CASE 1 – CRL 2008-1 (AFIP 3104062)

Signalment: Mouse (Mus musculus), strain unknown (homozygous for foxn1<sup>nu</sup>), age and gender unknown.

History: Submitted to necropsy for evaluation of scaly skin.

Gross pathologic findings: Skin over the entire body surface is hyperkeratotic, with white flakes.

Laboratory results: PCR of skin surface swabs positive for Corynebacterium bovis.

Histopathologic description: Skin (nude): The epidermis is diffusely acanthotic, approximately 3-fold thicker than normal. Abundant keratinaceous debris covers the surface (diffuse orthokeratotic hyperkeratosis) (Fig. 1-1). Numerous colonies of small bacteria mix with hair and keratinaceous debris on the surface of the hyperkeratotic layer (Fig. 1-2). The bacteria appear coccoid in some areas, and as short bacilli in irregular clusters.

1-1 Haired skin, mouse. Diffusely there is epidermal hyperplasia with orthokeratotic hyperkeratosis and there are scattered lymphocytes, plasma cells, histiocytes and fewer neutrophils within the superficial dermis. (HE 200X).
branching cl usters i n a few ot hers. T he de rmis i s
diffusely m idly infiltrat ed by m ononuclear leukocytes
and a few neutrophils. Mast cells are prominent.

**Brown and Brenn Gram ’s stain:** The bacte ria are
Gram p ositive. Th e predominant typ e is sho rt
coryneforms, arranged in typical corynebacterial arrays.

**Contributor’s morphologic diag nosis:** Dermatitis,
subacute, diffuse, m ild, with e pithel ium acanthosis,
hyperkeratosis and coryneform bacteria.

**Contributor’s Co mment:** Corynebacterial
hyperkeratosis, the s o-called “sca ly skin dis ease’’,
remains fairly common, with ap proximately 2% of nude
mice submitted t o our diagnostic laboratory fr om non-
vendor sources being confirmed with the disease.

Corynebacterial hyperkeratosis has bee n re cognized for
decades in athym ic nude m ice, but also occurs in SCID
mice and can be experimentally reproduced in euthymic
hairless mice.(1) Morbidity varies, but can b e h igh.
Mortality is u sually v ery low e xcept in su ckl ing m ice,
which can have high mortality. Immunocompetent mice,
other t han hairless st rains, m ay have asymptomatic
infection, but evidence suggests that infection is cleared
in these mice.(2,3)

Clinical si gns include hyperkeratosis, decreased act ivity
and a wrinkled a ppearance which prob ably indicates
dehydration. Signs typically appear in s usceptible mice
about one week a fter expos ure, persist for a week or
more, and then usually di sappear. M icroscopically, the
acanthosis remains after t he hyperkeratosis resolves; the
infection also p ersist. Th e m echanism b y wh ich C.
bovis, a lipophilic bacterium which colonizes the stratum
corneum, causes ac an thosis a nd hyperkeratosis is
unknown, as is th e r eason for r esolution of th e
hyperkeratosis.

Histologic feat ures of this c ase are ty pical.
Hyperkeratosis can be difficult to assess microscopically,
is transitory in this disease, and is non-specific; it may be
caused by various conditions. Thus, g reater diagnostic
significance should be given to the acanthosis. Di ffuse
acanthosis, with a mild non- suppurative dermatitis and
the presence of Gram positive coryneform bacteria in the
stratum corneum is sufficient for a diagnosis, although in
most si tuations co nfirmation by c ulture an d or PC R i s
preferred(4). Other corynebacteria may also colonize the
skin surface; our laboratory has identified C. jeikeium,
C. minutissimum and Gro up F2. Th e latter t wo or ganisms
are now included in C. amycolatum. None of these are
thought to cause skin disease in mice.

In a ddition t o mortality in su ckl ing im munodeficient
mice, C. bo vis has been r eported t o s l ow xenograft
growth and i ncrease toxicity observed after
chemotherapeutic agents. T he m echanism o f th is is
unknown, but the contributor speculates that it might be
related to de hydration (sy mptomatic mice virtu ally
always appear markedly dehydrated, conceivably due to
alterations in epidermal barrier function from the diffuse
skin disease). R etarded xenograft growth co uld al so
possibly be d ue to n on-specific stimulation of h ost
defense m echanisms such a s NK cell act ivy. Non-
specific antitumor effects have previously been described
for C. kutscheri.(6)

Control of C. bovis infection is difficult. T he bacterium
is read i ly tr ansmitted b y fomites an d is resistan t t o
drying. Our diagnostic labo ratory has fou nd po sitive
PCR samples o n sw abs fr om cage exter ior s, do or kn o bs
and even tumor l ines p assaged as tumor fragments.

**AFIP Morphologic Diagnosis:** Sk in: Hyperkeratosis,
orthokeratotic, di ffuse, m oderate, with e pidermal
hyperplasia and mild subacute dermatitis.

**Conference Comment:** The contributor did an excellent
job of s ummarizing t he cl inical m anifestations, st rains
affected, and histologic features of Corynebacterium
bovis infections i n m ice. This c omment will briefly
touch on t he r esults a nd hi stologic features of
Corynebacterium kutscheri, another i mportant or ganism
in laboratory animals.

**Mice and Rats:** *Corynebacterium kutscheri* is a Gram-positive, diphtheroid bacillus that is the causative agent of “pseudotuberculosis” in both mice and rats. The normal route of entry of *C. kutscheri* is through the oral or gastrointestinal mucosa with subsequent hematogenous spread throughout the body. An immunosuppressive event usually precedes clinical disease. (5)

Common gross findings include suppurative bronchopneumonia with randomly distributed caseopurulent nodules; raised, multifocal to coalescing caseopurulent nodules in the heart, liver, or kidney; reactive hyperplasia in lymph nodes near an active site of infection; and pedal arthritis. (5)

On histological section, the bacteria form large colonies surrounded by abundant neutrophils and necrotic debris. Because the disease is spread via sepsis, the suppurative lesions in the lung are randomly distributed. The large, bactrial colonies of *C. kutscheri* are pat hognomonic and resemble Chinese letters. Interstitial pneumonia is also commonly present in the heart, liver, and kidney; reactive hyperplasia in lymph nodes near an active site of infection; and pedal arthritis. (5)

**Hamsters:** These rodents are considered carriers of *C. kutscheri* but are typically resistant to systemic disease. (5)

**Contributing institution:** Charles River; www.criver.com

### References

6. Russell S, Riley LK, Maddy, AC, clifford, CB, Russell, RJ, Franklin, CL, Ho ok, RR, an d Besch-Williford, CL Identification of *Corynebacterium bovis* as the etiological agent of hyperkeratosis in nude mice and development of a diagnostic polymerase chain reaction assay. Laboratory Animal Science 48 [4], 412. 1998

### CASE II - 07-276-3 (AFIP 3102251)

**Signalment:** 10-month-old female C57BL/6 mouse (Mus sp.) #202, with homozygous point mutation in Mcm4 gene (Mcm4Chaos3/Chaos3).

**History:** The mouse was euthanized after developing an enlarged abdomen and lethargy.

**Gross pathologic findings:** The liver was pale brown, markedly enlarged and had rounded edges. The spleen was diffusely enlarged and also had rounded edges.

**Histopathologic description:** Liver is diffusely hypercellular and the capsular surface has slightly irregular contour. Normal hepatic architecture is disrupted by filling of multifocal sinusoids and veins with small to moderate numbers of neoplastic cells arranged ed individually and sm all cl usters (Fig 2-1). The neoplastic cells are moderately sized, round to oval or elongate, with distinct cytoplasmic margins and moderate amount of eosinophilic cytoplasm that occasionally contains a few sm all cl umps of hemosiderin. Occasionally, there are RBCs within neoplastic cell cytoplasm (erythrophagocytosis). The nucleus is round or oval, often eccentrically placed and cl umps chromatin in a nd indistinct nucleolus. There is mild anisocytosis and anisokaryosis and rares foci of extramedullary hemato poiesis co ntaining e rythroid a nd granulocyte precursors and low numb bers of megakaryocytes.

### References

6. Russell S, Riley LK, Maddy, AC, clifford, CB, Russell, RJ, Franklin, CL, Ho ok, RR, an d Besch-Williford, CL Identification of *Corynebacterium bovis* as the etiological agent of hyperkeratosis in nude mice and development of a diagnostic polymerase chain reaction assay. Laboratory Animal Science 48 [4], 412. 1998
intracytoplasmic inclusions in some hepatocytes and occasional hepatocytes filled with moderate amount of green-brown granular pigment.

Laboratory results: Immunohistochemistry for macrophage marker Mac-2 was done using M3/38 antibody clone from Cedarlane. The cytoplasm of intrasinusoidal neoplastic round cells was strongly positive for Mac-2 (Fig 2-2).

Contributor’s morphologic diagnosis: Liver: Histiocytic sarcoma. Spleen (tissue not included): Erythroid hyperplasia, diffuse, marked.

Contributor’s Comment: Histiocytic sarcoma (HS) in mice arises from the cells of mononuclear phagocytic system and most commonly affects liver and uterus with less frequent involvement of spleen, lung, lymph node, ovary, kidney and bone marrow. The incidence is dependent on strain, sex, age, nutrition, and varies from study to study. For example, HS incidence in C57BL/6 mice is higher than in most other strains of inbred mice, and the disease occurs rarely before 12 months of age. One of the highest incidences of HS has been reported by Blackwell in a study that determined effect of dietary restriction on the incidence of tumors in C57BL/6 mice. HS was the most prevalent neoplasm in this study that involved almost 1000 mice over period of 3 years. Overall lifetime incidence of HS was ~30% in ad libitum fed C57BL/6 female mice compared with ~55% in similarly fed male mice. 40% reduction in the feed resulted in slight decrease in incidence of HS in male mice and increased HS incidence in female mice to 50%.

The gross and microscopic appearance of HS depends on organs involved. The liver involvement is the most common manifestation of HS regardless of the mouse strain. The liver with HS is severely, diffusely enlarged and without focal lesions. Histologically, tumor cells are present diffusely or multifocally with sinusoids and vascular spaces. Progressive growth of neoplastic cells leads to compression of hepatic cords and hepatocyte atrophy. Uterine involvement is strong in strain-dependent in mice: uterine HS is rare in C57BL/6 mice but common in CBA mice. HS in uterus may present as diffuse thickening of both horns or as 1 to several variably sized nodules. Histologically, neoplastic histiocytic cells that infiltrate uterine wall tend to be elongated to fusiform.
be associated with the neoplastic infiltrates, particularly in the liver and multinucleated giant cells may be present in some tumors.\(3, 7\) Immunohistochemical stains that aid identification of neoplastic cells as histiocytes include Mac-2, F4/80, and lysozyme.\(9\)

Recent publications have linked development of HS in the liver in C57BL/6 mice with concurrent hepatic (but not splenic) extramedullary hematopoiesis (EMH) and hematologic abnormalities such as anemia.\(1, 5\) At this point it is not clear whether HS and hepatic EMH are co-incident lesional or if one of them leads to the other. Concurrent hemolytic abnormalities may suggest genetic abnormality in myeloid stem cells.

The submitted case is from a mouse with homozygous, single base mutation in \(Mcm4\) gene known as Chaos3. This mutation induced high incidence of amnary adenocarcinomas in C3H mice.\(7\) In contrast, C57BL/6 mice with Chaos3 mutation have high prevalence of histiocytic sarcoma with shortened tumor latency of less than 12 months. Diffuse liver involvement is noted most commonly in these mice but nodular tumor infiltrates and marked destruction of hepatic parenchyma by HS is seen occasionally. All mice with hepatic HS have extramedullary hematopoiesis in the liver and marked erythroid hyperplasia in the spleen. Some mice with HS have intra-abdominal solid masses in the peripancreatic omentum and elsewhere diagnose as HS base on presence of Mac-2 positive cells with histiocytic appearance (spindle to polygonal cells, abundant cytoplasm, and oval to indented nucleus) and neoplastic features (moderate mitotic figure rate and bizarre mitoses).

Gene complexes \(MCM2-7\) which includes \(MCM4\) encodes protein co-plex that is recruited to DNA replication origins and ensu res a single initiation of DNA synthesis during S phase restricting genome replication to once per cell cycle.\(7\) Chaos3 mutation was first identified in the screen for chromosomal instability and is known to have high prevalence of histiocytic sarcoma with short tumor latency of less than 12 months. Diffuse liver involvement is noted most commonly in these mice but nodular tumor infiltrates and marked destruction of hepatic parenchyma by HS is seen occasionally. All mice with hepatic HS have extramedullary hematopoiesis in the liver and marked erythroid hyperplasia in the spleen. Some mice with HS have intra-abdominal solid masses in the peripancreatic omentum and elsewhere diagnosed as HS based on presence of Mac-2 positive cells with histiocytic appearance (spindle to polygonal cells, abundant cytoplasm, and oval to indented nucleus) and neoplastic features (moderate mitotic figure rate and bizarre mitoses).

**AFIP Morphologic Diagnosis:**

1. Liver: Histiocytic sarcoma.
2. Liver, hepatocytes: Microvesicular lipidosis, multifocal, moderate.

**Conference Comment:** The contributor provided a thorough overview of the gross and histologic lesions of histiocytic sarcoma in mice. This brief discussion will focus on histiocytic sarcoma in rats.

In rats, the most commonly affected strain is Sprague–Dawley, and tumors are seen in animals over 12 months of age with no gender preference. The organs affected in rats are simlar to those in mice; the most common sites are liver, lymph nodes, spleen, mediastinum, retroperitoneum, and subcutaneous tissue. Affected rats most commonly have nodules of tumor cells that displace normal organ parenchyma, whereas in the mouse liver, neoplastic cells typically in infiltrate sinusoids with no distinct mass formation.\(6\)

Histologically, tumor cells are polymorphic with vesicular nuclei, prominent nucleoli, and abundant cytoplasm. Multinucleate giant cells are a common component of tumors with a predominant histiocytic makeup. Because of the resemblance to histiocytic sarcomas in mice with Hertwig’s anemia, Exp. Hemtol. 33: 1118-2911, 2005

**Contributing Institution:** Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota.

**References:**


viable allele of Mcm4 causes chromosome instability and mammary ade nocarcinomas in m ic e. Nature Genetics 39: 93-98, 2007

CASE III – 08014 (AFIP 3103042)

Signalment: 27-year-old, male, cy nomol oug m acaque (Macaca fascicularis), nonhuman primate

History: This animal arrived at Wake Forest University from Ind onesia ab out 9 y ears before de ath. It was diagnosed with type 2 diabetes on 3/6/2008 (glucose 328 g/dL), and on the following day, became lethargic and dehydrated. Insulin treatment and supportive fluid therapy were attempted, but the animal died on 3/9/2008.

Gross pathologic findings: The major gross lesion was in the lung. The entire left lung was four times larger than normal, firm, and tan to yellowish-brown, with adherence to the body wall in some areas (Fig. 3-1, 3-2). Similar changes were present in the middle right lung lobe. Cut surfaces revealed suppurative exudate (Fig. 3-3), and 10-20% of the parenchyma was replaced by dense fibrous connective tissue.

In addition to the pulmonary lesion, the coronary arteries were segmentally thickened by yellow plaques on the intimal surfaces (atherosclerosis).

Microbiology:
Pleural fluid and a lung swab were submitted for bacterial culture. A heavy pure growth (4+) of Corynebacterium ulcerans was recovered.

---

3.1. Lung, Cynomolgus macaque. Suppurative pleuropneumonia.
3.2. Lung, Cynomolgus macaque. Necrosuppurative pleuropneumonia with lymphadenitis and mediastinal edema.

Gross photographs courtesy of Animal Resources Program, Wake Forest University Health Sciences, Winston-Salem, North Carolina.

3-4 Lung, Cynomolgus macaque. The normal lung architecture is lost and there are large coalescing areas of lytic necrosis admixed with a cellular infiltrate. (HE 40X).

3-5 Lung, Cynomolgus macaque. Normal lung parenchyma is necrotic and replaced by fibrin admixed with cellular and karyorrhectic debris. (HE 200X).

3-6 Lung, Cynomolgus macaque. Scattered throughout the areas of necrosis there are large colonies of 1-2 micron diameter bacilli. (HE 400X).

**Histopathologic description:** Lung: The pulmonary architecture is extensively distorted (Fig. 3-4) by a necrotizing inflammatory reaction composed of abundant neutrophils and protein-rich edema fluid, which fills alveoli and bronchioles. Alveolar walls are often effaced (Fig. 3-5) and bronchiolar epithelium is often absent. Alveolar spaces measuring up to 500um in width contain fibrin and edema fluid. Vessels are variably infiltrated by neutrophils and mononuclear phagocytes, contain thrombi, and are surrounded by myriads of short bacterial rods. Similar bacterial colonies are also scattered throughout the pulmonary parenchyma and subpleural space (Fig. 3-6). The pleura is thickened up to 1.5mm owing to the presence of variably mature fibrous connective tissue and suppurative inflammation. Gram staining demonstrates mats of pleomorphic Gram positive bacilli throughout the lung.

**Contributor’s morphologic diagnosis:** Pneumonia, diffuse, chronic, severe, fibrinosuppurative with intralesional bacteria (Etiology: Corynebacterium ulcerans)

**Contributor’s Comment:** Corynebacteria are Gram positive, non-motile, pleomorphic bacilli. (2) Corynebacterium ulcerans was first isolated in 1926 from a human throat lesion, and has since been considered a common cause of laryngitis and cutaneous granulomas in humans. (2) It has also been isolated from abscesses and
Causes pneumonia and mastitis in nonhuman primates. It is considered a commensal of horses and cattle, although it can cause mastitis and cutaneous infections in cattle, and is widely distributed in soil and water. It is often isolated from non-pasteurized milk, the drinking of which has been linked to human infections. Fat al pneumonia caused by C. ulcerans has been reported in non-human primates. A retrospective study of respiratory disease in 272 nonhuman primates (75 cynomolgus macaques, 97 rhesus macaques, 100 vervets) indicated that C. ulcerans and Streptococcus pneumoniae were more common causes of winter respiratory infections in non-human primates.

**Contributing institution:** Animal Resources Program, Wake Forest University Health Sciences, Winston-Salem, NC; [http://www1.wfubmc.edu/pathology/training/index.htm](http://www1.wfubmc.edu/pathology/training/index.htm)

**References:**

**CASE IV – 08012 (AFIP 3103041)**

**Signalment:** 7-year-old, female, African green monkey, Chlorocebus aethiops, non-human primate

**History:** The animal failed to recover from sedation for routine tuberculosis testing.

**Gross pathologic findings:** The animal was se very dehydrated and had multiple g astric ulcerations in the fundus. Both thyroid glands were unremarkable, each weighed 0.17 g and 0.23 g.

**Histopathologic description:** Mostly lymphocytes admixed with fewer plasma cells in the infiltrate of the interstitium diffusely, with multifocal in the infiltration, separating and sometimes sequestering the follicles (Fig. 106).
In some instances the lymphocytes form follicles with germinal center formation (not present in all slides). The thyroid follicles range from 60-150 micro nm in diameter, and are often devoid of colloid. The follicular epithelial cells range from cuboidal to tall columnar, and are usually h ypertrophied, f oming stratified layers, o r papillary pro jections i nto t he lumina. Ten to twenty percent of the em are slig h tly sh runken, hypereosinophilic, and have deeply basophilic nu clei with condensed chromatin (apoptosis). Regularly these follicular epithelial cells are separated from the basement membranes by clear spaces, despite being attached to one another along their lateral sides. So metimes the l umina are filled with shed cells mixed with f amy macrophages and cellular debris enmeshed in amorphous, pale eosinophilic material (Fig. 4-2).

**Contributor’s morphologic diagnosis:** Thyroiditis, diffuse, chronic, marked, lymphocytic, Thyroid Gland.

**Contributor’s Comment:** Chronic lymphocytic thyroiditis is known in humans and non-human primates is commonly recognized as an autoimmune disorder with a poorly und erstood pathogenesis. Diagnoses are made based on the thyroid t est panel, cl inical s igns, a nd histologic findings. Spontaneous thyroiditis h as been reported in la boratory rats, o bese strains of f c hickens, dogs, and a cynomolgus monkey.(2,3)

Two types of au toimmune thyroiditis are described in humans, Graves’ disease and Hashimoto’s thyroiditis, the latter being more frequent.(3,4,6,8) In Graves’ disease, the inflammation is mild, while the thyroid glands are enlarged due to proliferation of thyocytes, resulting in follicular hyperplasia and hypertrophy. Graves’ disease accounts for 50-80% of hyperthyroidism cases in humans, and results from circulating IgG antibodies that bind to and activate the G-protein-coupled thyrotropin receptor. Clinical pathological findings include decreased serum thyrotropin, elevated serum triiodothyronine (T3) and thyroxine (T4), with an increased fraction of T3 relative to T4.(1,3,6)

In Hashimoto’s thyroiditis, the lymphocytic infiltration is prominent, often with germinal center formation, causing enlargement of the thyroid glands and destruction of the thyroid follicles, leading to hypothyroidism. The pathogenesis of Hashimoto’s thyroiditis involves a delayed hypersensitivity reaction to thyroid epitopes. Sensitization of autoreactive CD4 + T-helper cells to these initiates the immunologic events leading to death of thyrocytes. The effector mechanisms include destruction of thyrocytes by CD8+ cytotoxic T cells by exocytosis of perforin/granzyme granules or engagement of the death receptor, and reproduction of inflammatory cytokines by CD4+ T cells, and the binding of antithyroid antibodies (anti-TSH receptor, antithyroglobulin, anti-thyroid peroxidase antibodies) followed by antibody-dependent cell-mediated cytotoxicity. Hypothyroidism usually develops gradually, although in some cases it may be preceded by transient thyrotoxicosis due to the disruption of thyroid follicles, with secondary release of...
thyroid hormones (hashitoxicosis).(2,3,6,8)

As there is relatively little literature on African green monkeys, normal reference ranges for humans (7) were used to confirm the presence of autoimmune thyroiditis in this case. Serum-binding assays to measure thyroxine (T4) and triiodothyronine (T3) autoantibodies revealed 15% and 3% more binding of the immunoglobulin to the T4 and T3, respectively, compare to each negative control. Low TT4 and FT4 support a diagnosis of hypothyroidism, which, combined with the histological appearance, resembles Hashimoto’s thyroiditis in humans. Values of thyroid stimulating hormone (TSH), autoantibodies against thyroid peroxidase, or TSH receptor are not available due to the limitation of serum available for testing.

**AFIP Morphologic Diagnosis:** Thyroid gland: Thyroiditis, lymphoplasmacytic, chronic, diffuse, marked, with follicular hyperplasia and colloid depletion.

**Conference Comment:** Other animal species with autoimmune thyroiditis resembling Hashimoto’s disease include dogs, obese strains of chickens, nonhuman primates, and Buffalo rats. The pathogenesis of autoimmune thyroiditis in the dog is not completely understood, but it seems to stem from production of autoantibodies directed against a variety of targets in the

4-1 Thyroid gland, African green monkey. Multifocally, expanding the thyroid interstitium is a cellular infiltrate that often forms lymphoid follicles with vague germinal centers. (HE 40X).
Migration of lymphocytes and plasma cells between follicles causes follicular cells to lose their attachment to the basement membrane, and this leads to sloughing of cells into the lumen of the follicle. Ly mphoid cells also migrate into the lumen, and this combination of changes leads to eventual death of the follicle. While the damage to follicles is ongoing, adjacent follicles undergo hypertrophy in an attempt to keep up with demand and in response to increased TSH secretion. Eventually the parenchyma of the thyroid gland is replaced by fibrous connective tissue. At this stage, only scant residual inflammatory cells and small, widely dispersed “end-stage” follicles with a small amount of vacuolated colloid remain. 

4-2 Thyroid gland, African green monkey. Multifocally thyroid follicles are lined by hypertrophic columnar epithelium with abundant, lightly eosinophilic, finely granular cytoplasm and often follicular lumina are devoid of colloid and contain histiocytes, lymphocytes and plasma cells. (HE 400X).

Contributing institution: Animal Resources Program, Wake Forest University Health Sciences

References: