The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 10
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Conference Moderator:
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CASE I – 08-956-1 (AFIP 3106956)

Signalment: Approximately 2-3 year old adult, male, Northern leopard frog (*Rana pipiens*)

History: The frog was euthanized and submitted for necropsy after presenting with a history of lethargy, distended abdomen and possible abdominal mass on palpation.

Gross Pathology: On external examination, the abdomen was severely distended and mild hyperemia and erythema were noted on the distal extremities. On incision, the ventral abdomen contained a large, approximately 4cm, space-occupying multilobulated, cauliflower-shaped pale pink soft tissue mass. The testes were positioned ventral to and in contact with the mass. The mass was not adhering to any viscera and caused displacement of the abdominal organs.

Laboratory Results: NA

Histopathologic Description: Abdominal Mass: The examined section is composed of part of the abdominal mass, small segment of renal parenchyma and testes. The partially encapsulated, multilobulated and moderately cellular neoplastic mass is well-differentiated and composed of a proliferation of closely packed cells arranged in irregularly-shaped tubules or papillary projections that are supported by a fine fibro-vascular stroma (**Fig. 1-1**). Neoplastic cells are variably sized, mostly large, cuboidal to columnar with distinct cell borders and contain moderate amounts of eosinophilic, granular cytoplasm that often contains eosinophilic to mucinous globular droplets. The cells frequently form piles of 4-12 cell-layers deep. The nuclei of the cells are round to oval, central to basally positioned, with coarsely stippled chromatin, and contain one or more prominent basophilic nucleoli. Rarely, the nucleus contains 2-4 µm diameter eosinophilic inclusion-like material with a clear halo and marginated chromatin. Mitotic figures are 22 per 10 high-powered fields. There is mild anisocytosis and anisokaryosis. In multiple foci, individual cells to aggregates of necrotic/ghost cells are present. Many tubules contain ectatic lumen filled with necrotic cells, few lymphocytes and eosinophilic proteinaceous material.

Kidney: Within the submitted small remnant renal tissue, islands of dysplastic convoluted tubules mostly in the renal pelvis are also lined by epithelium with morphological features similar to those observed in the adjacent neoplasm. The neoplastic cells variably contain faintly visible micro-villi.

Contributor's Morphologic Diagnosis: Renal mass (presumed): Adenocarcinoma, well-differentiated, tubulo-papillary with rare eosinophilic intranuclear inclusion-like material

Contributor's Comment: This fairly large abdominal mass is suspected to be of renal origin, though no remnants of renal parenchyma were present within the actual mass. However, the presence of islands of tubules with features similar to those observed in the mass is highly indicative of renal origin along with the massive growth of the tumor effacing the normal renal parenchyma. Rarely, indistinct eosinophilic inclusion-like material was observed within the nucleus and rarely in the cytoplasm. The inclusions, though not of typical size, are considered to be herpes viral inclusions. Additional electron microscopic evaluation may be needed for confirmation. The morphological features are most consistent with that of Ranid herpesvirus 1 (RaHV-1) induced adenocarcinoma of leopard frogs.

RaHV-1 is the etiologic agent of Lucké renal adenocarcinoma and occurs spontaneously in *Rana pipiens* typically in frogs aged 2 years or older.(1) Tumor incidence can be as high as 50% in laboratory populations living at 25°C.(2) The viral replication and growth kinetics of the tumor are dependent on temperature and season. High environmental temperature during summer is permissive for viral invasion and rapid growth of tumor with very few inclusion bodies. During cooler, winter temperatures invasion is restricted but viral replication occurs in the convoluted tubules of the kidney with dormant phase of tumor growth.(1) When frogs are hibernating or maintained at low temperature, tumor cells contain intranuclear inclusions whereas frogs in summer months or maintained at 25°C do not contain virus or inclusions in tumor cells.(2) Frogs may not show clinical signs of lethargy, emaciation and ascites until the disease is well advanced.(1) Whitish tumors can be seen at necropsy on the kidneys, though tumors can grow very large and metastasize.(1) There is no treatment and affected animals should be euthanized.(1) The other well-known spontaneous amphibian tumor is lymphosarcoma, occurring in *Xenopus laevis*.(3)

AFIP Diagnosis: Kidney: Adenocarcinoma, tubulopapillary

Conference Comment: Lucke's tumor, or renal adenocarcinoma of frogs, is commonly found in the northern and northeastern United States. It can be found in up to 10% of frogs captured in the wild.(2) The Lucke tumor herpesvirus, (LTHV), is the causative agent of the tumor, and ultrastructurally the virions are icosahedral and 95-100nm.

Frogs shed this virus in the colder months of the year, and it travels via water to infect frog eggs during spawning season. Mortality due to renal adenocarcinoma usually occurs after spawning when temperatures are warmer and the tumor has grown considerably within the coelomic cavity. Frogs often seem clinically normal until just prior to death. Neoplastic cells can often be isolated from ascitic fluid to aid in an ante-mortem diagnosis.(1)

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CASE II – 04/3025 D (AFIP 3102369)

Signalment: 3-year-old male Tasmanian Devil (*Sarcophilus harrisii)*

History: Wild male Tasmanian Devil, condition score 1.5 out of 5, trapped from the wild.

Gross Pathology: There were multi-centric tumors on the face, 6 in total, labeled T1 to T6. The mass labeled T4 was submitted to the Wednesday Slide Conference. T1 was a mass on the lower right lip measuring 6.2 x 4.4 x 2.8 cm. T2 was a mass at the right commissure of the lips measuring 1.5 x 0.7 cm. T3 was on the upper right lip measuring 0.5 x 0.2cm. T4 was a mass on the left side of the forehead measuring 5 x 4 x 1.7 cm. T5 was near the lateral canthus of the left eye measuring 1.2 x 0.7cm. T6 was a mass on the left cheek measuring 0.5 x 0.4 cm. There were no other significant internal findings at post mortem. There were fly larvae and eggs around the rump area (cutaneous myaisis).

Laboratory Results:
Significant clinical chemistry abnormalities included:

AST (IU)	436	(39.0-206.0)
Creatinine (umol/L)	28	(44.0-106.0)
Protein (g/L)	75.6	(52.0-69.0)
Albumin (g/L)	24.3	(28.0-37.0)

Globulin (g/L)	51.4	(18.0-37.0)

Hematological abnormalities included:

WBC (x 10^9/L)	59.5	(3.4-13.2)
Neutrophils (x 10^9/L)	55.93	(0.82-9.33)
Monocytes ((x 10^9/L)	1.78	(0.04-0.26)
Fibrinogen (g/L)	12.0	(0.0-3.0)

Urinalysis

Volume (ml)	10
Color	yellow
Turbidity	clear
Specific Gravity	1.058
pH	7.0
Protein	3+
Glucose	nil
Ketones	nil
Bilirubin	nil
Blood (heme)	2+
Urobilinogen	nil
Cells (per hpf)	0
Debris	1+

Comment: The serum biochemistry changes indicate a moderate increase in AST, moderate decrease in Creatinine, mild hyperproteinemia characterized by a mild hypoalbuminemia and a moderate hyperglobulinemia. The leukon changes demonstrate a marked leukocytosis characterized by a marked neutrophilia and a moderate monocytosis. There is a marked hyperfibrinogenemia, moderate hemoglobinuria, and marked proteinuria. The increase in AST in this case could be attributed to a myopathy related to exertion or capture, since the hepatic function was normal. Although CK was within the normal reference range, AST can remain elevated for up to 5-6 days once released from the muscle, whereas CK can diminish rapidly (within 2 days). Moreover, a delay in sample collection may result in there being no apparent change in activity of CK. The decrease in serum creatinine can occur with severe cachexia and subsequent significant loss of muscle. Low albumin could be due to decreased intake (starvation) or reduced synthesis to maintain oncotic pressure (since globulins are increased). The hypoproteinemia and hyperglobulinemia are consistent with neoplasia and chronic inflammatory disease. The leukon changes (marked neutrophilia, moderate monocytosis and hyperfibrinogenemia) are consistent with severe inflammatory disease.

Histopathologic Description: The dermis and hypodermis are expanded by a partially circumscribed, nodular, neoplastic proliferation of epithelial cells. The neoplastic cells are divided into lobules by fibrous connective tissue. Within the lobules the neoplastic cells are arranged in streams and nests, supported by delicate fibrovascular stroma (**Fig. 2-1**). Scattered amongst the intact neoplastic cells are occasional, individual apoptotic and necrotic neoplastic cells. Within the center of many of the lobules are extensive areas of necrosis. Most of the neoplastic cells have a round to polyhedral or cuboidal shape with a moderate amount of eosinophilic cytoplasm. Nuclei are round to ovoid, with a stippled, basophilic chromatin pattern. Nucleoli are often multiple and mitoses range from 0-12 per field of 400x magnification. The epidermis overlying the neoplasm is extensively ulcerated. The exposed dermis is superficially necrotic and expanded and covered by a proteinaceous and neutrophilic exudate containing multifocal colonies of bacteria.

Contributor's Morphologic Diagnosis: Haired skin: Carcinoma "Devil Facial Tumor Disease"

Contributor's Comment: The history, gross and histopathological findings are consistent with Devil Facial Tumor Disease, a debilitating transmissible neoplastic condition affecting significant numbers of the wild population of Tasmanian Devils.

Out of all of Australia's unique wildlife, Tasmanian Devils are the largest living dasyurids, or carnivorous marsupials. They are iconic natives of Tasmania, where they inhabit the coastal forests, scavenging on

dead or dying animals. A debilitating disease with proliferating facial masses was detected in wild populations of Tasmanian Devils in the mid 1990's.(1,5) Dubbed the Devil Facial Tumour Disease, this emerging new disease was quickly increasing in prevalence, with population declines of up to 80% in some areas.(1,2) Affected animals develop large, multicentric, flat soft tissue masses, often with ulcerative and exudative centers.(2) The tumors first develop around the face, mouth, and neck, but may spread to elsewhere in the body. As they enlarge, the tumors interfere with feeding, and the devils quickly lose condition, and usually succumb to the disease within 6 months.

A multi-disciplinary approach was taken to investigate and characterize this disfiguring, fatal disease. The main goal of this research was to maintain an ecologically sustainable population of Tasmanian Devils in the wild.(5) Standard cytology, histopathology, and electron microscopy were used to characterize these facial tumors. Loh et al (2006a) discovered that the neoplasm originated within the dermis, and was composed of dense, multinodular proliferations of pleomorphic round to polyhedral cells, with a high nuclear to cytoplasmic ratio.(2) These cells had a fibrillar, eosinophilic cytoplasm, indistinct cytoplasmic margins, and single, basophilic nuclei with no obvious nucleoli.(2) The mitotic rate ranged from 0-12 per 400x magnification, and necrosis was observed in most samples.(2) Metastasis was also reported in 65% of the cases, with frequent involvement of the regional lymph nodes, and distant metastases mainly to the lungs, but also the spleen, heart, ovary, serosal surface of ribs, kidney, mammary, pituitary and adrenal glands.(2)

The pleomorphic neoplastic cells were difficult to characterize, and immunohistochemistry was utilized for final confirmation. The cells stained negative for cytokeratin, epithelial membrane antigen, von Willebrand factor, smooth muscle actin, desmin, glial fibrillary acid protein, CD 16, CD 57, CD3, and LSP1, but stained positive for vimentin, S-100, melan A, neuron specific enolase, chromogranin A, and synaptophysin 3. With this information, together with the morphological and ultrastructural features of the cells, it was concluded the neoplasm was consistent with a malignant neuroendocrine tumor.

In parallel with this work, cytogenetic studies were performed, and a unique, aneuploid karyotype was identified, which was shared by tumors from animals in many geographical locations within the state. These genetic rearrangements were identical in male and female animals from a range of ages, indicating that cytogenetically, DFTD is relatively stable.(4) Such findings, combined with the frequent habit of the devils engaging in "jaw wrestling" and the propensity for the tumors to arise in the lips, oral mucosa, or the face, is consistent with disease being spread as allografts. The most significant confirmative indicator that DFTD is a transmissible neoplasm has been the successful experimental transfer of DFTD cells derived from cell cultures and natural tumors to healthy devils with the subsequent variable development of DFTD. This work has fulfilled elements of Koch's postulates, confirming the transmissible neoplasm hypothesis. (5)

A collaborative effort to save the Tasmanian Devils has been established with the goal to investigate disease control mechanisms and identify management options to prevent further population decline.

AFIP Diagnosis: Haired skin: Malignant neuroendocrine neoplasm (Tasmanian Devil Facial Tumor Disease)

Conference Comment: There was considerable discussion at the post-conference meeting about the proper classification of this tumor. Based upon positive immunohistochemical staining for vimentin, S-100, melan A, neuron specific enolase, chromogranin A, and synaptophysin 3, we favor the diagnosis of malignant neuroendocrine tumor.

The contributor did an outstanding job of reviewing DFTD including transmission, gross and histologic findings and tumor karyotype.

Additionally, some attendees noted the incidental finding of intrafollicular mites consistent with *Demodex* in their sections.

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CASE III – 60136 (AFIP 3102636)

Signalment: Adult, male American horseshoe crab (*Limulus polyphemus*)

History: This horseshoe crab was part of an aquarium touch-tank exhibit for two years and developed mild gill and shell lesions. The gill and shell lesions progressively worsened; the crab became moribund and was euthanized.

Gross Pathology: The dorsal carapace (prosoma) had multifocal partial thickness pitting lesions with black and pale tan discoloration (**Fig. 3-1**). Book gill coverings had multifocal areas of black discoloration and several pale tan proliferative lesions along the caudal edges (**Fig. 3-2**). Individual book gill leaflets were very friable, tearing easily, and were opaque and pale tan (**Fig. 3-2**).

Laboratory Results: *Fusarium* sp. was isolated from the gill lesions, and it was speciated to *F. solani* using polymerase chain reaction (PCR).

Histopathologic Description: The cuticle of both the carapace and the book gill cover is multifocally thickened and the chitinous layers of the carapace are multifocally infiltrated and disrupted by fungal hyphae seen in longitudinal and cross section (**Fig. 3-4**). Fungal hyphae occasionally penetrate through the carapace into the underlying tissues (striated muscle and spongy parenchyma with prominent hemolymph channels). The affected tissues are hypereosinophilic (necrotic) with associated viable and degenerate amebocytes (hemolymph cells with eosinophilic cytoplasmic granules) (**Fig. 3-4**). Fungal hyphae are septate with mostly parallel appearing walls, and range from 4 to 7 um in diameter with occasional acute and right angle branching (**Fig. 3-5**). Some of the hyphae are poorly stained and appear swollen and non viable. The more viable forms stain with PAS and GMS fungal stains. Individual hyphae are sometimes seen on the outer surface of the cuticle and are pigmented brown (dematiaceous). Areas of disrupted cuticle have variably sized accumulations of basophilic granular material (bacteria, confirmed by Gram stain).

The cuticle of individual gill leaflets is multifocally thickened and penetrated by fungal hyphae. The central vascular channel of individual leaflets is expanded with hypereosinophilic and necrotic material, viable and degenerate amebocytes and bacteria (**Fig. 3-3**). There is necrotic debris mixed with bacteria between leaflets and sometimes pigmented fungal hyphae. Occasionally, there are some fungal hyphae on the surface of affected gill leaflets that are smaller (2-3 um diameter) than the invasive fungi.

Slides occasionally have metazoan parasites within the parenchyma and degenerate parasites associated with the surface of the carapace and/or gill leaflets.

Contributor's Morphologic Diagnosis: Carapace: Shell disease, acute and chronic, necrotizing, multifocal, severe, with intralesional fungal hyphae and bacteria
Gills: Branchitis, acute and chronic, necrotizing, multifocal, severe, with intralesional fungal hyphae and bacteria

Contributor's Comment: The American horseshoe crab is of extreme importance to the medical field because of the lysate extracted from amebocytes (hemolymph blood cell), which is used to test pharmaceuticals for bacterial endotoxin contamination (limulus amebocyte lysate assay). Despite the importance of the horseshoe crab, there is a paucity of published information regarding its diseases. Infectious disease agents of horseshoe crabs include algae, fungi, cyanobacteria, Gram negative bacteria and many different parasites, with diseases of the shell being the most common manifestation.(8,9)

Mycotic shell disease has been reported only in captive horseshoe crabs. Specifically, mycotic infections of the carapace are reported in juvenile horseshoe crabs and most often in those housed without a sand substrate.(8,9) Gill and shell lesions similar to the ones seen in this crab are reported in a group of horseshoe crabs in a touch-tank at Ripley's Aquarium of the Smokies.(1) Single dose itraconazole therapy is well tolerated by horseshoe crabs, (1) but the efficacy of this treatment for the fungal lesions has not been investigated.

Fusarium species are saprophytes found ubiquitously within the environment and are the cause of diseases of both plants and animals including humans. *Fusarium* can cause disease in animals both by ingestion of mycotoxins and by fungal invasion of body tissues. Several examples of diseases caused by *Fusarium* infection in humans include keratitis, onychomycosis, dermatitis and disseminated disease.(3) Two recent reports of disease caused by *Fusarium* species reported in veterinary species include keratitis in a Holstein cow and intracranial fusariosis in a German Shepherd Dog.(4,5)

Invertebrate animals lack an adaptive immune system and respond to microbial antigens with a variety of innate immune responses including hemolymph coagulation, toll-like receptor mediated antimicrobial peptide production, melanin formation and lectin-mediated complement fixation. In horseshoe crabs, the hemolymph contains soluble antimicrobial proteins including C-reactive protein, alpha-2 microglobulins, lectins and hemocyanins. The granular amebocytes (also called hemocytes), which make up more than 99% of the circulating cells in the hemolymph, also contain antimicrobial proteins and coagulation proteins. Exposure to microbes causes degranulation of amebocytes and formation of a hemolymph clot. (6)

AFIP Diagnosis: 1. Carapace: Shell disease, necrotizing, acute and chronic, multifocal, severe, with fungal hyphae and bacteria
2. Gills: Branchitis, necrotizing, acute and chronic, multifocal, severe, with fungal hyphae and bacteria

Conference Comment: Another Fusarium species of importance in veterinary medicine is *Fusarium moniliforme* because certain strains of this mold release the toxin fumonisin B1. Fumonisin B1 is a potent mycotoxin that induces hepatocellular carcinoma in rats and leukocencephalomalacia in horses. Pigs get pulmonary edema and hydrothorax from fumonisin B1 ingestion. This mycotoxin has also been shown to be hepatotoxic to pigs.(10) Horses and pigs are exposed to this toxin when they eat corn infected with the mold *Fusarium moniliforme. Fusarium proliferatum and Fusarium verticilliodes* are also listed as producers of the mold fumonisin B1 in the newest edition of Jubb, Kennedy, and Palmer.(7)

Morphologically, the *Fusarium* sp. are identified by hyaline, septate hyphae measuring 4 to 7um in width with frequent, usually right angle branching. This is important in helping to distinguish these from *Aspergillus* sp*,* which tend to have dichotomous branching and are 3-5um in width.(2)

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CASE IV – GF-D90-3 (AFIP 3102368)

Signalment: 6-month-old, female, *Cavia porcellus*, Hartley guinea pig

History: Experimental animal, infected via aerozolization with *Mycobacterium tuberculosis.* This animal was euthanized after being infected for 90 days.

Gross Pathology: The left mammary gland is markedly enlarged and exudes necrosuppurative material upon mild palpation. This affected region may incorporate and efface normal inguinal lymphoid architecture as this node is neither palpable nor observed. Approximately 30-40% of pulmonary parenchyma is replaced by primary tuberculoid lesions with the remaining lung parenchyma affected by secondary lesions. The primary lesions are depressed centrally and are surrounded by a dense fibrotic parenchyma. The mediastinal lymph nodes are diffusely enlarged with loss of lymphoid architecture and are further expanded by fibrinous adhesions which connect with the ventral aspect of the thoracic pleura. Multifocally, the spleen and the liver contain multifocal to coalescing granulomas and are markedly enlarged. The spleen is approximately 10x larger than normal.

Laboratory Results: Lung, viable cell count; approximately 9.8×10^6

Histopathologic Description: Haired skin, subcutis, and mammary glandular tissue. The deep subcutaneous tissue is effaced by large coalescing nodules of epithelioid macrophages, admixed with heterophils, rare lymphocytes and multinucleated giant cells (**Fig. 4-1**). Multifocally, these nodules contain central areas of necrosis characterized by degenerate heterophils, hypereosinophilic debris (necrosis), and karyorrhectic debris. In some sections, large areas of cavitation of these necrotic regions are observed. Admixed within this extensive inflammatory response are rare entrapped adipocytes and remnant mammary glands. There are no remaining ductules. Within the superficial subcutis, subjacent to the deep dermis, there are small granulomas comprised of macrophages and lymphocytes. There is superficial expansion of the epidermis by a moderate amount of orthokeratotic hyperkeratosis. An acid-fast stain revealed few positive bacilli within the necrotic centers.

Contributor's Morphologic Diagnosis: Mammary gland: Mastitis, necrosuppurative, granulomatous, regionally extensive, with intralesional acid- fast bacilli

Contributor's Comment: The guinea pig is used extensively as an animal model of human tuberculosis. (5) The primary lesion complex in both humans and guinea pigs are similar and comprises multifocal granulomatous inflammatory lesions within the lung and draining lymph nodes. Similar to humans, guinea pigs develop small foci of mixed inflammation which subsequently develops into the characteristic granuloma which is comprised predominantly of macrophages and occasionally granulocytes.(8) As seen in this case, these large granulomas develop a central zone of necrosis, which is where the highest concentrations of bacterium can be observed in both humans and in guinea pigs.(1)

The guinea pig in this case was experimentally infected via aerosolization with *Mycobacterium tuberculosis* to evaluate pulmonary tuberculosis. Diagnosis of tuberculosis in the mammary gland was an incidental finding. To the contributor's knowledge, tuberculosis mastitis has never been reported in an experimentally

infected guinea pig. In human patients, involvement of the mammary gland is a rare manifestation of the disease.(2) Clinically, tuberculoid granulomas of the mammary gland are unilateral and may mimic breast cancer and/or breast abscesses which are all managed differently making accurate diagnosis crucial.(3,9) The reliable diagnostic tests include bacteriologic culture, histopathology, and guinea pig inoculation.(6)

Tuberculosis mastitis (TM) in humans can occur as primary or secondary disease. Secondary involvement of the mammary gland is more common than primary infection of the mammary gland.(6) Common routes of infection include the lymphatic route, hematogenous route or from direct spread from local organs. The direct form of spread in humans may occur from an infected rib, cartilage, or joint.(6)

In humans, there are three forms of mammary tuberculosis: nodular, diffuse, and sclerosing. The nodular form is the most common presentation in women and is typically slow growing and often develops a caseating center.(7) The sclerosing form develops excess fibrous connective tissue and is often the most difficult to differentiate from mammary gland carcinoma.(7)

AFIP Diagnosis: Mammary gland: Mastitis, pyogranulomatous, focally extensive, severe with acid-fast bacilli

Conference Comment: Mycobacteria are non-motile, non-spore forming organisms with a lipid-rich cell wall that stains poorly with gram-stain. Acid-fast stains are commonly used to identify mycobacteria in tissue sections. The term "tuberculosis" is now conventionally used to describe only infections caused by *Mycobacterium bovis* and *Mycobacterium tuberculosis*, whereas diseases caused by other mycobacterial species are referred to as mycobacteriosis or atypical mycobacteriosis.(4)

Susceptibility to tuberculosis and organ system affected varies greatly among domesticated animal species. The hallmark lesion of tuberculosis is the granuloma. These granulomas may be in different organs with slightly different histologic appearances based on the route of entry and species affected. Below is a brief list of animal species, susceptibility, and organ systems affected by tuberculosis.

Species	**Susceptibility**	**Organ System Affected**
Cat	More susceptible to *M. bovis*	Gastrointestinal disease – ingestion of contaminated wildlife or milk
Dog	Susceptible to *M. bovis* and *M. tuberculosis*	Respiratory form
Cattle	More susceptible to *M. bovis*	Respiratory and gastrointestinal forms – calcification can also occur
Pigs	Susceptible to both *M. bovis* and *M. tuberculosis*	Systemic infection
Small ruminants	Rare cases	Similar to cattle when infected
Horses	Rare; usually *M. bovis*	Gastrointestinal; also see lesions in retropharyngeal and mesenteric lymph nodes
Non-Human Primates	Very susceptible to *M. tuberculosis*	Infected humans transmit respiratory form to NHP's
Birds (Psittacines)	Only birds to get tuberculosis; get both *M. tuberculosis* and *M. bovis*	Respiratory form - transmitted from humans

(4)

Contributing Institution: 1619 Campus Delivery, Colorado State University, Microbiology, Immunology, and Pathology, Fort Collins, CO 80523-1619; http://www.cvmbs.colostate.edu/

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