3. Lung, sheep. There is smooth muscle hyperplasia, lymphohistiocytic infiltration of the alveolar septa, and small amounts of fibrosis. Multifocally within alveoli there are few alveolar macrophages. (HE 400X)

plasma cells, endothelial cells, fibroblasts, adipocytes, microglial cells, pericytes, epithelial cells of bronchi, alveoli, mammary glands, thyroid follicles, choroid plexus, small intestine, renal tubules, and third eyelid. However, viral antigen has only been found in macrophages and monocytes, suggesting these cells may be the more exclusive replication sites for the virus complex.[1] The characteristic histopathologic finding for maedi is a pulmonary lymphocytic proliferation, predominantly of T cells.[4] Opportunistic bacterial infections can also occur, making a precise diagnosis of the disease sometimes difficult.

AFIP Diagnosis: Lung: Pneumonia, interstitial, lymphohistiocytic, diffuse, mild to moderate, with peribronchiolar lymphoid hyperplasia and smooth muscle hypertrophy.

Conference Comment: The contributor provides a useful synopsis of this widespread entity. Lentiviral infections in small ruminants differ from those in felids and primates in that they do not result in immunosuppression. Progressive pneumonia is the most common manifestation of MVV infection in sheep; less frequent manifestations include encephalitis, arthritis, mastitis, and glomerulonephritis. Because it is closely related to MVV, CAEV not surprisingly produces a similar constellation of disease manifestations in goats (see WSC 2009-2010, Conference 1, case III), with several notable differences. Chief among these, arthritis in adults and neurologic disease in kids are far more common manifestations of CAEV in goats than is pneumonia. Additionally, CAEV pneumonia in goats is characterized by well-demarcated areas of type II pneumocyte hyperplasia, alveolar septal expansion by lymphocytes, and alveolar flooding with eosinophilic proteinaceous fluid. By contrast, type II pneumocyte hyperplasia is not a prominent feature of ovine progressive pneumonia, which is instead typified by interstitial pneumonia with the formation of lymphoid nodules with germinal centers, smooth muscle hypertrophy, and interstitial fibrosis.[2] In the present case, lymphoid proliferation and smooth muscle hypertrophy are the most striking lesions, although there is considerable slide variability with respect to severity, ranging from mild lymphocytic peribronchiolitis and perivasculitis to moderate lymphohistiocytic interstitial pneumonia as described above. There is infrequent type II pneumocyte hyperplasia, which participants attributed to local compression by immediately adjacent large lymphoid nodules.

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CASE II: NIAH-1 (AFIP 3133944).

Signalment: 50-day-old piglet (*Sus scrofa*).

History: The piglet was euthanized because of severe emaciation.

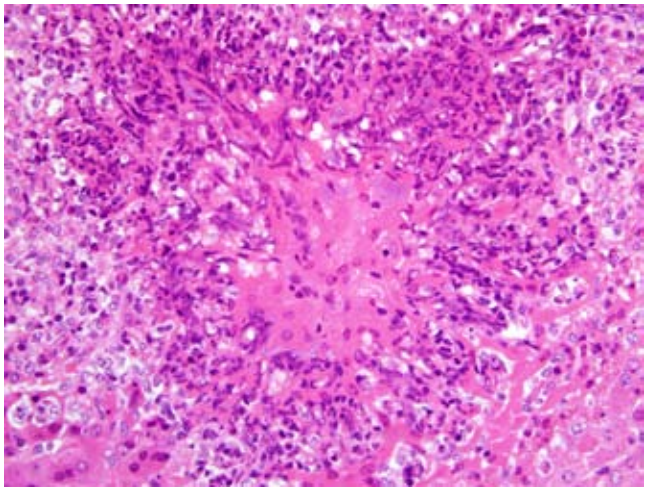
Gross Pathology: Consolidation with multiple microabscesses was found in the cranial-ventral portions of the lung. Multiple petechiae and microabscesses were present throughout the liver. The inguinal lymph node was swollen and edematous.

Laboratory Results: *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) and *Pasteurella* spp. were isolated from the lung. *Pasteurella* spp. and *Haemophilus parasuis* were isolated from the pulmonary lymph node.

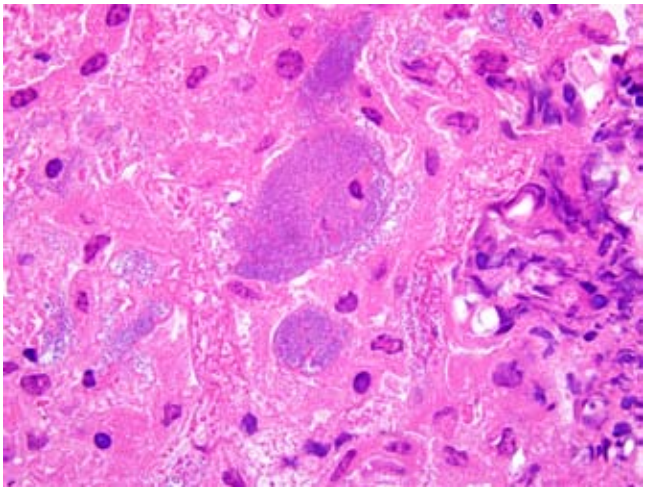
Histopathologic Description: Multiple microabscesses with bacterial colonies at the center of the lesion were found in the liver, lung, spleen, lymph nodes and the serosa of the urinary bladder (**figs. 2-1 and 2-2**). The infiltrating inflammatory cells surrounding the bacterial colonies had a spindle-shaped nucleus and cytoplasm which were morphologically different from neutrophils usually seen in a typical abscess. In addition, thrombi often containing bacteria were observed in hepatic portal veins. In the lung, similar lesions of the microabscesses with bacterial colonies were formed in the alveolar space and bronchiolar lumen. Immunohistochemical examination with rabbit hyperimmune serum against *A. pleuropneumoniae* type 2 revealed a positive reaction of the bacteria in the liver and lung (**fig. 2-3**). Other than the lesions caused by *A. pleuropneumoniae*, lymphocytic depletion was found in the various lymphoid tissues. In the tonsil, porcine circovirus type 2 antigens were detected by immunohistochemistry. Intense plasma cell infiltration was observed in the interstitial tissue of the kidney. *Balantidium* species infected the colon. No lesions were found in the heart, stomach, duodenum, pancreas, ileum and brain.

Contributor's Morphologic Diagnosis: Liver: Multiple microabscess formation with bacterial colonies and thrombi.

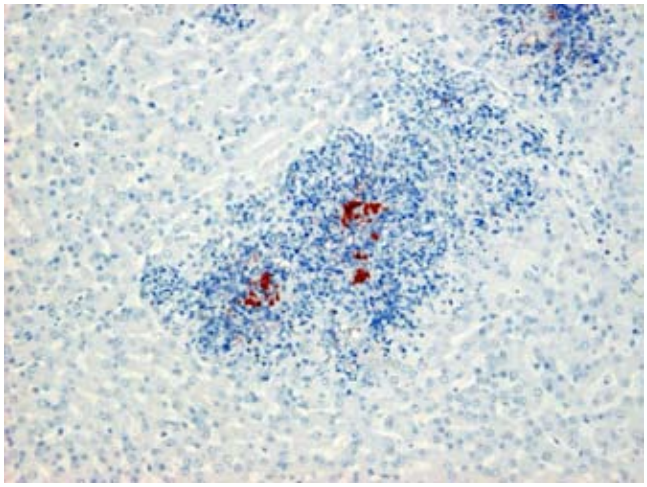
Contributor's Comment: *A. pleuropneumoniae* is a pathogenic agent of the respiratory disease called porcine pleuropneumonia. In a typical case of actinobacillosis, the histopathological changes are characterized by necrosis, hemorrhage, neutrophil infiltration and widespread edema with fibrinous exudates. However, the present case is characteristic in that multiple microabscesses were observed in various organs. Ohba et al [2] reported that *A. pleuropneumoniae* produced granulomatous lesions with asteroid bodies in the liver, showing a possibility of *A. pleuropneumoniae* as a pathogenic agent affecting not only the lung but also the liver and other organs. In the present case, asteroid bodies were not observed. However, bacterial colonies positive for *A. pleuropneumoniae* type 2 were found at the center of microabscess, indicating that these microabscesses could lead to granulomatous lesions later in



2-1. Liver, pig. There are multifocal random areas of necrosis surrounded by numerous viable and degenerate neutrophils and fewer histiocytes. Within the central areas of necrosis there are frequently large colonies of 1 x 2 um coccobacilli. (HE 400X)



2-2. Liver, pig. Large colonies of coccobacilli within random areas of acute coagulative hepatocellular necrosis. (HE 1000X)



2-3. Liver, pig. Colonies of bacteria are immunohistochemically positive for Actinobacillus pleuropneumonia type-2 antigen. Photomicrograph courtesy of 3-1-5 Kannondai, Tsukuba, Ibaraki, 305-0856, Japan, yyu@affrc.go.jp.

the course of the disease.

AFIP Diagnosis: Liver: Hepatitis, random, necrotizing and histiocytic, acute, multifocal, moderate, with fibrin thrombi and colonies of coccobacilli.

Conference Comment: This very interesting case underscores the importance of *A. pleuropneumoniae* not only as a cause of pleuropneumonia in pigs, but as a potential cause of necrotizing lesions in other organs as well. Less common lesions associated with *A. pleuropneumoniae* in pigs include meningitis, nephritis, osteomyelitis, arthritis, tenosynovitis, endocarditis, and pericarditis. In a recent case report of 11 pigs with granulomatous hepatitis caused by *A. pleuropneumoniae*, 7 had concurrent pleuropneumonia, which the authors concluded was likely the primary site of infection, with secondary dissemination to the liver; a similar pathogenesis is plausible in the present case.[2]

While conference participants generally concurred with the contributor's histopathologic description, most could not definitively identify neutrophils either within or adjacent to the lesions, and thus preferred the above morphologic diagnosis over the use of the term "microabscess." Participants interpreted the necrotic cells with streaming nuclei as predominantly Kupffer cells and hepatocytes. Despite the rather unusual anatomic location, most participants suspected *A. pleuropneumoniae* as the etiology based on 1) the characteristic nuclear streaming in foci of necrosis, 2) the presence of small to occasionally large colonies of bacilli, and 3) the abundance of fibrin within affected areas, particularly in blood vessels and sinusoids.

Nuclear streaming of leukocytes is typical in pleuropneumonia caused by *A. pleuropneumoniae*, and is attributed to secreted cytotoxins belonging to the repeats in toxin (RTX) family, namely Apx I, II, and III. These potent virulence factors are critical to the development of disease, and mutants lacking the Apx toxin have reduced virulence. Other virulence factors include a capsule that impedes phagocytosis by macrophages, lipopolysaccharide which induces macrophage activation, enzymes (e.g. superoxide dismutase, catalase, and hydroperoxide reductase) that protect against oxidative killing by leukocytes, metalloproteinase, urease, outer membrane proteins, and iron-binding proteins.[1]

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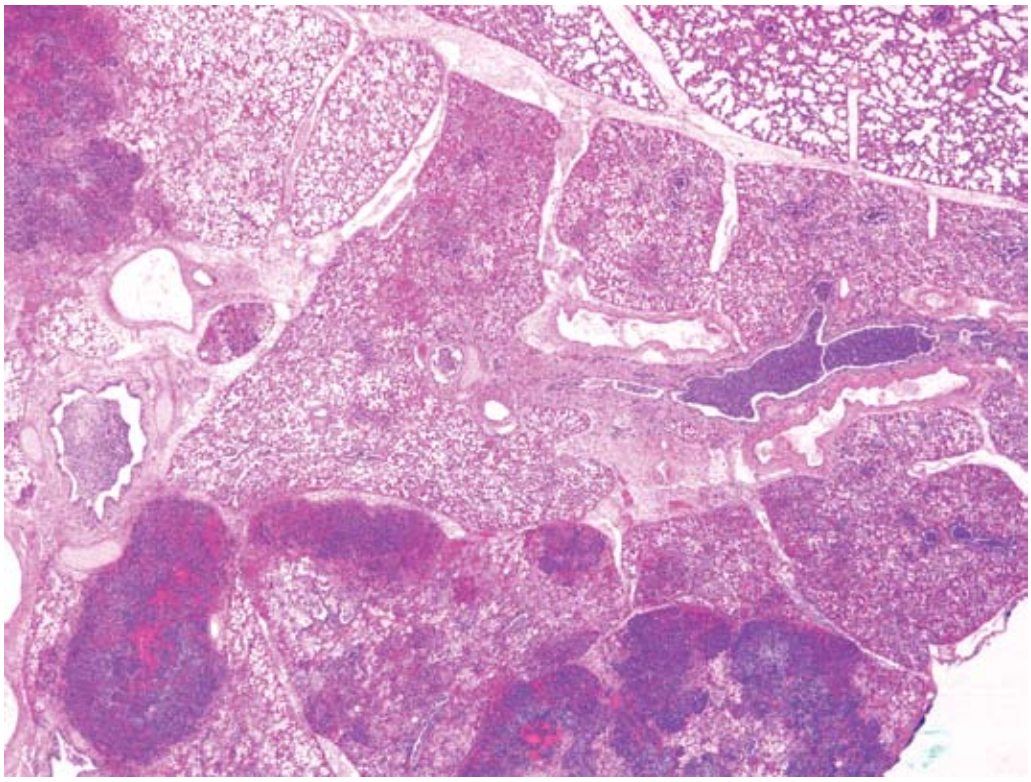
CASE III: 9-35-A (AFIP 3134628).

Signalment: Two (one male and one female) 3-week-old, Holstein calves (*Bos taurus*).

History: These two calves had been housed in separate calf pens in a calf barn with 14 other calves. The calves were fed 2 liters of milk replacer twice per day and heifer calves received Deccox® in the milk daily. The calves received Calf-Guard®, Colimune® and 3 to 4 liters of colostrum at birth. These two calves became ill at 7 to 10 days of age with mildly elevated temperature, poor drinking behavior and scours and subsequently developed abnormal breathing. Despite treatment with antibiotics (Nuflor®) and tube feeding of milk and electrolytes, the calves became progressively weaker and died.

Gross Pathology: Both calves exhibited mildly sunken eyes with a moderate reduction of internal fat stores. The lungs of both calves exhibited dark red to plum purple lobular discoloration of the intermediate lobes, anteroventral aspect of the diaphragmatic lobes and ventral aspect of the anteroventral lobes. Affected lobules were depressed, firm on palpation, and on cut surface exhibited dark red to plum purple discoloration with purulent exudate in airways. The female calf also exhibited mild fibrin exudation over the pleural surface of the discolored lobes, fibrinous adhesions of these lobes to the pericardial sac and increased clear yellow



3-1. Lung, ox. Multifocally there is a lobular bronchopneumonia with marked expansion of interlobular septa by edema. (HE 20X)

fluid in the pericardial sac with fibrin strands. Intestinal content was watery.

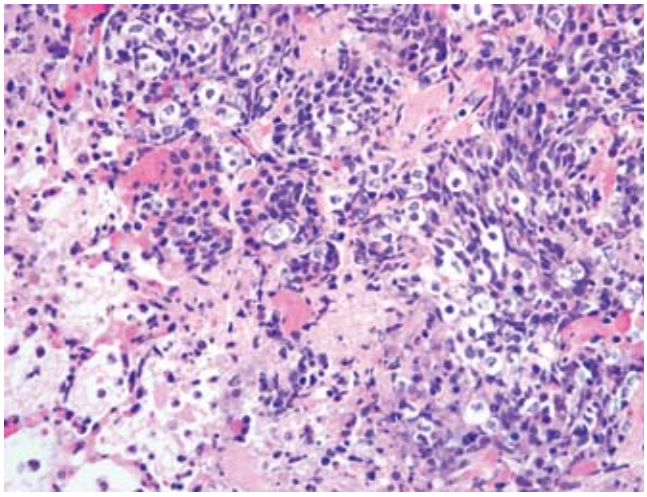
Laboratory Results: *Mannheimia granulomatis,* 4+, in lungs of both calves; *Mannheimia granulomatis*, few, in pericardial sac of female calf; bovine respiratory syncytial virus (BRSV) positive on lung by PCR and immunohistochemistry; rotavirus and bovine coronavirus positive on intestinal contents by PCR.

Histopathologic Description: There was moderate to marked pulmonary lobular consolidation characterized by macrophages and neutrophils intermixed with edema fluid, red blood cells and fibrin strands within alveolar spaces and airway lumina. Variably sized, multifocal aggregates of highly condensed leukocytes with nuclear streaming obscured parenchymal architecture and were observed throughout the sections (**fig. 3-1**). These foci were often associated with colonies of coccobacillary bacteria, hemorrhage and fibrin (**fig. 3-2**). Depending on the section examined, foci of bronchiolar epithelial necrosis and attenuation, with and without syncytical cells, were occasionally observed (**fig. 3-3**). Interlobular interstitium and lymphatics were mildly to markedly dilated with edema fluid, fibrin strands and a mild to moderate number of neutrophils and macrophages. Fibrin was focally present on the pleural surface and there were subpleural inflammatory infiltrates similar to the interlobular interstitium. There were scattered areas of atelectasis.

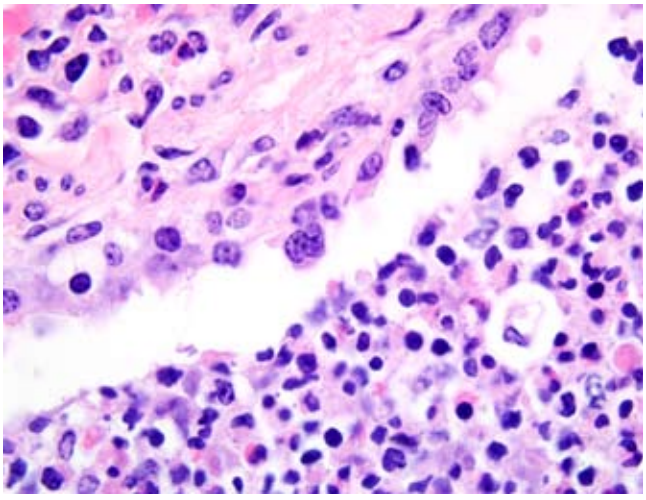
Contributor's Morphologic Diagnosis: Lung, bronchopneumonia, anteroventral, marked, subacute, fibrinosuppurative with intralesional coccobacilli and focal bronchiolar necrosis with syncytial cells.

Contributor's Comment: *Pasteurella multocida* and *Mannheimia hemolytica* are the most common Pasteurellaceae bacteria isolated from bovine pneumonia.[3] These organisms are commensals of the bovine nasopharynx which, during periods of stress or viral infection, can overwhelm host defense mechanisms establishing infection in the lower respiratory tract.[3] *M. granulomatis* is one of five recognized species of the genus *Mannheimia*.[1] This bacterium has been reported in association with oral abscesses and suppurative bronchopneumonia in Australian cattle [1], purulent bronchopneumonia, pleuritis, stomatitis and abscesses in Danish Roe deer [2] , purulent bronchopneumonia and conjunctivitis in European Brown hares [5] and a fibrogranulomatous panniculitis in Brazilian cattle known as lechiguana.[6]

The bronchopneumonia in the two Holstein calves reported here was similar to pneumonia described with *M. haemolytica* infection in that there was significant leukocyte necrosis as evidenced by oat cells, widespread accumulation of edema fluid, neutrophils, macrophages, fibrin, foci of hemorrhage and bacterial aggregates within alveolar spaces and airway lumina and interlobular septae were distended with serofibrinous exudate.[3] A similar lesion to *M. haemolytica* would be anticipated as it has been shown that *M. granulomatis* has leukotoxin activity in vitro.[2,7] However, large areas of coagulation necrosis surrounded by a thick rim of leukocytes, as is frequently observed with *M. haemolytica* pneumonia [3], were lacking in the *M. granulomatis* lesion. These observations suggest that, although both *M.*



3-2. Lung, ox. There are multifocal patchy areas of coagulative necrosis which are bordered by numerous degenerate leukocytes, many of which have streaming nuclei. Rarely, there are moderate numbers of 1 x 2 um bacteria within areas of coagulative necrosis. (HE 400X)



3-3. Lung, ox. Within degenerate, attenuated bronchiolar epithelium there are rare multinucleate syncytial cells. Bronchiolar lumina contain a cellular exudates composed of neutrophils, histiocytes and fewer lymphocytes admixed with low numbers of bacteria. (HE 1000X)

granulomatis and *M. haemolytica* share virulence factors [2,7], there may be subtle differences in pathological expression. However, more cases of *M. granulomatis* pneumonia would need to be examined before any conclusions could be drawn with regard to expression of virulence.

These calves also had focal infection with BRSV which was confirmed by PCR and immunohistochemistry. Viral BRSV lesions were not present in all tissue sections submitted. Pulmonary BRSV infection likely predisposed these calves to bacterial pneumonia. While *P. multocida* and *M. haemolytica* are reported to be commensal in the bovine nasopharynx [3], it is unclear how these two calves became infected with *M. granulomatis*.

AFIP Diagnosis: Lung: Bronchopneumonia, fibrinosuppurative, multifocal to coalescing, marked, with edema, hemorrhage, and coccobacilli.

Conference Comment: The conference moderator emphasized the importance of additional diagnostic testing when assessing the histomorphology present in this case; the two most likely etiologies include *P. multocida* and *M. haemolytica*, as noted by the contributor, and definitive determination necessitates additional diagnostics, such as microbial culture, immunohistochemistry, in situ hybridization, and PCR, as employed in this case.

Leukotoxin, mentioned by the contributor, is an important virulence factor of *M. haemolytica*. A member of the repeats in toxin (RTX) family, leukotoxin is secreted during the log phase of growth, and its receptor is CD18.[3] At low concentrations, leukotoxin activates bovine platelets and leukocytes or induces their apoptosis [4], while at high concentrations it induces leukocyte lysis, resulting in the characteristic nuclear streaming noted microscopically in areas of necrosis.[3] Lipopolysaccharide, another important virulence factor, is synergistic with leukotoxin because it induces increased expression of β2-integrins on leukocytes which contain the CD18 receptor. Other important bacterial virulence factors include a polysaccharide capsule that assists in attachment and impairs phagocytosis, iron-regulated outer membrane proteins that bind transferrin and alter neutrophil function, adhesins that mediate attachment, and neuraminidase that decreases both respiratory mucus viscosity and repellant negative charge on host cells. Cytokines TNF-α, IL-1β, and IL-8 are particularly important components of the complex inflammatory milieu produced in pneumonic mannheimiosis.[8]

Bovine parainfluenza virus 3, bovine herpesvirus 1, and BRSV all increase susceptibility to *M. haemolytica*, and presumably other respiratory pathogens, by infecting ciliated epithelium and reducing mucociliary clearance; the latter two also infect and impair alveolar macrophages. Of note, bovine herpesvirus 1 is also thought to increase susceptibility to the effects of *M. haemolytica* by upregulating CD18 expression in neutrophils.[3]

As mentioned by the contributor, there is some slide variation with respect to the presence of viral syncytia. In a few sections, participants noted viral syncytia in bronchioles with multifocal bronchiolar epithelial necrosis, consistent with bronchointerstitial pneumonia due to BRSV infection. Additionally, lymphatic vessel lumina within interlobular septa are multifocally occluded by coagula of fibrin and mixed inflammatory cells. There are rare intravascular megakaryocytes in some sections, which participants speculated may be associated with terminal bone marrow hypoxia. Intravascular megakaryocytes in

the human lung were first described in 1893, and it has since been demonstrated that under normal conditions, intact megakaryocytes leave the bone marrow and reach the pulmonary capillary bed, where they release platelets by fragmentation of their cytoplasm.[9]

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CASE IV: UFSM-1 (AFIP 3134867).

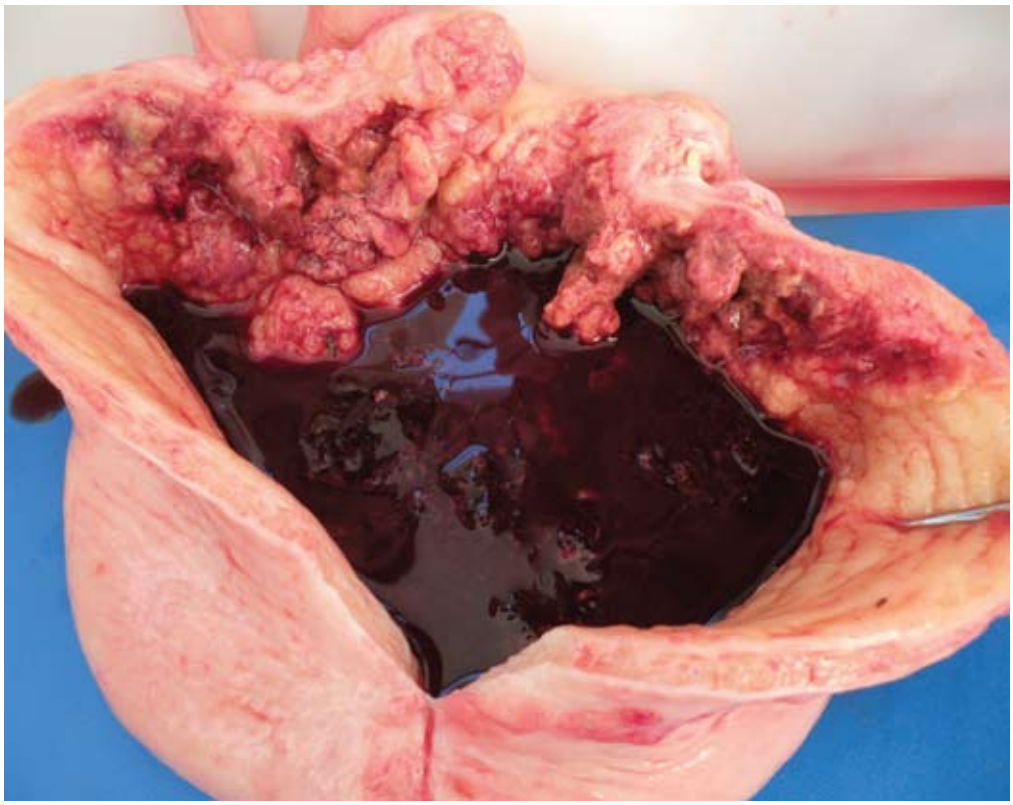
Signalment: 7-year-old, female, mixed breed ox (*Bos taurus*).

History: This cow had chronic intermittent hematuria, anorexia, and marked weight loss over the last several weeks. During the three days before its death it assumed a lateral recumbency.

Gross Pathology: The cow was in poor body condition. There was pallor to the external mucous membranes. The urinary bladder was distended by and full with urine admixed with blood and blood clots. The bladder wall was approximately 1.5 cm in thickness and in the ostium of the ureters there was an ulcerated area of 19 x 6 cm covered with red-brown fibrin. There were also two 2.5 x 1.5 x 3 cm ulcerated areas and several 0.5 cm in diameter nodules randomly distributed throughout the vesical mucosa, but mainly localized around the ulcerated areas (**fig. 4-1**). The ureters were distended (hydroureter). The left kidney was 24 x 14 x 68 cm, had a thick and adhered capsule and multifocal irregular white-yellowish areas covered by pus and fibrin could be observed throughout the capsular surface. At cut surface pyelonephritis was evident. Hydronephrosis, pyelonephritis, and hydroureter could be observed in the contralateral kidney, where these lesions were less marked.

Histopathologic Description: There are areas of hyperplasia of the lining urothelium with clusters of transitional epithelial cells in the lamina propria (Brunn's nests). Some nests are solid and others have central empty spaces. Underneath the areas of hyperplastic urothelium there are developed acini and tubules lined by well differentiated columnar epithelial and goblet cells (glandular metaplasia = cystitis glandularis). From the lamina propria to the muscle layer there is a proliferation of neoplastic epithelial cells arranged as islands, ribbons, small clusters, or as isolated individual cells (**fig. 4-2**). There is necrosis, mainly at the center of several islands. The neoplastic cells are large, polyhedal, with eosinophilic cytoplasm and open faced and markedly pleomorphic nuclei; the nuclear chromatin is either finely loose or clumped and there are 1-2 mitoses per high power field (**fig. 4-3**). Several neoplastic emboli can be observed within lymphatic vessels and moderate desmoplasia is apparent (**fig. 4-4**).

Contributor's Morphologic Diagnosis: 1. Urinary bladder, nonpapillary and infiltrating transitional cell carcinoma; Etiology: chronic ingestion of *Pteridium aquilinum*.
2. Kidney, pyelonephritis, chronic, suppurative, severe (slides not included).
3. Kidney, diffuse cortical atrophy (slides not included).



4-1. Urinary bladder, ox. The urinary bladder is distended and filled with urine admixed with blood and blood clots. The bladder wall is multifocally thickened, ulcerated, or covered with red-brown fibrin. There are several variably sized nodules which protrude into the bladder lumen which are both concentrated around areas of ulceration and randomly distributed throughout the mucosa. Photograph courtesy of Universidade Federal de Santa Maria, Departamento de Patologia, 97105-900, Santa Maria, RS, Brazil, claudioslbarros@uol.com.br.

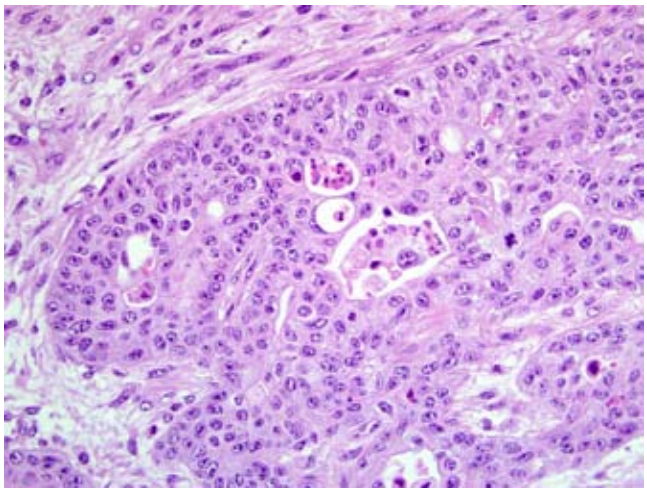
Contributor's Comment: Bracken fern (*Pteridium aquilinum*) is the second most important plant poisoning in southern Brazil. It is responsible for 12% of all deaths of cattle caused by poisonous plants in this region.[13] The ingestion of bracken fern results in three forms of clinical disease in cattle, one acute and two chronic. The acute form occurs when cattle ingest amounts equal to or above 10 grams per kilogram of body weight during 2-11 weeks. Clinical signs associated with acute form of bracken fern poisoning in cattle include high fever, severe hemorrhages in various tissues, and neutropenia and thrombocytopenia due to bone marrow aplasia [1] (please see also WSC 2008-2009, Conference 19, case I).

Chronic forms of disease are characterized by development of neoplasms: 1) squamous cell carcinomas in the upper digestive tract (base of tongue, esophagus and entrance of the rumen) [15] (please see also WSC 2004-2005, Conference 12, case IV) and 2) several types of the neoplasms in the urinary bladder.[5,12] This latter condition, the one of this report, is associated with bleeding from the urinary bladder and is universally known as bovine enzootic hematuria (BEH). Ptaquiloside is reportedly the active toxic principle in bracken fern; however, as a complexing factor, bovine papillomavirus type 4 (BPV-4) is frequently associated with bracken-induced tumors of upper digestive system [4] and bovine papillomavirus type 2 (BPV-2) is frequently associated with bracken-induced tumors of the urinary bladder.[3,5]

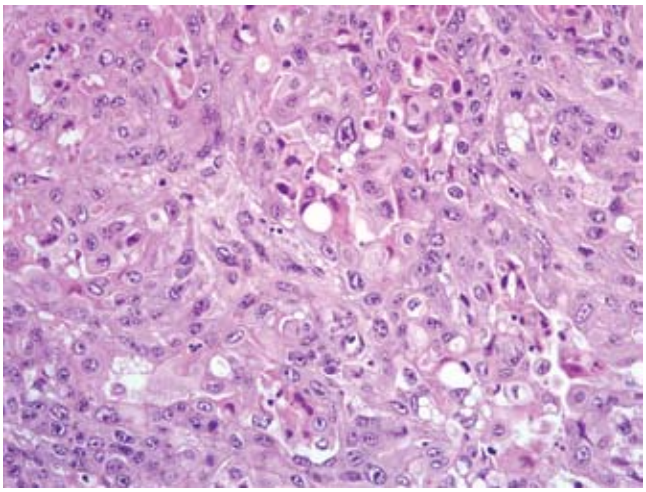
The prevalence of BEH varies depending on the region. Morbidity rates could be up to 10% and lethality rates are 100%.[8] BEH is observed in adult cattle and the most frequent clinical signs include intermittent or continuous hematuria, weight loss, pale mucous membranes, and anemia. In lactating cows there is a severe drop in milk yield.[16] Less frequently urinary incontinency, polyuria and dysuria are seen. The amount of daily loss of blood with urine varies from 0.01 to 1.0 L [5] and results in chronic anemia; in some cases a mass or thickening of the wall can be felt in the urinary bladder by rectal palpation. Cases of BEH can go through intervals of weeks and even months in which clinical signs become inapparent, but reccurrences are the rule and death of affected animals will occur months or even up to one year after the onset of clinical signs.[7]

Due to the ureteral obstruction caused by tumors, hydronephrosis, hydroureter and pyelonephritis are common complications observed in BEH. Laboratory findings in cattle affected by BEH include lymphocytosis, neutropenia, progressive anemia, decreased albuminemia, increased globulinemia, decreased calcium and phosphorous serum levels and increased serum activities of creatinine kinase and alkaline phosphatase.[7]

Gross findings in the urinary bladder of BEH affected cattle include dilatation of the bladder, with thickening of the wall and urine admixed with blood and blood clots. In the bladder mucosa there is congestion, markedly conspicuous vascularization, hemorrhagic foci, vascular growths,



4-2. Urinary bladder, ox. Malignant neoplastic transitional epithelial cells are arranged in islands, nests, and occasional papillary projections supported by a moderate to dense fibrous stroma (desmoplasia). The mitotic rate is high, with bizarre mitoses. Multifocally there is necrosis of neoplastic cells. (HE 400X)



4-3. Urinary bladder, ox. Neoplastic cells have generally distinct cell borders and moderate to abundant eosinophilic cytoplasm. Multifocally neoplastic cells contain a large clear cytoplasmic vacuole which peripheralizes and compresses the nucleus (signet ring cells). (HE 400X)

cauliflower-resembling nodules, red, white or yellow masses, pedunculated or sessile, frequently multilobulated and ulcerated and multiple translucent nodules arranged as bunches of grapes.[7,16]

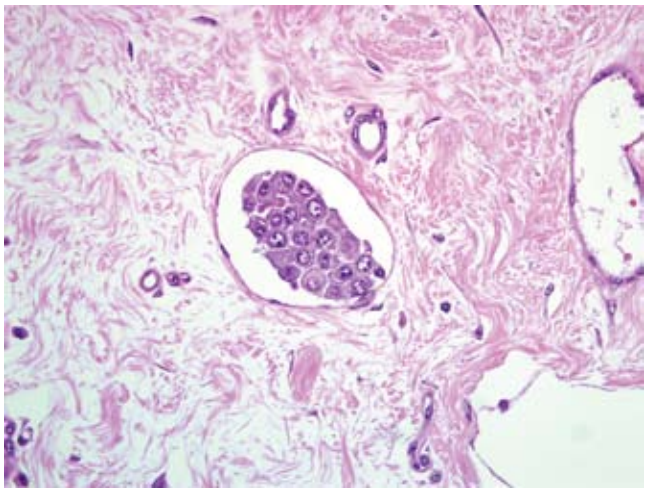
Histologic examination of the bladder tumors associated with BEH reveals a large spectrum of tumor types, both of epithelial and mesencyhmal origin [7,12] and although mestastases are rare they are known to occur mainly to the lumboaortic lymph node.[5] In some cases the hematuria is not associated with the presence of neoplastic lesions, and the only lesions observed in the bladder mucosa are congestion and blood vessel ectasia.[7]

Other species, like sheep and horses, might be poisoned by *Pteridium* spp. Sheep, although much less frequently, are also affected by enzootic hematuria. In Australia the disease in sheep was attributed to the ingestion of *P. esculentum*. Changes in the bladder epithelium were metaplastic and neoplastic (carcinomas).[9] The acute hemorrhagic form of the disease, similarly to the one described in cattle, is also known to occur occasionally in sheep.[1] Three other conditions, namely intestinal tumors, the so called "bright blindness" and polioencephalomalacia are described in sheep that consume *P. aquilinum*.[10] Bright blindness is a form of retinal degeneration in which rods and cones are almost completely destroyed. Polioencephalomalacia is reportedly caused by the high thiaminase I contained in the plant.[10] Polioencephalomalacia has been reproduced in sheep by the feeding of bracken fern. A form of neurological disease due to the thiaminase action is also described in horses but the evidences for this disease are less convincing than the other forms of intoxication associated to this plant. Reportedly neurointoxication of the horse occurs more frequently in stabled horses receiving fodder contaminated with bracken fern, with clinical signs occurring 3-6 weeks after continuous exposure to the plant. Although the disease has been termed polioencephalomalacia, noteworthy pathological changes have not been described associated to presumptive bracken poisoning in horses. Horses fed for more than two months with dry bracken fern had no clinical or pathological findings in southern Brazil (unpublished data).

AFIP Diagnosis: Urinary bladder: Transitional cell carcinoma, nonpapillary and infiltrating.

Conference Comment: The contributor provides a complete review of bracken fern toxicosis, and in a well-constructed description of this neoplasm, mentions the presence of necrosis, frequently at the center of islands of neoplastic transitional epithelial cells. This histomorphologic feature, often referred to as the "comedo pattern," is frequently seen in transitional cell carcinoma (TCC). Additional features typical of TCC, including signet ring cells and desmoplasia, were also noted in several sections by conference participants.

Roperto et al [14] recently classified 400 bovine urothelial tumors in accordance with the World Health Organization morphological classification for human urothelial tumors, which is based on four distinct patterns of growth: flat, exophytic or papillary, endophytic, and invasive. In this series, urothelial tumors were usually multiple, and among flat urothelial lesions, carcinoma in situ (CIS) was the most common, accounting for 4% of the series, and occurring adjacent to papillary and invasive neoplasms in 80-90% of examined cases. The authors preferred the use of the term "urothelial CIS" to such synonyms as "non-papillary and non-infiltrating TCC"; urothelial CIS is characterized by marked local aggressive behavior, formation of von Brunn's



4-4. Urinary bladder, ox. Lymphatic vessels in the submucosa occasionally contain neoplastic cells. (HE 400X)

nests, pleomorphism, and frequent microinvasion into the lamina propria.[14] In the present case, participants noted CIS adjacent to the invasive neoplasm in many sections. In the same series, low-grade papillary urothelial carcinoma, consisting of papillary fronds lined by variably orderly urothelium, was the most common urothelial tumor recorded. Invasive urothelial tumors, most of which were high-grade carcinomas as with the present case, were less common than papillary urothelial neoplasms.[14]

Ptaquiloside is the major carcinogen in bracken fern. In alkaline environments, such as the urinary bladder or ileum of cattle, ptaquiloside is activated to its unstable reactive dienone form, which induces DNA alkylation.[14] The plant also contains thiaminase, tannins, phenolic acid, and several other carcinogens (e.g. quercetin, ptaquiloside Z, aquilide A, shikimic acid, and prunasin).[2,14] Bracken fern is also of significant public health interest in certain geographic locations due to epidemiological studies linking bracken fern consumption and cancer, either directly or indirectly through the milk of animals grazing on the plant or contaminated water.[14]

The contributor mentioned the role of BPV-2 and BPV-4 in association with bracken fern-induced tumors of the urinary bladder and upper digestive system, respectively. BPV-2 also is associated with cutaneous papillomas and fibropapillomas of the skin, esophagus, esophageal groove, and rumen.[2] The only BPVs known to infect the bladder urothelium, BPV-1 and BPV-2 are capable of infecting mesenchymal tissues and exhibiting cross-species transmission, and encode three oncoproteins (i.e. E5, E6, and E7) that initiate cellular transformation by a variety of mechanisms.[14]

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