CASE I: A (IPC 4048645).

Signalment: 9-week-old mixed sex commercial meat rabbits, *Oryctolagus cuniculus*.

History: Some members of this group of 9-week-old rabbits were being raised on the barn floor because of insufficient numbers of cages. The rabbits raised on the floor were bedded on shavings. The bedding was changed weekly, and the floor was limed before applying new shavings. Only the rabbits being raised on the floor were dying. The rabbits were initially treated with amprolium for two weeks because of bloody diarrhea but the rabbits developed a bloated appearance, and so were retreated with amprolium for an additional two weeks. Two days following the last treatment, three rabbits were submitted for postmortem examination.
Gross Pathology: The rabbits were in very poor body condition with reduction in muscle mass and marked reduction in external and internal fat stores. Similar internal changes were noted for each. There was increased clear fluid in the abdominal cavity. Stomachs contained feed and the small intestine, cecum and sacculated colon had normal contents. The distal colon and rectum contained normal fecal pellets. The liver was dark red, enlarged with an irregular bosselated capsular surface and large numbers of variably sized, pale, cystic, round to elongate corded nodules filled with turbid pale green yellow fluid were scattered throughout the hepatic parenchyma. The gallbladder was thickened and contained similar cloudy fluid.

Laboratory Results: Parasitology: Oocysts with measurements consistent with *Eimeria stiedae* were identified in the biliary fluid collected from the cystic hepatic lesions. Fecal flotation revealed large numbers of a mixture of *Eimeria* spp. oocysts.

Histopathologic Description: Liver: There is generalized marked dilation of bile ducts causing compression of the surrounding hepatic parenchyma. Hyperplastic biliary epithelium forms papillary projections into duct lumens. Most epithelial cells are filled with asexual and sexual developmental stages of coccidial organisms and cystic duct lumina contain numerous oocysts. Few to moderate numbers of plasma cells and lymphocytes infiltrate within increased periductal fibrous tissue and within the connective tissue stroma of the proliferative biliary epithelium. Other portal tracts have mild cholangiolar proliferation, mildly increased periportal fibrosis and few to moderate numbers of mixed mononuclear cells within and around biliary epithelium. Occasional portal and hepatic veins contain low numbers of oocysts. Hepatocyte cords are markedly atrophied, and sinusoids are moderately congested and contain increased numbers of circulating neutrophils.

Contributor’s Morphologic Diagnosis: 1. Marked proliferative and nonsuppurative cholangitis with large numbers of intralobular coccidial organisms. 2. Marked hepatic atrophy. 3. Ascites.

Contributor’s Comment: While enteric coccidiosis continues to be one of the important potential causes of enteritis in commercial rabbitries in Ontario, hepatic coccidiosis is seldom identified in commercially raised rabbits submitted to our laboratory for diagnostic workup.
of diarrhea. However, increasingly over the last few years, smaller collections of rabbits raised for meat or as pets are being housed on or provided access to the ground. Owners of these small rabbitries seek veterinary assistance for morbidity and mortality concerns and in turn, the diagnostic laboratory has received increased numbers of phone calls and pathology submissions from veterinary practitioners related to the diagnosis of hepatic coccidiosis in these rabbits.

Infection with *Eimeria stiedae* is usually subclinical and historically, hepatic coccidiosis has been associated with significant condemnations of livers from meat-type rabbits at processing but not with elevated mortality during grow-out. However, if young, naïve rabbits are exposed to high enough levels of sporulated oocysts, clinical disease including anorexia, poor weight gain, weight loss, development of a distended abdomens, diarrhea and elevated mortality can occur. In this particular situation, only the rabbits being reared on the floor were clinically affected and although the rabbits submitted for postmortem did not have diarrhea, histologically, they also had significant numbers of coccidial organisms within the intestinal mucosa and results of flotations conducted on feces from all rabbits indicated large numbers of mixed *Eimeria* spp. oocysts. Despite weekly cleaning and rebedding, the environment was heavily contaminated with coccidial oocysts and these rabbits were being continually challenged with both intestinal and hepatic coccidia.

Recommendations for the control of hepatic coccidiosis parallel the recommendations for control of intestinal coccidiosis, and sanitation is of great importance. Coccidial oocysts are extremely resistant to environmental influences and no commonly available disinfectants will kill them. Removal of organic material from cages, feed pans and around waterers where oocysts can reside can help reduce the challenge. Rabbits can develop long-lasting immunity to *Eimeria stiedae* as long as they are not exposed to an excessively high dose initially and are immunocompetent.

With the advent of increased interest in raising small groups of rabbits and allowing them access to the ground, hepatic coccidiosis may re-emerge as a clinical disease.

**JPC Diagnosis:** Liver: Cholangitis, proliferative, multifocal to coalescing, chronic, severe, with intra-epithelial coccidia.

**Conference Comment:** There are over a thousand species of *Eimeria*, the vast majority of which are known to primarily infect the epithelial cells lining the gastrointestinal tract. Hepatic coccidiosis is commonly reported in rabbits, known to occur in ferrets (see WSC 2009-2010, Conference 12, Case 2), and reported in some avian species which acquire extra-intestinal coccidiosis to include the liver and kidneys, albeit infrequently. Each species has a host specific direct life cycle which originates with the unsporulated oocyst shed in the feces.
The oocyst initially contains a single cell called a sporont, and through a process known as sporulation or sporogony, the sporont develops into four sporocysts, each with two sporozoites. This appearance of the sporulated oocyst (4 sporocysts and 8 sporozoites) is a distinguishing feature of *Eimeria* spp. from other coccidian apicomplexans. The sporulated oocyst is now infectious, and sporozoites will be released when ingested by the host. The sporozoites invade epithelial cells and round up to become trophozoites which are the growing forms. Trophozoites undergo asexual nuclear division, called both schizogony and merogony, to form a schizont/meront. These two terms are used synonymously in most references of this stage, with the term schizogony referring to multiple nuclear divisions (as occurs in *Eimeria* spp. while merogony equates with nonspecific asexual division. Each schizont develops within it numerous merozoites, which following rupture of the host epithelial cell, are released to infect additional cells and subsequently develop into additional schizonts. This process repeats over a variable number of generations depending on the individual species. For reasons still unknown, eventually some merozoites will infect epithelial cells and begin the sexual phase of the life cycle called gametogony. The majority become females (macrogametocytes), while some become males (microgametocytes) which form numerous biflagellate microgametes within epithelial cells. Each microgamete can fertilize a macrogamete to form a zygote known as the oocyst, which are then shed in the feces. Among the phases of this elaborate life cycle, conference participants identified oocysts, schizonts containing merozoites, micro- and macrogametocytes, and micro- and macrogametes in abundance in this case.

In hepatic coccidiosis, this life cycle still begins within gastrointestinal epithelial cells, particularly in the duodenum. Following exposure, sporozoites have been documented in the regional lymph nodes within 12 hours, in bone marrow...
within 24 hours, and in the liver within 48 hours. Thus, spread by both hematogenous and lymphatic routes have been proposed. Additionally, the organism may infect mononuclear cells which aid in their dissemination. Following gametogony in the biliary epithelium, the oocysts are shed in the bile and passed to the intestines. Numerous oocysts are present in multiple blood vessels in many sections, a finding most participants attributed to an artifact of processing.

_Eimeria stiedae_ have a characteristic appearance to the oocyst, with a thinning of the opercula that distinguishes it from all other coccidians in rabbits. There are eight other species of _Eimeria_ known to infect rabbits and mixed infections are common, as demonstrated by the fecal results obtained in this case.

**Intestinal Coccidia of Rabbits**

_E. flavescens_
_E. magna_
_E. media_
_E. intestinalis_
_E. irresidua_
_E. perforans_
_E. piriformis_
_E. neoleporis_

**Contributing Institution:** Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada. http://ahl.uoguelph.ca

**References:**
CASE II: B (JPC 4048646).

Signalment: 1.5-year-old female New Zealand white rabbit, *Oryctolagus cuniculus*.

History: In August of 2007, a pet rabbit was presented to an Ontario veterinary clinic with a 3-day history of lethargy, anorexia and facial swelling. The affected animal was one of a group of six housed in a large hutch on a grassy enclosure surrounded by a chain link fence. Chipmunks, other small mammals and occasionally birds were seen within the enclosure on a number of occasions and direct contact with other forms of wildlife could occur across the fence; two dogs also resided on the property.

During the examination, the rabbit was noted to be tachypneic. A 1-cm crust was noted on the nasal planum and skin over the right nares and upper lip was swollen. Excessive waxy debris was present in the left ear canal.

A presumptive diagnosis of *Pasteurella multocida*-induced pneumonia was made and oral antibiotic therapy (chloramphenicol palmitate) was prescribed and initiated. Mineral oil was infused in the left ear and when the luminal debris was removed, mild bleeding and ulceration of the epithelium was noted. Antibiotic ointment was instilled into the ear canal and the animal was discharged. Approximately 30 minutes after leaving the clinic, the owner reported that the animal began choking and bleeding from the nose. Shortly afterwards, the rabbit died and the body was submitted for postmortem examination.

Gross Pathology: The rabbit was in good body condition with abundant internal fat stores. Blood stained the inside of the left pinna and a blood clot was present at the base of the ear canal. A 1 cm round raised reddened area was noted on the nasal planum. Blood stained the perinasal and dewlap fur. The tracheal mucosa was mildly congested and there was generalized purple red mottling of the lungs with numerous, up to 3 mm, foci of hemorrhage on the pleural surface and in the parenchyma. Within the abdomen, the spleen was enlarged and congested. There was transmural reddening of the caudal 2 cm of the ileum and the lumen contained a blood clot.

Laboratory Results: Low numbers of *E. coli* were isolated from the lung. An alphaherpesvirus isolated from the lung and skin by cell culture was further identified by electron microscopy and

2-1. Lung, rabbit (fixed specimen): There are multifocal mottled areas of hemorrhage scattered randomly through the parenchyma. (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada; http://ahl.uoguelph.ca)
ribonucleotide reductase gene sequencing as *leporid alphaherpesvirus*4 (LHV-4).1

**Histopathologic Description:** There is marked generalized acute necrotizing and hemorrhagic bronchopneumonia with segmental bronchial epithelial proliferation and necrosis, large areas of alveolar necrosis and hemorrhage and flooding of alveoli by edema fluid, fibrin, heterophils and large macrophages. Numerous bronchiolar epithelial cells, bronchiolar epithelial syncytia, pneumocytes, endothelial cells and macrophages contain prominent glassy eosinophilic intranuclear viral inclusions. Several vessels have perivascular hemorrhage, edema, mural fibrinous necrosis, vasculitis and thrombosis. There is patchy alveolar overinflation and emphysema.

**Contributor’s Morphologic Diagnosis:** Marked acute generalized necrotizing and hemorrhagic bronchopneumonia with syncytia containing numerous eosinophilic intranuclear viral inclusions.

**Contributor’s Comment:** In the early 1990s, two herpes-virus like outbreaks with high mortality characterized by ulcerative dermatitis, pneumonia, splenic necrosis, and gastrointestinal hemorrhage were reported from commercial rabbitries in Alberta4,6 and British Columbia.4 In both cases, herpes-like viral particles were identified in formalin-fixed, paraffin-embedded tissue sections and herpesvirus was isolated from affected tissues, but neither isolate was further characterized. However, the disease was experimentally reproduced in meat-type rabbits using one of the isolates. In the summer of 2006, a commercial pet and agricultural rabbitry in Alaska also reported high morbidity and mortality associated with systemic herpesvirus infection.3 Rabbits were housed outside in open-sided hutches, where mosquito and biting fly activity was high. Snowshoe hares were present in the surrounding area and feral domestic rabbits had been in close proximity to the hutches earlier in the spring. In the following spring and summer, several rabbits from this same rabbitry developed conjunctivitis and skin lesions; and one breeding rabbit that had recovered from clinical infection in the previous year experienced perinatal mortality. The herpesvirus was isolated and characterized as leporid herpesvirus-4.2

The case presented here is the first documented and characterized case of leporid herpesvirus-4 infection in a pet Canadian rabbit.1 This viral disease should be included in the list of differential diagnoses for acute morbidity and mortality in domestic rabbits presenting with epistaxis or respiratory distress, which presently includes rabbit hemorrhagic disease virus (RHDV), peracute *Pasteurella multocida* septicemia or chronic *Pasteurella multocida* pleural/pulmonary abscessation with rupture.

**JPC Diagnosis:** Lung: Pneumonia, bronchointerstitial, necrohemorrhagic, multifocal to coalescing, acute, severe, with intranuclear viral inclusions and syncytia.
Conference Comment: There are four known herpesviruses of rabbits, two of which are gamma herpesviruses that produce lymphoproliferative disease and neoplasia (Leporid herpesvirus 1 and Leporid herpesvirus 3). Leporid herpesvirus 2 is also a gammaherpesvirus, but is capable of inducing encephalitis. Additionally, natural infections of Human herpesvirus 1 (herpes simplex) have been reported in rabbits causing a fatal encephalitis. The virus demonstrated in this case, Leporid herpesvirus 4 (LHV4), is a novel herpesvirus with rare reports in the literature as the contributor highlighted.

LHV-4 is classified as an alphaherpesvirus on the basis of its rapid growth and cytopathic effect in cell culture. This case illustrates the severity of bronchopneumonia which results from infection and leads to the reported 50% morbidity and 29% mortality rates. Additionally, ulcerative rhinitis and splenic necrosis has been observed in experimental infections, and hemorrhagic dermatitis and myocarditis in natural infections.

Contributing Institution: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada; http://ahl.uoguelph.ca

References:


CASE III: 13-1079 (JPC 4031940).

Signalment: 2-year-old female rabbit, *Oryctolagus cuniculus*.

History: There is a ten-day history of increasing bloody discharge from the vulva. Abdominal palpation and abdominal radiographs revealed an enlarged uterus. An ovariohysterectomy was performed and the uterus was submitted.

Gross Pathology: Uterus was received in formalin. Uterine horns are enlarged and filled with brown to black watery fluid and blood clots.

Laboratory Results: The PCV before surgery was 14%.

Histopathologic Description: Uterus: A blood vessel in the endometrium is markedly dilated and filled with blood, laminated fibrin, and neutrophils mixed with karyorrhectic debris (thrombus). The endometrium overlying this area is thin with only 1 layer of low cuboidal cells and 2 to 3 layers of collagen separating it from the endometrium of the dilated vessel. The uterine lumen is markedly compressed by the aneurysmal vessels. In one section, the thrombus is adhered to the wall of the vessel (will vary with section). There are hemosiderin laden macrophages focally in the adjacent endometrium (in some sections).

Contributor’s Morphologic Diagnosis: Uterus: Endometrial venous aneurysms.

Contributor’s Comment: Hematuria in rabbits has been associated with uterine adenocarcinoma, uterine polyps, renal infarction, urolithiasis, cystitis, bladder polyps, pyelonephritis and uterine endometrial venous aneurysms. The most common clinical signs with venous aneurysms are hematuria or urogenital bleeding. Occasionally, the aneurysms are associated with mild anemia and proteinuria. Varices and aneurysms of uterine subserosal and myometrial venous plexi, but not of endometrial vessels, have been reported in women. Similar endometrial aneurysms have been seen in rats and mice. In rabbits, at necropsy, clotted blood may be found within the uterine...
Non-pregnant multiparous does are most affected. The defect is likely congenital. JPC Diagnosis: Uterine horn: Endometrial venous aneurysm, with thrombosis.

Conference Comment: In rare sections in this case, the origin of the venous aneurysm is visible within the vessel wall. By definition, an aneurysm is a localized dilatation of a vessel due to widening of the lumen which causes an abnormal attenuated vascular wall. In normal branching vessels, the elastic fibers contract but are continuous when the area of branching is cut into histologic section. In this case, when present, a small vessel empties into this large aneurysm and the elastic fibers are lost at this point of transition, enabling participants to definitively identify it as the aneurysmal origin. Aneurysms should be contrasted with false aneurysms or dissections, which are a defect in the vascular wall leading to an extravascular hematoma. Both aneurysms and dissections can rupture, often with catastrophic consequences.

Aneurysms occur when the structure or function of the connective tissue within the vascular wall is compromised, often during the continuous remodeling process it undergoes to maintain structural integrity. These defects can occur as a congenital condition or be acquired over time with progressive weakening of the wall. Increased expression of matrix metalloproteases (MMPs) and decreased expression of tissue inhibitors of metalloproteinases (TIMPs) often contributes to this degradation. MMPs, which require metal ions such as zinc for their activity, are instrumental in the process of remodeling the extracellular matrix, to include vascular remodeling. Additionally, MMPs play a prominent role in tumorigenesis due to their prominent role in cell turnover and migration, and regulating signal pathways of cell growth, inflammation and angiogenesis leading to a large body of research on these proteinases. Specific to angiogenesis, MMP-2 and MMP-9 are cited as both pro and anti-angiogenic while MMP-1, MMP-7, and MMP-14 are specifically pro- while MMP-12 is anti-angiogenic. MMPs are produced by a variety of cell types and regulated by growth factor and cytokine secretion. Their activity is...
tightly controlled as they are rapidly depleted by TIMPs produced by most mesenchymal cells.\textsuperscript{5} When this MMP/TIMP balance is altered, such as occurs with inflammation in atherosclerosis or vasculitis, the risk of aneurysm formation increases.\textsuperscript{6}

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**References:**

**CASE IV:** N2013-1019 (JPC 4048674).

**Signalment:** Adult male chinchilla, *Chinchilla lanigera.*

**History:** The chinchilla was found dead with no reported premonitory signs. This animal was one of seven chinchillas submitted for necropsy during an approximately 3-week period that were either noted to exhibit respiratory distress and tachypnea or found dead without premonitory signs.

**Gross Pathology:** The caudal portion of the right cranial lung lobe was firm and mottled red to tan. Small amounts of soft to gelatinous, pale tan material were adhered to the pleural surface of the affected lung lobe. All lung lobes oozed a small volume of clear fluid on section.

**Laboratory Results:** Aerobic bacterial culture of the lung yielded the growth of many *Bordetella bronchiseptica*; *B. bronchiseptica* was also identified in three additional chinchillas with similar gross and histologic lesions. *Mycoplasma* culture and pneumovirus PCR were negative.

**Histopathologic Description:** In the most severely affected sections, 50-75% of airways and alveolar spaces are multifocally obscured and expanded by large numbers of heterophils that are often degenerate with poorly demarcated, round to streaming nuclei (oat cells), macrophages, small numbers of lymphocytes and plasma cells, aggregates of homogenous, eosinophilic material (fibrin), wispy to granular eosinophilic material (fibrin and proteinaceous fluid), and variable amounts of cellular and karyorrhectic debris (necrosis). Large numbers of small, <1 x 2 µm coccobacilli form dense intra- and extracellular colonies. Similar inflammation and necrosis also obscures and replaces alveolar septa. Bronchial and bronchiolar epithelial cells are often hypertrophic (reactive) with occasional areas of attenuation and infrequent piling of cells (hyperplasia). There are multifocal areas of acute alveolar hemorrhage and erythrophagocytosis. The adventitia surrounding pulmonary vessels and airways is often expanded by clear space with wispy, eosinophilic material and small numbers of heterophils, macrophages, lymphocytes and plasma cells. The alveolar spaces surrounding areas of intense inflammation contain copious amounts of wispy, eosinophilic material (edema) and moderate numbers of macrophages with foamy cytoplasm and fewer heterophils and lymphocytes. Moderate amounts of fibrin with small numbers of associated heterophils, macrophages and lymphocytes are adhered to the pleural surface multifocally; the underlying pleura is lined by plump, reactive mesothelial cells. In less severely affected sections, small to moderate numbers of heterophils infiltrate the bronchial and bronchiolar mucosa and are associated with small amounts of fibrin and edema. Alveolar septa are multifocally fragmented with the formation of large alveolar spaces (emphysema). Small numbers of lymphocytes, plasma cells and heterophils mildly expand perivascular spaces.

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4-1. Lung, chinchilla: The right lung lobes were firm and mottled red to tan. (Photo courtesy of: Wildlife Conservation Society, Zoological Health Program, Bronx, NY. www.wcs.org

4-2. Lung, chinchilla: The filling of airways with exuberant suppurative exudate clearly defines this pattern as a bronchopneumonia. (HE 9X)
**Contributor's Morphologic Diagnosis:** Lung: Bronchopneumonia, necrosuppurative, fibrinous, acute, severe with oat cells, pulmonary edema, alveolar hemorrhage, fibrinous pleuritis and intralesional, intra- and extracellular coccobacilli colonies.

Nasal cavity (tissue not submitted): Rhinitis, mucopurulent, diffuse, moderate, subacute with intralesional, intra- and extracellular coccobacilli colonies and multifocal, mild mucosal erosion and ulceration.

**Contributor's Comment:** Gross, histologic and ancillary findings were consistent with a diagnosis of pulmonary and upper respiratory bordetellosis. *Bordetella bronchiseptica* was isolated from samples of lung for all chinchillas (*n*=4) that were submitted for aerobic bacterial culture, and all 7 chinchillas showed similar gross and histologic features. While *B. bronchiseptica* can commonly be a secondary, opportunistic pathogen, as lung samples were negative for consistent growth of other significant bacteria, *Bordetella* is believed to be the primary cause of disease in these chinchillas. Other significant associated findings in the affected chinchillas included rhinitis (*n*=3), tracheitis (*n*=2) and otitis media (*n*=1). Though not present in the submitted case, affected chinchillas with a more prolonged disease period demonstrated marked pneumocyte hyperplasia and hypertrophy; lung samples were negative for pneumovirus by quantitative PCR.

In small mammals, respiratory bordetellosis is most commonly reported in guinea pigs and rabbits; however, outbreaks in commercial chinchilla operations have been documented. The contributor has also observed similar histologic changes, including the presence of prominent colonies of coccobacilli, in wild eastern gray squirrels (*Sciureus carolinensis*) with pulmonary bordetellosis. Other species in which *B. bronchiseptica* can be a significant pathogen include dogs (infectious tracheobronchitis), cats (tracheitis and bronchopneumonia) and pigs (atrophic rhinitis and neonatal pneumonia).

Like *Mannheimia* and *Actinobacillus spp.*, *Bordetella bronchiseptica* encodes a pore-forming, “repeat in toxin” (RTX) family virulence factor, adenylate cyclase toxin (ACT). ACT is an essential virulence factor that can: i) disable innate immunoprotective functions, including phagocytosis, chemotaxis, and superoxide production, ii) modulate immune responses through alterations in cytokine secretions, and iii) trigger apoptosis in macrophages. Other important virulence factors of *B. bronchiseptica* include filamentous hemagglutinin (FHA), dermonecrotic toxin (DNT), tracheal cytotoxin, osteotoxin, fimbriae and a Type-III secretion system.

**JPC Diagnosis:** Lung: Pleuropneumonia, necrotizing and fibrinosuppurative, acute, multifocal to coalescing, severe, with numerous bacilli.
Conference Comment: This is a great descriptive case with an impressive abundance of fibrin within most alveolar airways and, in some sections, causing a thickening of the pleura. *Bordetella bronchiseptica* has an array of virulence factors as explained by the contributor, yet in many domestic animal species it is a known commensal organism of the upper respiratory tract. Its presence is not typically equitable with clinical disease unless an immune compromising event takes place in the host. Of particular importance in laboratory species is its ability to take refuge within clinically normal rabbits, and subsequently spread to and wreak havoc in guinea pigs if housed in close proximity. Pulmonary lesions are typically supplicative in most species, though fibrinous exudation within alveoli has been described in acute cases. Fibrinous bronchopneumonias or pleuropneumonias are the result of severe pulmonary injury and, thus cause death earlier in the sequence of the inflammatory process than supplicative pneumonias. Dramatic clinical signs and death can occur even in cases involving only 30% or less of the total pulmonary surface area. Although lesions in this case had areas of suppurative inflammation, fibrin predominated and this finding correlates with the rapid onset and death provided in the case history.


References: