The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2004-2005

CONFERENCE 3
22 September 2004

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CASE I – CRL2 (AFIP 2936451)

Signalment: Female, 4-month-old, RAG1 mouse (*Mus musculus*).

History: Formalin-fixed tissues from a 4-month-old female RAG1 mouse were submitted for evaluation because of increased mortality in the colony.

Gross Pathology: None given

Laboratory Results: None

Contributor's Morphologic Diagnoses: Lung: 1. Acidophilic macrophage pneumonia, multifocal, moderate.
2. Intraalveolar *Pneumocystis carinii*, multifocal, mild.
3. Bronchopneumonia, subacute, suppurative, multifocal, marked.

Contributor's Comment: In the sections of lung, many alveoli are filled with macrophages, some multinucleated, with prominent intracytoplasmic eosinophilic acicular crystals. Although in this case the crystals are easily visualized on the H&E-stained section, their appearance can be enhanced with Grocott's methenamine silver stain or Gram stain (Figure 1). There is some alveolar septal thickening, type II pneumocyte hyperplasia, and a mild to moderate neutrophilic, histiocytic, and eosinophilic infiltrate in the areas with the acidophilic macrophage pneumonia. Scattered among the foci of acidophilic macrophage pneumonia, as well as in unaffected regions of the lung, is intraalveolar granular eosinophilic extracellular material typical of *Pneumocystis carinii* (Figure 2), with minimal associated inflammation. Bronchi are distended with an exudate of neutrophils and fewer macrophages with multifocal necrosis of epithelium, and extension into the adjacent pulmonary interstitium. On some of the sections, there is a well-developed abscess with mineralization where an airway has been destroyed. A tissue Gram stain demonstrates intralesional gram-negative bacilli (Figure 3).

RAG1 [V(D)J recombination activating protein 1]-deficient mice do not produce mature B or T lymphocytes, and are therefore deficient in both humoral and cell-mediated immunity.[1] Loss of the RAG1 gene prevents rearrangement of both immunoglobulin and T cell receptor genes early in lymphocyte differentiation. RAG1-deficient mice have a greatly increased susceptibility to murine pathogens of all types.

Pneumocystis carinii is an opportunistic fungal pathogen of many species, which can cause fatal pneumonia in immunocompromised individuals. There are two tissue forms of *Pneumocystis carinii* – trophozoites, and cysts containing sporozoites, which can be demonstrated in tissues sections by immunohistochemistry or special stains, such as GMS.[2] This case is typical of the minimal inflammation associated with *Pneumocystis carinii* pneumonia in an immunodeficient mouse.

Acidophilic macrophage pneumonia is characterized by alveolar accumulations of macrophages containing eosinophilic crystals. The crystals may also be found free in alveoli and in the cytoplasm of airway epithelial cells. The macrophage infiltrates are accompanied by variable numbers of eosinophils, neutrophils and lymphocytes. Acidophilic macrophage pneumonia is often found in association with other pulmonary lesions, such as *Pneumocystis carinii* pneumonia or lung tumors. The development of this lesion is strain and age dependent, with lesions commonly found in nude mice, C57BL/6 or Sv/129, or genetically engineered mice, particularly those generated on a C57BL/6 or Sv/129 background. Ultrastructurally the crystals are indistinguishable from Charcot-Leyden crystals. However, biochemical analysis indicates that they are composed of Ym-1 protein, a member of the chitinase family. This protein is also known as T lymphocyte-derived Eosinophilic Chemotactic Factor (ECF-L).[3,4]

Bacterial culture could not be performed, as the tissues were fixed in formalin. *Pasteurella pneumotropica* has been associated with bronchopneumonia in association with *Pneumocystis carinii* in immunocompromised mice,[2] but other gram negative bacilli are possible etiologies as well.

AFIP Diagnoses: 1. Lung: Pneumonia, acidophilic macrophage, diffuse, mild to marked, RAG1 mouse, rodent.
2. Lung: Bronchopneumonia, suppurative, lobar, severe.
3. Lung: Intra-alveolar fungal organisms, multifocal, etiology consistent with *Pneumocystis murina*.

Conference Comment: Studies have recently show that the *Pneumocystis* found in laboratory mice is phylogenetically distinct from *P. carinii*; it was named *Pneumocystis murina* sp. nov., and was formerly known as *Pneumocystis carinii* f. sp. *muris*.[5]

The first transgenic (introduction of ectopic DNA) mouse was created in 1980 by random insertion, a process that remains common today. However, as technology has

evolved, transgenes are now often targeted to specific sites on the genome for either gain of function (knock in) or loss of function (knockout or null mice). Gene alteration, both random and targeted, can lead to unexpected phenotypes, severe immunodeficiency, and embryonic or fetal death.[2]

Primary and opportunistic pathogens often significantly affect transgenic mice, not only because they cause overt disease, but they may also alter the biological responses of the mice to experimental pathogens. The pathologist must be aware of strain-related patterns of pathology in relation to spontaneous and infectious disease, developmental and comparative pathology, and the predicted and unexpected outcomes of gene alteration.[2]

Contributor: Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA 01887 (www.criver.com)

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CASE II – RT04-1470 (AFIP 2937327)

Signalment: 14 week-old, male, Norwegian Rat, (*Rattus norvegicus*), Strain: Sprague-Dawley

History: This 14 week-old rat underwent surgery using an anesthetic combination of pentobarbital, magnesium sulfate and chloral hydrate of an unknown concentration. The anesthetic, "Equithesin", was injected intraperitoneally. The procedure implanted a microdialysis guide cannula in the ventral tegmental area, a region of the mesencephalon. Over the next five days, the rat's weight dropped from 273 grams to 247 grams. Four days after the operation, the animal looked depressed with "ruffled fur". Palpation revealed dilated gut loops, especially in the left ventral abdominal quadrant. Five days following the operation, an eight-hour period passed with no fecal

pellets. The animal was euthanized. The attending veterinarian performed the necropsy and submitted tissue in formalin.

Gross Pathology: Gross pathology findings reported by the clinician were as follows: The animal had very little fat stores and the stomach and duodenum had very little content. The ileum, cecum and colon were dilated and filled with ingesta. Contents of the colon were dry and firm. The dilated portion of digestive tract ended abruptly at an area of adhesion between body wall and colon. The terminal colon and rectum were only moderately dilated. The anterior surface of the liver had round, blunted edges and multiple adhesions to the stomach and diaphragm. The lungs were moderately congested.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Colon: Peritonitis, fibrosing, diffuse, with moderate leiomyositis.

Etiologic Diagnosis: Chloral hydrate induced peritonitis.

Contributor's Comment: Chloral hydrate administered intraperitoneally to rodents can injure abdominal organs. Documented effects include gastric mucosal injuries, adynamic ileus, and peritonitis,[1,2] fibrosis of the serous membranes, and steatitis.[3] A study in 1999 documented a marked decrease in intestinal peristalsis and acetylcholine-induced contractions in rats following intraperitoneal injections of chloral hydrate.[4]

In this case, histopathological changes include fibroplasia of the colonic serosa and muscularis, giving it a pleated form. Individual myofibers of the muscularis externa are necrotic. There is inflammation of the serosa of the colon with neutrophils, lymphocytes, macrophages and plasma cells with small numbers of inflammatory cells extending into the muscularis externa. Hemorrhage is present in muscular layers. Some sections of colon have ulcers. Gastric ulcers often accompany intraperitoneal (IP) injections of chloral hydrate.[2] However, no gastric sections were submitted in this case.

Differential diagnoses included peritonitis secondary to perforating ulcer of the colon, or a needle perforation of the bowel with leakage of contents. A perforating ulcer or other cause of leakage of intestinal contents would be expected to cause more suppurative inflammation with peritoneal effusion and large numbers of intraperitoneal bacteria. Although not used in this case, the anesthetics tribromoethanol (Avertin) and pentobarbital have been reported to produce peritonitis and ileus in rats.[5, 6]

In 1961, The Walter Reed Army Institute of Research reported losing some rats and guinea pigs to ileus and intestinal blockage. They attributed it to a side effect of intraperitoneal injections of chloral hydrate.[1]

A 1977 study reporting chloral hydrate toxicity is commonly cited in literature. The study involved the implantation of brain electrodes into rats. Several rats became ill and died. Symptoms were lethargy, anorexia, abdominal distension, and constipation. After an infectious etiology was ruled out, chloral hydrate was administered in the second leg of the experiment. Of 27 rats, 20 developed typical signs of gastric distress seen in the initial study. Fourteen of the 20 rats died.[7]

Chloral hydrate usage is controversial but it remains widely used in surgical procedures. The drug is often chosen for intra-cranial surgeries because inhalation anesthetics present access problems. Barbiturates often depress respiratory and cardiovascular systems and require long recovery times.[8] Regardless of the drug, intraperitoneal injections in rats are often utilized because of their small muscular mass and relatively inaccessible vasculature.[6]

The pathogenesis of colonic ileus is unclear but it may involve a decrease in parasympathetic activity coupled with an increase in sympathetic inhibition of the colon. The most common cause of paralytic ileus is abdominal surgery. Abdominal trauma, serum electrolyte imbalance, septicemia, and intrathoracic conditions such as pneumonia, myocardial infarction and lower rib fractures have contributed to paralytic ileus. Narcotics, calcium channel blockers, and anticholinergic medications can also cause this condition. Treatment usually involves discontinuing use of narcotic and anticholinergic drugs and correction of electrolyte imbalance.[9]

Chloral hydrate is thought to be a chemical irritant. In a study that tested chloral hydrates' anesthetic effect, 83% of rats that were injected intraperitoneally had mild to moderate inflammation of the abdominal area involving the splenic capsule and peritoneal surface of the body wall.[6] Intestinal inflammation was not reported in this study.

Some studies attribute disease to the concentration of the drug. However, ileus has been reported to occur sporadically even at lower concentrations.[4] Preceding its use with a low dose of barbiturates, opioids, alpha-2 agonists, or phenothiazine tranquilizers has been reported to reduce negative side effects of chloral hydrate.[2] A study in 1995 concluded that "Equithesin" without chloral hydrate is an effective anesthetic that can be maintained for several hours by supplemental doses.[8]

AFIP Diagnosis: Colon, serosa and tunica muscularis: Serosal fibrosis, diffuse, mild, with leiomyocyte degeneration and necrosis, and neutrophilic inflammation, Sprague-Dawley rat, rodent.

Conference Comment: As many pathology residents realize, it is often more difficult to recognize the absence of cells or a structure than the addition of cells or a change in morphology. This is especially true in this case. Many conference participants did not recognize the focally extensive loss of smooth muscle that normally comprises the

external longitudinal layer of the tunica muscularis and replacement by fibrous connective tissue. This histologic feature is highlighted with a Masson's trichrome stain. Of interest to many of the conference participants, is the relative paucity of inflammation. This is somewhat surprising given the extent of myocyte degeneration and necrosis present in most tissue sections and the short clinical history of only five days post injection. Whether these changes are due to species, strain, individual, anesthetic, or inflammatory mediator differences is uncertain. Nonetheless, these findings are consistent with those in published reports.[4,5,6,7]

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CASE III – CF13 (AFIP 2935883)

Signalment: 4.33 year-old male rhesus macaque (*Macaca mulatta*).

History: This animal was one of a group used to evaluate viral dynamics in acute simian immunodeficiency virus (SIV) and recombinant simian-human immunodeficiency virus (SHIV) infection and the role of $CD8^+$ T cells. It was inoculated with SHIV162p3 six months prior to necropsy. Multiple biopsies of peripheral lymph nodes were

performed at determined intervals prior to euthanasia. Due to the development of severe emaciation, weight loss, and dehydration this animal was euthanized.

Gross Pathology: Approximately 10 ml of pale yellowish fluid was present in the peritoneal cavity. Fibrous adhesions of the colon to the abdominal wall were observed in moderate severity. Yellow fluid feces were observed in the colon. The walls of the gallbladder and bile ducts were extremely thickened, and folds of mucosa protruded into the lumina, which contained cloudy bile. The pancreas was slightly firmer than normal. Mesenteric, colonic and iliac lymph nodes were enlarged four times normal size, and the palatine tonsils were prominent.

Laboratory Results: None

Contributor's Morphologic Diagnoses: 1. Gallbladder and liver: Marked diffuse chronic lymphoplasmacytic and suppurative cholecystitis with adenomatous hyperplasia and intralesional protozoal parasites (*Cryptosporidium sp.*).
2. Moderate widespread chronic lymphoplasmacytic and neutrophilic cholangiohepatitis with bile duct hyperplasia.
3. Marked focal intimal myocyte hyperplasia with minimal lymphocytic and neutrophilic phlebitis.

Contributor's Comment: Major bile ducts are markedly expanded by a proliferation of epithelium that occludes the lumen, by cellular inflammatory infiltrates of the lamina propria, and by variable degrees of periductal fibrosis. The epithelium is thrown into deep folds and crypts are lengthened, giving the tissue a multilocular appearance. The tall columnar ductular epithelium has more variability in its height, more prominent nucleoli, and markedly increased mucus production when compared to normal macaque bile duct epithelium. Segmentally, the apical epithelial surface has numerous 2-6 _m diameter lightly basophilic spherical bodies interpreted as *Cryptosporidium sp.* The lumina of the ducts and crypts contain increased mucus and scattered foci of neutrophils and necrotic cellular debris. The lamina propria is markedly expanded by infiltrates of plasma cells, neutrophils, lymphocytes, and eosinophils. Scattered lymphoid nodules are present. The tunica muscularis is incorporated into hyperplastic folds and is discontinuous. There is variable fibrosis surrounding the muscularis. Within the hepatic parenchyma there are portal infiltrates of lymphocytes, histiocytes, eosinophils, and plasma cells that rarely cross the limiting plate and there is multifocal marked hyperplasia of bile ducts. Cryptosporidia were not detected in small bile ducts. Rare random foci of lymphocytic inflammation are present in sinusoids. A prominent hepatic vein has a well-demarcated plaque-like thickening of the intima due to myocyte hyperplasia and deposition of matrix with a high content of ground substance. A few scattered lymphocytes and neutrophils are present within the lesion. In tissues not represented on the distributed slide, there was minimal cryptosporidiosis of the pancreatic duct, mild chronic myelitis, and lymphoid depletion. There was also mild chronic colitis, characterized by lymphoplasmacytic infiltration of the lamina propria, crypt dilatation, and the accumulation of necrotic debris in crypt lumina, but no cryptosporidia were detected. Colitis is a frequent finding in SIV-infected macaques.

The proliferative cholangitis and cholecystitis observed in this SHIV-infected macaque resembles the cholangiopathy previously reported in SIV-infected monkeys[1,2] and described in human AIDS patients.[3] AIDS-cholangiopathy, characterized by sclerosing cholangitis and acalculous cholecystitis is presumptively due to *Cryptosporidium* infection. Unlike primary cryptosporidial infections in immune competent human hosts, which are usually limited to the small intestine, cryptosporidial infections in AIDS result in persistent infections and extension beyond the intestine to include the stomach, and biliary and pancreatic ducts, lung, and inner ear.[3] Among human immunodeficiency virus (HIV)-positive persons with diarrhea, *Cryptosporidium* infection was present in 14-24%.[4] *Cryptosporidium* infection of nonhuman primates immunosuppressed by either SIV or simian type D retrovirus occurs in the small intestine, biliary and pancreatic tracts, conjunctiva, and lung.[5,6,7] Intestinal infection is associated with a profuse persistent watery diarrhea leading to dehydration and mortality.[8,9,10]

Primary intestinal cryptosporidial infections occur in neonatal macaques[11], as in many other mammalian species, and are associated with diarrhea that resolves within 16 days. Neonatal cryptosporidiosis is an important cause of diarrhea in cattle and humans worldwide and is usually due to sporocyst-containing fecal contamination of water supplies. Reportedly, 20% of US adolescents have been infected while over 90% of children living in an urban shantytown in Northeast Brazil were seropositive.[4] The resistance of sporocysts to chlorination, their small size (4-6_m), and their low infective dose (as low as 10 sporocysts) challenge the ability of water treatment plants to remove this agent from drinking water supplies.

Cryptosporidium spp. cause primary infections in immunocompetent hosts across many, if not all, vertebrates.[12] The separation of *Cryptosporidium* into species remains a controversial task and is based on morphology, host specificity, virulence, and genomic characterization. Currently, at least two mammalian (*C. parvum*, *C. muris*), two avian (*C. meleagridis*, *C. baileyi*), one reptilian (*C. serpentis*), and one fish (*C. nasorum*) species are established, although mammalian infections with avian cryptosporidia are documented.[4,13] The gastric cryptosporidia, *C. muris*, *C. serpentis*, and *C. baileyi* have larger cysts and are distinct from other cryptosporidia. *C. parvum* is divided into two genotypes where type 1 is restricted in host range to humans and nonhuman primates and type 2 is more commonly associated with bovine infections.[13]

Resolution of cryptosporidial infection depends upon interferon gamma (IFN-gamma), interleukin 12 (IL-12) and CD4+ T cells in mouse models.[14] However, differences in immune responses to *Cryptosporidium* infection between species limit further generalizations regarding the mechanisms of immune response. Secretory immunoglobulins can provide protection at the sporozoite attachment and entry stages.[14] The lack of effective chemotherapeutics has led to investigation of maternal vaccines for protection through maternal antibody transfer in cattle and humans and immunoglobulin therapeutics for immunocompromised patients.

The recent sequencing of the genome for *Cryptosporidium* parvum type 2 revealed a paucity of metabolic and mitochondrial enzymes, indicating that the organism depends heavily on the host cell for nutrients. However, the identification of metabolic enzymes that resemble enzymes of plant and bacterial origin has provided new targets for drug development.[15]

Cryptosporidial infection causes epithelial hyperplasia in a variety of species, but the mechanism remains unknown. Cryptosporidial infection also results in villus blunting, which may be due in part to induction of apoptosis in epithelial cells.[16] Recently, oligonucleotide microarrays identified host cell genes which are differentially regulated during infection of epithelial cells in culture. Several genes involved in control of cell proliferation and apoptosis were significantly modulated[17], which may lead to a better understanding of this characteristic pathological effect of *Cryptosporidium sp.* infection.

The intimal myocyte hyperplasia and minimal phlebitis are interpreted as a SIV-related change, similar to the arteriopathy that has been described.[18]

AFIP Diagnoses: 1. Liver: Choledochitis, proliferative, chronic-active, diffuse, severe, with mucus cell metaplasia, apical protozoa, and multifocal mild cholangiohepatitis with bile duct hyperplasia, etiology consistent with *Cryptosporidium* sp., rhesus macaque, primate.
2. Large muscular vein, intima: Fibromyxomatous proliferation, focally extensive, moderate.

Conference Comment: The contributor provides a thorough overview of cryptosporidiosis in several species. Transmission is direct with ingestion or inhalation as the main routes of infection. Upon ingestion of oocysts, sporozoites excyst, invade mucosal epithelial cells, and undergo both asexual (merogony) and sexual (gametogony) replication resulting in meronts or macro- and microgametocytes respectively. Oocytes sporulate internally to contain four sporozoites that then either contribute to autoinfection or exit the body.[19]

The ultrastructure of *Cryptosporidium* sp. in tissue is unique. In the intestine, the organism attaches to enterocytes via a specialized feeder organelle, displaces microvilli, and is surrounded by a host cell derived membrane (parasitophorous vacuole). This results in the organism being intracellular but extracytoplasmic and is characteristic for *Cryptosporidium* sp.[20]

Contributor: Tulane National Primate Research Center, Division of Comparative Pathology, 18703 Three Rivers Road, Covington, LA (http://www.tpc.tulane.edu)

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CASE IV – PA-4039-1 or PA-4039-4 (AFIP 2888624)

Signalment: 8 week-old New Zealand White (NZW) rabbit (*Oryctolagus cuniculus*).

History: Because of recent unexplained deaths, 6-week-old sentinel animals were placed in this rabbit room. They were exposed to dirty bedding from other cages and euthanized after two weeks. At the time of their sacrifice the animals were clinically healthy and normal in appearance.

Gross Pathology: The terminal ileum was enlarged and prominent in appearance. The mucosal surface was markedly thickened and had a corrugated appearance.

Laboratory Results: None

Contributor's Morphologic Diagnoses: 1. Enteritis, proliferative, diffuse, moderate-marked, with severe crypt epithelial hyperplasia and focal crypt abscesses.
2. Villous blunting, focal, mild (some sections).
3. Central lacteal dilatation, patchy, mild.
4. Intraepithelial protozoa consistent with *Eimeria*, villous epithelium, multifocal, minimal-mild (some sections).

Contributor's Comment: Multiple sections of bowel were cut to provide slides for the conference. Lesions vary slightly from block to block. Histological changes were limited to the ileum and consisted of a significant thickening of the mucosa associated with hyperplasia of both villous and crypt epithelium. The crypt epithelium was severely crowded with a marked increase in mitotic rate and crypts occasionally demonstrated branching. Focal areas of crypt luminal dilatation with attenuated epithelium and central necrotic debris were observed (crypt abscesses). A moderate mixed inflammatory infiltrate was observed in the deep mucosa that in areas included a significant histiocytic component. Areas of villous shortening & blunting (some sections) and dilatation of the central lacteal structure were occasionally observed.

In some of the H&E stained sections, numerous small punctate structures consistent with coccoid and small bacilliform bacteria were observed adjacent to villous and crypt epithelium, extending in some sections into the submucosal regions.

A Warthin-Starry silver stain revealed the presence of myriads of short, curved rod-shaped argyrophilic bacterial organisms primarily within the apical cytoplasm of hyperplastic crypt epithelium. These organisms were strongly positive on immunohistochemical staining with a porcine *Lawsonia intracellularis* -specific monoclonal antibody. Additionally, the presence of this organism was further confirmed by amplification of 319-base-pair-(bp) and 182-bp products specific for porcine *L. intracellularis* chromosomal DNA and 16S rRNA genes, respectively.

The gross and microscopic findings, in conjunction with the immunohistochemical and molecular verification are consistent with a diagnosis of *Lawsonia intracellularis* induced subclinical proliferative enteritis.

Lawsonia intracellularis is a recently identified intracellular pathogen, phylogenetically unrelated to other pathogens.[1] It is an obligate intracellular organism and has been associated with hyperplastic intestinal processes in a growing number of mammalian and avian species. Although the disease can manifest sub-clinically in many animals, moderate to severe clinical disease is often seen in a wide variety of hosts, most notably in pigs and hamsters.

Infection in rabbits with *Lawsonia* has been well documented,[2,3,4,5] including dual involvement with enteropathogenic *E. coli*. Some of these reported outbreaks have been associated with high mortality and the presence of lesions including severe necrosis, ulceration and suppurative inflammation in association with the proliferative changes. In some reports, histiocytic enteritis is described as a result of *Lawsonia* infection.[2,6] In other sentinel animals from this cohort sacrificed at later time points, a distinctly granulomatous pattern of inflammation was noted - suggesting that the histiocytic response may be associated with a later stage/resolution of infection.

The *Eimeria* organisms noted in this case were thought to be incidental. Concurrent infection of this animal with other enteric organisms and agents was not excluded.

AFIP Diagnoses: 1. Small intestine: Enteritis, proliferative, histiocytic and heterophilic, diffuse, moderate, New Zealand White rabbit, lagomorph.
2. Small intestine: Intraepithelial protozoa, multifocal, few, etiology consistent with *Eimeria* sp.

Conference Comment: As the contributor mentioned, *Lawsonia intracellularis* has been identified as the causative agent of a proliferative enteropathy in a number of species, including rabbits, hamsters, guinea pigs, rats, ferrets, non-human primates, swine, sheep, horses, white-tailed deer, dogs, Arctic and blue fox, emus, and ostriches.[1,7] Regardless of the species affected, two hallmarks are constant, namely proliferation and intracellular bacteria.[1]

The gross lesions in pigs, hamsters, foals, white-tailed deer, and guinea pigs are similar. They predominately occur in the distal ileum, beginning as small raised opaque

islands that progress to a confluent, irregularly nodular, surface which may have areas of hemorrhage and/or necrosis. Histologically, the thickened epithelium results from expansion and elongation of the crypts, with actively dividing epithelial cells, and an absence of goblet cells.[1]

Lesions in other species differ in location and histologic changes. In ferrets, rabbits, and blue foxes lesions are most commonly found in the cecum and proximal colon. In ferrets, histologic changes may include organisms that penetrate the muscular tunics and appear in the serosa or draining lymph nodes. In rabbits, infiltrating histiocytes may expand the lamina propria, and the organism often has a single polar flagellum and is located in a membrane-bound vacuole, a feature not found in pigs. [1]

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