



WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 14

9 January 2018

CASE I: NE 17-176 (JPC 4101745).

Signalment: 6-year-old neutered male
brindle Mastiff dog, *Canis familiaris*



History: This dog had a 10-minute seizure at home and afterwards he was blind. He had had a similar, but less severe episode of altered mental status several weeks prior. Physical exam findings included dull mentation, circling to the right, and negative menace response. After premedication for imaging, the dog developed ventricular tachycardia and was euthanized.

Gross Pathology: The dog was in obese nutritional condition (body condition score 4.5/5) and the tail was alopecic. The thyroid glands were small (right 3x2x32mm; left 5x3x30mm). The walls of many vessels including the coronary arteries, prostatic vessels, and meningeal vessels had white opaque firm walls. The myocardium adjacent to affected coronary vessels was often red.

Laboratory results: None.

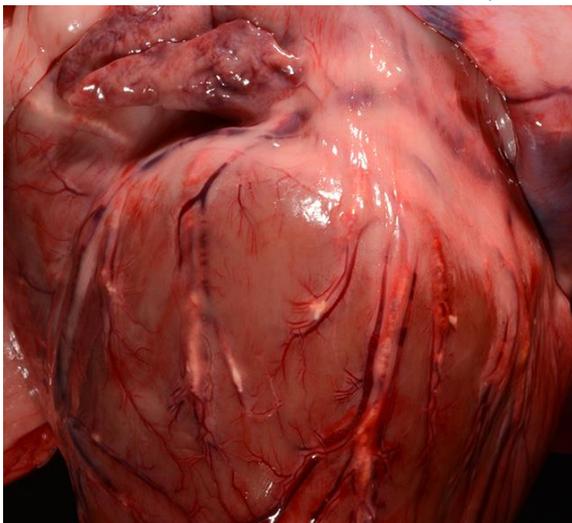
Microscopic Description:

Thyroid: The thyroid glands are small and lack follicles. The follicles are replaced by dense infiltrates of small lymphocytes and plasma cells. The remaining C (parafollicular) cells are prominent. The

Thyroid gland, dog. The thyroid glands were small. Vessels within the gland and fibroadipose tissue are occasional opaque and whitish-yellow prominent due to mural accumulation of cholesterol and inflammatory cells. (Photo courtesy of: University of Tennessee College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences, 2407 River Drive, Room A205, Knoxville, TN 37996, https://vetmed.tennessee.edu/departments/Pages/biomedical_diagnostic_sciences.aspx)

vessels within and around the gland have foamy macrophages and acicular (cholesterol) clefts replacing and expanding their walls, often resulting in occlusion of the lumen. The parathyroid gland is unremarkable.

Artery and surrounding vessels: The muscular artery at the center of the section (taken from the hilus of the liver) has mild multifocal expansion of the tunica muscularis by foamy macrophages and small acicular clefts (not present in all sections). The surrounding smaller vessels have severe replacement and expansion of their walls by foamy macrophages and acicular (cholesterol) clefts. The deposits often narrow or occlude the lumens, which



Heart, dog. Atherosclerotic plaques are prominent within coronary arteries. (Photo courtesy of: University of Tennessee College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences, 2407 River Drive, Room A205, Knoxville, TN 37996, https://vetmed.tennessee.edu/departments/Pages/biomedical_diagnostic_sciences.aspx)



occasionally contain thrombi. There is mild lymphoplasmacytic inflammation within the adventitia of the affected vessels.

Contributor's Morphologic Diagnoses:
Severe chronic diffuse lymphocytic thyroiditis with follicular atrophy
Severe multifocal chronic vascular atherosclerosis with thrombosis.

Contributor's Comment: Lymphocytic thyroiditis in dogs is similar to Hashimoto's disease of humans. Both are thought to have a polygenic inheritance. Predisposed dog breeds include: boxers, bulldogs, dachshunds, great Danes, Doberman

Prostate gland. Atherosclerotic plaques are prominent within prostatic and paraprostatic arterioles. (Photo courtesy of: University of Tennessee College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences, 2407 River Drive, Room A205, Knoxville, TN 37996, https://vetmed.tennessee.edu/departments/Pages/biomedical_diagnostic_sciences.aspx)

pinschers, golden and Labrador retrievers. Circulating autoantibodies to thyroglobulin are present in affected humans and dogs. Interestingly, the infiltrating lymphocytes and plasma cells are thought to mechanically dislodge the thyroid follicular epithelial cells from their basement membranes resulting in destruction of the follicle.³

Knoxville, TN 37996

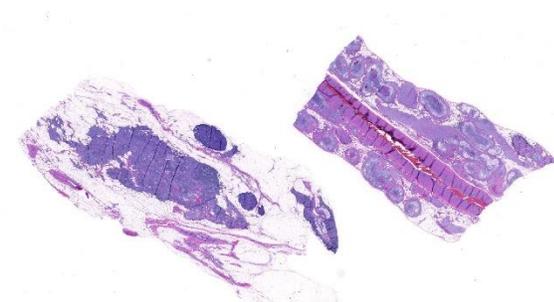
The vessel (and nerve) bundle on the submitted slides was taken from the hilus of the liver. The central vessel is presumed to be the hepatic artery (although pretty small for a mastiff). The surrounding more severely affected vessels were presumed to be veins, but veins are reportedly unaffected in canine atherosclerosis.⁴ Grossly, they resembled lymphatic vessels, but those are also presumably unaffected by atherosclerosis.

This dog had a history of hypothyroidism and had been recently restarted (too late) on his thyroid supplementation in response to his clinical signs. The seizures were likely due to ischemic brain lesions; meningeal and cerebral vessels were occluded by atherosclerosis. Coronary artery atherosclerosis and the resulting myocardial ischemia likely caused the arrhythmia. Externally the findings consistent with hypothyroidism were obesity and tail alopecia.

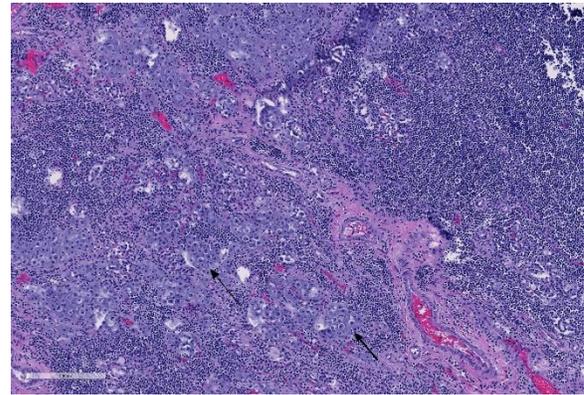
In summary, this was a case of lymphocytic thyroiditis which led to hypothyroidism which led to hyperlipidemia which led to widespread atherosclerosis which led to cerebral and myocardial ischemia which led to seizures and arrhythmia, respectively.

Contributing Institution:

University of Tennessee College of Veterinary Medicine
Department of Biomedical and Diagnostic Sciences
2407 River Drive, Room A205



Thyroid gland (left) and hepatic hilar vessels: The thyroid gland (left) is largely effaced by an inflammatory infiltrate. At right, hepatic hilar arterioles are tortuous and markedly expanded. (HE, 5X)

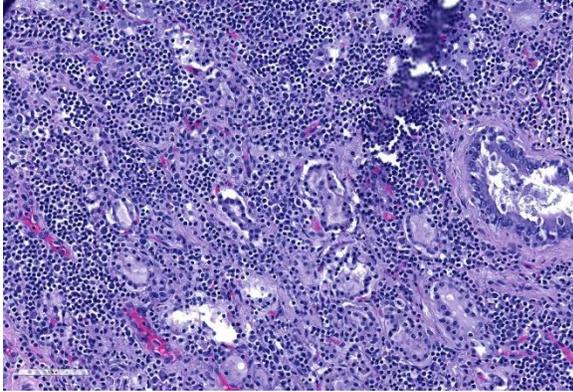


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Thyroid gland: Follicular architecture is lost and replaced by marked lymphoplasmacytic inflammation. Large nests of interfollicular cells remain (arrows). (HE 154X)

JPC Diagnosis: 1. Thyroid gland: Thyroiditis, lymphoplasmacytic, diffuse, severe with severe follicular atrophy and moderate parafollicular hyperplasia.
2. Thyroid gland, adjacent perivascular fibroadipose tissue, arterioles: Atherosclerosis, diffuse, severe with thrombosis and recanalization.

JPC Comment: As discussed above, this case is an excellent example of primary hypothyroidism due to canine lymphocytic thyroiditis (CLT) in the dog. According to various studies, 65-80% of cases are the result of autoimmune disease, with resulting lymphoplasmacytic thyroiditis and destruction of the thyroid gland.² A number of breeds show a breed predisposition for hypothyroidism, including dobermans, Great Danes, poodles, Irish Setters, miniature and giant schnauzers, boxers, golden retrievers, dachshunds, Shetland Sheepdogs, Pomeranians, Cocker Spaniels and Airedales and Hovawarts.¹ Both cellular and humoral mechanisms of immunity are involved in this pathogenesis. Affected animals generally exhibit decreased thyroxine and

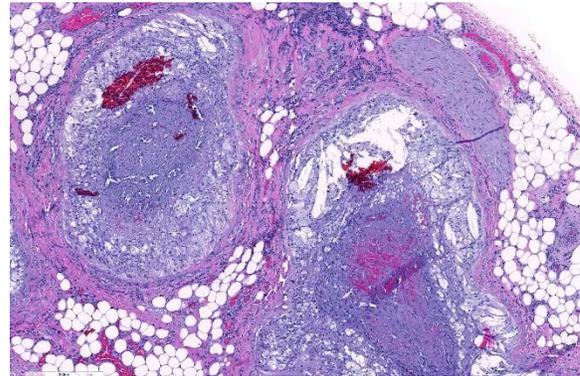


Thyroid gland: Remaining follicles are lined by cuboidal, often detached epithelium and do not contain colloid. A cross section through a Kursteiner's cyst is present at right. (HE, 190X)

75% will demonstrate decreased serum TSH levels. Autoantibodies against thyroglobulin, thyroxine, and triiodothyronine correlate well with lymphocytic thyroid inflammation. In humans, immune complexes containing thyroglobulin have been identified in the basement membrane of thyroid follicles, which may induce NK cell activity and/or complement activation.¹

A sensitive assay for thyroglobulin autoantibodies (TGAA) is available and may be positive in animals at a young age, but progression of the disease is variable and cannot be accurately predicted. TGAA positivity has been identified as the first stage of subclinical canine lymphocytic thyroiditis.¹ Even when up to 60%-70% of the thyroid has been replaced by lymphocytic inflammation, hypothyroidism may yet be subclinical as a result of overstimulation of remaining follicles by elevated TSH.¹ In end stage hypothyroidism, in which almost all thyroid follicles are destroyed (similar to that seen in this case), TGAA may decrease, but TSH, T3, and T4 levels remain abnormal.¹ Boxers, Giant Schnauzers, and Hovawarts have been shown to develop CLT with elevated TGAA levels.¹

The foamy basophilic to amphophilic cells within the thyroid were discussed during the conference. Some participants thought they represented "oncocytes" (thyroid follicular cells), others believed they were macrophages, while others still thought there were parafollicular c-cells. Participants reviewed a battery of immunohistochemical stains (TTF-1, Iba-1, and synaptophysin and chromogranin-A). The cells in question are diffusely immunoreactive to neuroendocrine markers (chromogranin-A) indicating c-cell origin. It was unclear to the participants whether these cells were hyperplastic, hypertrophic, or simply remnants of normal cells.



Hepatic hilus, dog: The walls of hilar arterioles is markedly expanded by plaques of foam cells and cholesterol clefts admixed with cellular debris. The tunica intima is effaced and the lumen has been remodeled and recanalized. (HE, 85X)

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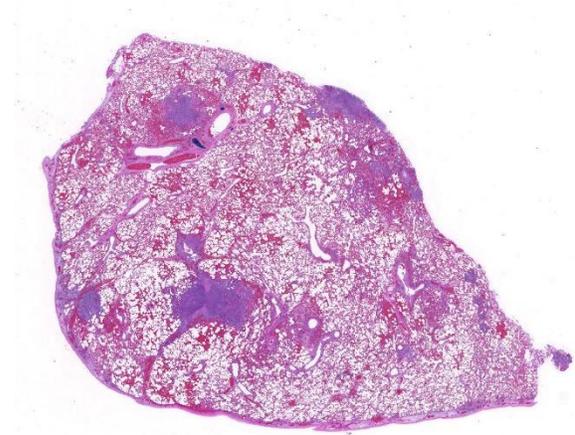
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CASE II: S1603556 (JPC 4084299).

Signalment: A 1-year-old Quarter horse filly

History: The filly had a history of 10-day lethargy. The owner was suspicious of a rattlesnake bite.

Gross Pathology: The lip was diffusely thickened with edema. Both submandibular lymph node were enlarged, firm and dark red. Bilaterally, the parietal pleura had multiple petechiae and ecchymoses. Multifocally in both lungs there were numerous white, firm nodules, approximately 2 to 3mm in diameter, surrounded by a dark red halo. The parenchyma between these nodules was dark red and firm. The pericardium, mainly in proximity to the coronary vessels and paragonal septum, also showed several multiple petechiae and ecchymoses. The kidneys had several multifocal white, firm cortical spots of approximately 1 to 3mm in diameter. The cortex was diffusely pale. The mesentery had multifocal random white firm nodules of approximately 3 to 5 mm in diameter surrounded by a dark red halo.



Lung, horse. At subgross magnification, areas of a dense cellular infiltrate as well as foci of hemorrhage are scattered randomly throughout the section. (HE, 4X)

Laboratory results: No bacterial pathogens were isolated from liver, lung, spleen and submandibular lymph node on aerobic culture.

Microscopic Description:

Lung: centering in small caliber vessels and extending into the adjacent alveolar spaces and septa, there is a multifocal and random inflammatory infiltrate composed by large numbers of viable and degenerated neutrophils, fewer histiocytes and occasional multinucleated giant cells that are admixed with basophilic cellular debris and several rounded eosinophilic protozoa of approximately 15 to 20 μm in diameter, with an eccentric single round nucleus. In addition, there are numerous fibrin thrombi and segments of alveolar necrosis. A few blood vessels display transmural necrosis, with fibrinoid degeneration and the presence of pyknotic debris. The remaining parenchyma shows moderate congestion, occasional small hemorrhages and multiple focal areas of intra-alveolar edema.

Contributor's Morphologic Diagnosis: Embolic pneumonia, necrosuppurative,

severe, multifocal, with numerous trophozoites.

Etiology: *Acanthamoeba sp.*

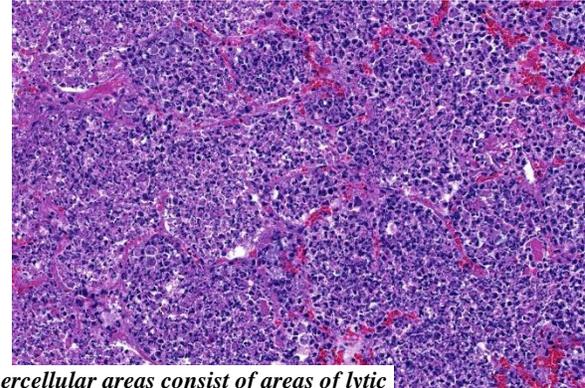
Additional Findings: Trophozoites were immunolabeled for *Acanthamoeba sp.* in the lung, kidney and lymph node via immunohistochemistry test. In addition, a lymphohistiocytic to granulomatous interstitial nephritis, myocardial necrosis and mineralization, focal suppurative myositis; moderate to severe suppurative, lympho-plasmacytic and histiocytic, necrotizing peritonitis, gastritis and hepatitis, with protozoa consistent with *Acanthamoeba sp.* were also observed.

Contributor's Comment: *Acanthamoeba sp.* are ubiquitous free-living amoebae distributed in the environment.^{2,3} Disease produced by these amoebae have been described in cows, dogs, pigs, rabbits, pigeons, sheep, reptiles, fish, turkeys, rhesus macaque and toucans.^{7,8} Amoebic infection in horses is very rare and it has been reported in Southern California, USA, causing pneumonia, meningoencephalitis⁴ or systemic infections, and in placentitis in the state of New South Wales, Australia¹. The reason for these presentations is still not clear. Amoebas can be easily missed in histology sections because of the morphology of their trophozoites, which resemble macrophages¹. Reports of human infection with *Acanthamoeba sp.* have traditionally been limited to cases of systemic disease in the immunocompromised, granulomatous encephalitis, keratitis and cutaneous and sinus lesions.²

Acanthamoeba has two stages in its life cycle, a vegetative trophozoite stage with a diameter of 13-23 µm and dormant cyst stage of 13-23 µm. During the trophozoite

stage, *Acanthamoeba* feeds on organic particles as well as other microbes and divides mitotically under optimal conditions.

⁵ It has been proposed as a reservoir of several



Lung, horse: Hypercellular areas consist of areas of lytic necrosis with discontinuous, thrombosed, and necrotic alveolar septa and expansion of alveoli by innumerable degenerate neutrophils admixed with cellular debris. (HE, 335X)

microorganisms as *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella spp.* and *Streptococcus pneumoniae*.²

Amoebae can be cultured from lesions by special methods, which are not available in most diagnostic laboratories. Speciation may be accomplished by fluorescent antibody testing and immunohistochemistry.³

Information on treating topical and systemic amoebic infections in animals is lacking.³

Contributing Institution:

California Animal Health and Food Safety Lab. San Bernardino Branch. University of California, Davis

JPC Diagnosis: Lung: Pneumonia, necrosuppurative, multifocal to coalescing, moderate, with numerous extracellular amebic trophozoites.

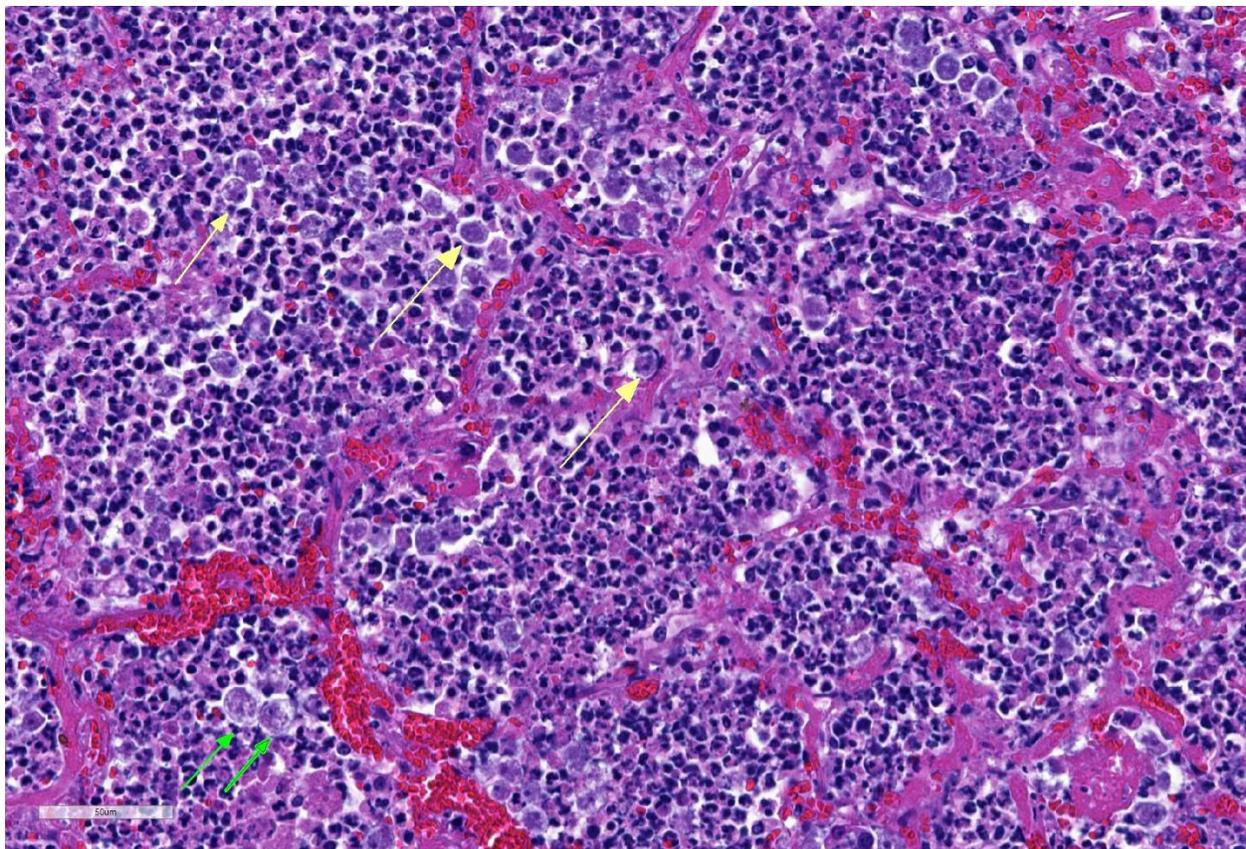
JPC Comment: Free-living ameba are ubiquitous protozoans in the environment, of which four generally are considered

pathogenic for humans and animals: *Acanthamoeba*, *Balamuthia*, *Naegleria*, and *Sappinia*.¹

Acanthamoeba appear to be most often associated with disease in humans and animals, with 18 distinct genotypes based on nuclear small-subunit ribosomal DNA rather than morphology. The most common condition associated with infection in humans is a chronic keratitis, seen in immunocompetent patients associated with improper handling of contact lenses, exposure to contaminated water, or trauma. Risk factors of contact lens users include the use of all-in-one solutions, showering while wearing contact lenses, and poor contact lens hygiene.⁵ Granulomatous amebic encephalitis is a well-documented syndrome

in humans resulting from hematogenous spread, often from the lower respiratory tract or skin lesions. It shows a chronic fatal progression with luckily only 150 documented cases worldwide.⁵ Due to its hematogenous origins, areas of granulomatous inflammation are seen throughout all parts of the brain. Cutaneous acanthamoebiasis is also an uncommon opportunistic condition primarily seen in immunosuppressed patients, resulting in erythematous sores and skin ulcers.¹

Primary amebic meningoencephalitis (PAM) is another rare fatal disease in humans caused by *Naegleria fowleri*. This condition generally occurs in healthy children and adults swimming or bathing in warm freshwater ponds. Infective amebae migrate



Lung, horse. Areas of necrosis contain numerous 15-20um amebic trophozoites with prominent nuclei and karyosomes (green arrow) and 20-30 amebic cysts. (HE, 400X)

along olfactory nerves from the nose to the brain; fatal infection proceeds rapidly and is almost always fatal. Due to this unique entry portal, areas of lytic necrosis are clustered at the base of the brain; hypothalamus, pons, and occasionally seen in posterior areas such as the medulla oblongata.

Balamuthia mandrillaris is also a cause of granulomatous amebic encephalitis which ranges in duration between *Acanthamoeba* and *Naegleria*, which usually results from hematogenous spread from soil-contaminated wounds.

Other species of free-living amoeba which may have been identified in cases of keratitis include *Hartmanella*, *Vahlkamphia*, and *Allovahlkampfi* *sp.*¹

As the contributor mentioned above, free-living amoebae may act as vectors for a wide range of pathogenic bacilli. They may also act as hosts for a range of viruses, including coxsackieviruses and adenoviridae pathogenic for humans. Other viruses, the so-called giant viruses, may act as endocytobionts, including representatives of the *Mimi*-, *Moumo*- and *Megaviridae*, as well as *Pandoviridae*. Co-cultivation in amoebae has been of great benefit in the eventual isolation of these putative human pathogens.

The moderator and participants could not definitively identify cysts in tissues although PAS and GMS were run, they were of no help in identifying cysts. Megakaryocytes were identified in the alveolar capillaries. The moderator noted that they can normally be found there as the lungs are a site of thrombopoiesis and as such can serve as reservoirs for platelet production and release them in response to various stimuli.⁹ Finally, the moderator reviewed the various types of

free-living amoebae, and participants discussed the associated syndromes in humans and animals.

References:

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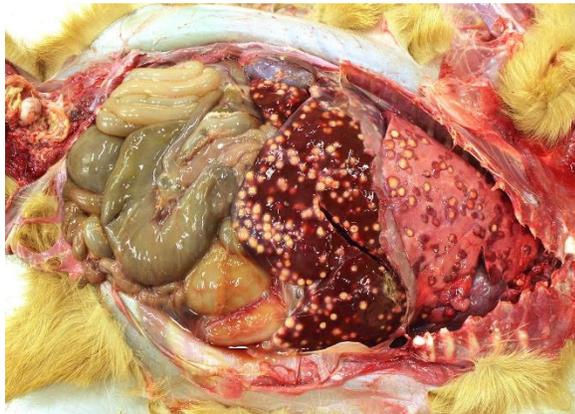
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CASE III: S1603556 (JPC 4084299).

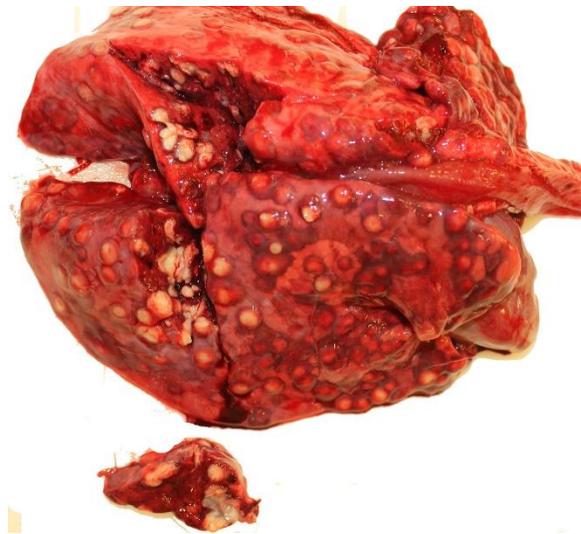
Signalment: A 14-month-old, 5 kg, female, Nigerian dwarf goat (*Capra hircus*)

History: A total of 4 goats in an original herd of 8, died in a period of 8 months with a history of limping, progressive loss of body condition, and difficulty in getting up. Three goats died naturally and one was euthanized. This goat died naturally.

Gross Pathology: The thoracic cavity contained approximately 25 mL of thin, pink fluid. The lungs and liver had innumerable disseminated, soft to semi-firm, pale yellow, 0.3-0.6 cm diameter, occasionally raised nodular foci scattered throughout the



Viscera, goat: The lungs and liver had innumerable disseminated, soft to semi-firm, pale yellow, 0.3-0.6 cm diameter, occasionally raised nodular foci scattered throughout the parenchyma. (Photo courtesy of: University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources <http://patho.uconn.edu/>)



parenchyma, which frequently oozed pale yellow, thick liquid upon sectioning. The spleen had a few similar foci.

Lung, goat. The lungs had innumerable disseminated, soft to semi-firm, pale yellow, occasionally raised nodular foci scattered throughout the parenchyma, which frequently oozed pale yellow, thick liquid upon sectioning. (Photo courtesy of: University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources <http://patho.uconn.edu/>)

Laboratory results: Bacteriology: aerobic bacterial culture of the liver yielded heavy growth of *Rhodococcus equi*, *Streptococcus sp.* and *Staphylococcus sp.*

Microscopic Description:

Lung: centering in small caliber vessels and extending into the adjacent alveolar spaces and septa, there is a multifocal and random inflammatory infiltrate composed by large numbers of viable and degenerated neutrophils, fewer histiocytes and occasional multinucleated giant cells that are admixed with basophilic cellular debris and several rounded eosinophilic protozoa of approximately 15 to 20 μ m in diameter, with an eccentric single round nucleus. In addition, there are numerous fibrin thrombi

and segments of alveolar necrosis. A few blood vessels display transmural necrosis, with fibrinoid degeneration and the presence of pyknotic debris. The remaining parenchyma shows moderate congestion, occasional small hemorrhages and multiple focal areas of intra-alveolar edema.

Contributor's Morphologic Diagnosis:

Lung: marked, multifocal to coalescing, pyogranulomatous, pneumonia with myriad intrahistiocytic, gram-positive, coccobacillary bacteria

Other tissues (Slides not submitted):

Liver: marked, multifocal to coalescing, pyogranulomatous, hepatitis with intrahistiocytic, gram-positive, coccobacillary bacteria

Spleen: 1. mild, multifocal, nodular, pyogranulomatous, splenitis with multifocal mineral deposition, intrahistiocytic, gram-positive, coccobacillary bacteria; 2. marked histiocytosis

Skeletal muscle: marked, focally extensive, pyogranulomatous, myositis with myriad



Contributor's Comment: The morphology and distribution of the lesions found in this goat were consistent with a disseminated rhodococcal infection. In goats, this is a recognized cause of disseminated pulmonary and hepatic abscesses^{2, 4}. *Rhodococcus equi* is classified largely as a soil organism, but has been isolated from the feces of birds and grazing herbivores. It has been suggested that *R. equi* may be widespread in *Lung, goat*. 75% of the section is replaced by discrete pyogranulomas. Intervening alveoli are expanded by edema, an inflammatory infiltrate, and multifocal emphysema. (HE, 5X)



Liver, goat. The liver had innumerable disseminated, soft to semi-firm, pale yellow, occasionally raised nodular foci scattered throughout the parenchyma. (Photo courtesy of: University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources <http://patho.uconn.edu/>)

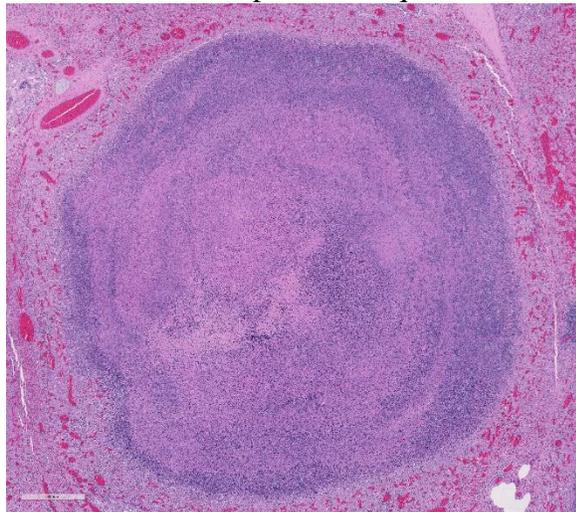
herbivores and their environment because their manure supplies the substrates on which the organism thrives⁵. Inhalation or ingestion of the bacteria is thought to be the major mode of transmission, causing the development of respiratory or enteric infections, respectively⁸. Subsequent bacteremia and hematogenous dissemination within macrophages to other sites in the body then occurs⁸.

Rhodococcus equi is a facultative intracellular gram-positive bacterium which proliferates in macrophages and multinucleated giant cells⁸. *R. equi* causes two primary forms of disease in foals which are pyogranulomatous bronchopneumonia

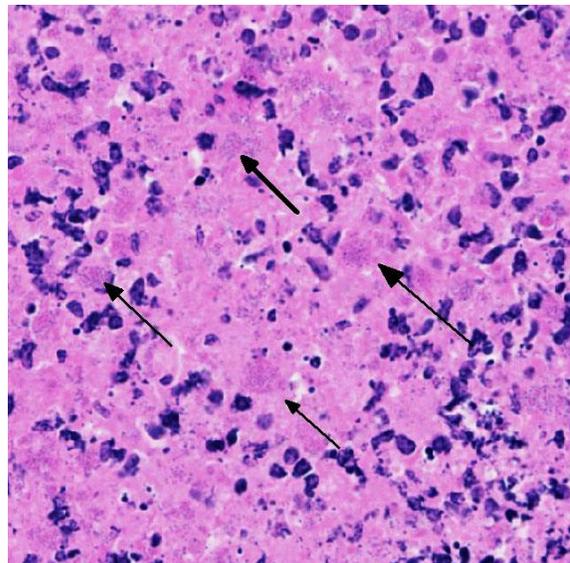
intra-histiocytic coccobacillary bacteria

and ulcerative enterocolitis. In addition, ulcerative typhlitis with suppurative or granulomatous lymphadenitis, osteomyelitis, synovitis, septic arthritis, and rarely abortion are reported in horses with *R. equi* infection^{6, 8}. *R. equi* has also been reported to cause pneumonia in immunosuppressed people, especially in AIDS patients³. *R. equi* infections are rarely reported in cattle, sheep, pigs, cats, dogs and camelids^{1, 8}. In goats, *R. equi* typically causes pyogranulomatous lesions in the liver and lungs^{2, 4}. Occasionally, osteomyelitis of the vertebra and skull and fibrinous enterocolitis have also been reported in goats⁴.

The virulent forms of *R. equi* contain a plasmid containing a vapA gene (virulence associated protein A) which encodes a 15-17 kDa cell surface lipid protein. This protein can elicit an intense humoral response^{7, 8}. The bacterium is able to prevent phagosome-lysosome fusion by inhibiting opsonization or activation of macrophages by IFN-gamma resulting in bacterial survival within the macrophage, therefore, an intense Th-1 response is required to clear



Lung, goat: Higher magnification of a pyogranuloma with a lamellated center of eosinophilic and basophilic cellular debris. Viable inflammatory cells populate the periphery, enmeshed in collagen and dilated capillaries. (HE, 34X)



Lung, goat: Scattered throughout the necrotic center are numerous macrophages containing 1-3 coccobacilli within their cytoplasm (arrows). (HE, 400X)

an established infection⁸.

Contributing Institution:

University of Connecticut
Connecticut Veterinary Medical Diagnostic
Laboratory,
Department of Pathobiology and Veterinary
Science
College of Agriculture, Health and Natural
Resources

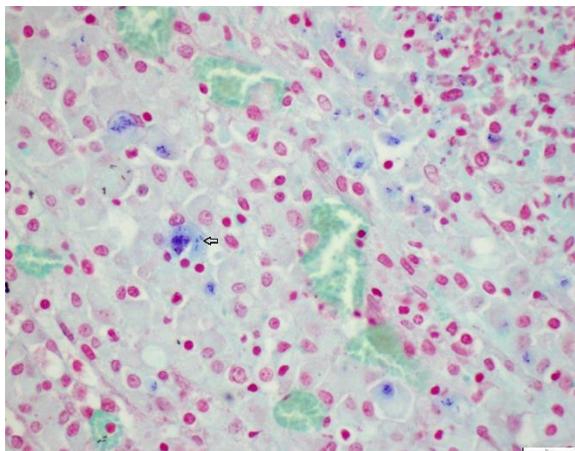
<http://patho.uconn.edu/>

JPC Diagnosis: Lung: Pyogranulomas, multiple, with numerous intrahistiocytic coccobacilli.

JPC Comment: *R. equi*'s virulence-associated proteins (vapA in virulent strains, and vapB in intermediately virulent strains) are encoded on circular and linear plasmids. Strains that do not contain these proteins are classified as avirulent. Recently, a novel *R. equi* host-adapted linear virulence plasmid, pVAPN was characterized in cattle isolates, which encodes another virulence-associated protein (vapN). VapG, another protein

identified in virulent strains has recently been identified as a potential vaccinal protein which has shown efficacy in the partial protection of *R. equi* in mice (in addition to current vaccinal work with vapA). In addition, a highly conserved conjugal transfer protein gene (*traA*) is common to all strains which carry these plasmids and may now be used to identify pathogenic strains.¹ In previous studies, the virulent and intermediately virulent strains are primarily identified in foals and pigs, while most reports of *R. equi* isolated from cattle and dogs appear to be of avirulent (non-vapA, non-vapB) strains.¹

A recent article by Bryan et al¹ describes the clinical syndrome associated with *R. equi* infection in five dogs. Two of the isolated strains were avirulent, one was vap-A positive, and one carried the vapN virulence-associated plasmid. Four dogs were on immunosuppressive drugs or had endocrinopathies. The varied presentation and distribution of disease in the five dogs reflects the systemic and almost random nature of *R. equi* infection of the dog, with granulomatous dermatitis, aortic vegetative valvular endocarditis, pulmonary abscessation, and lymphadenitis the most common presentation, but each only being present in two out of five cases.



Lung, goat: The bacteria within the macrophages (arrow) stain positively with a tissue Gram stain (Twort's, 600X)

Considered an emerging pathogen in humans, over 100 cases of *R. equi* infection have been reported since the first description of its occurrence in 1967, likely as a result of improvement in isolation techniques and recognition of this bacterium as a human pathogen.⁹ The vast majority of cases have occurred in immunosuppressed patients with over 66% in cases in AIDS patients, and an additional 10% in transplant recipients as a late complication (mean 49 months post-transplant) of immunomodulation. 80% of cases involved pulmonary infection, most commonly abscesses with necrotic centers, although pulmonary disease characterized by microabscesses is also common. As in other species, pyogranulomatous infection may occur in a wide range of organs. Treatment involves regimes of multiple antibiotics (often including rifampin) and is complicated by the typical presence of immuno-suppression in affected individuals.⁹

The moderator reviewed the differences between *Corynebacterium pseudotuberculosis* and *Rhodococcus equi*. Additionally, he identified several recent review articles discussing rhodococcal infection in several atypical species such as dogs¹ and goats^{2,4}.

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CASE IV: Case 1 (JPC 4103659).

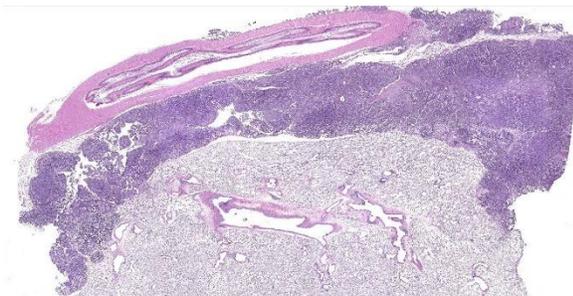
Signalment: 2-year old, male, NCTR Sprague Dawley (CD) rat, (*Rattus rattus*)

History: This male rat was part of a 2-year carcinogenesis study that included prenatal and perinatal exposure to the test article. The animal survived to terminal sacrifice and was euthanized via CO₂ inhalation.

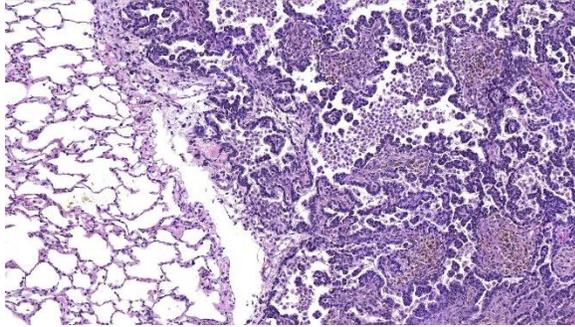
Gross Pathology: At necropsy, an extensive tumor was identified within the thoracic cavity that involved the lung, pleura, pericardium, diaphragm, and rib.

Laboratory results: None.

Microscopic Description:



Lung, NCTR Sprague Dawley (CD) rat. Subgross image of an expansile neoplasm involving the lung, pleura, and adjacent rib. (HE, 6X) (Photo courtesy of: EPL, Inc., P.O. Box 12766, Research Triangle Park, NC 27709 <http://epl-inc.com/>)



Lung, NCTR Sprague Dawley (CD) rat. The neoplasm is composed of cuboidal to columnar cells arranged in tubules and cords as well as papillary and alveolar-like structures. (HE, 100X) (Photo courtesy of: EPL, Inc., P.O. Box 12766, Research Triangle Park, NC 27709 <http://epl-inc.com/>)

Lung: a multifocal, unencapsulated, expansile, neoplasm is diffusely expanding the pleural surface, compressing the adjacent lung parenchyma and adhering to the costal pleura of the adjacent rib. The neoplasm is composed of epithelial cells, arranged in tubules and cords, as well as papillary and alveolar-like structures that are supported by small amounts of fine, fibrovascular stroma. Neoplastic cells are cuboidal to columnar, with moderate amounts of finely fibrillar, eosinophilic cytoplasm and a single, round to oval, central to basilar nucleus, with 1-3 variably distinct nucleoli and stippled chromatin. Anisocytosis and anisokaryosis are minimal and the mitotic rate is low with 0-2 mitotic figures per 40X field. Some areas within the neoplasm contain abundant fibrous connective tissue (schirrous response) infiltrated by large numbers of hemosiderin-laden macrophages. Large influxes of foamy, alveolar macrophages are frequently observed within the neoplasm. Multifocal areas of hemorrhage, emphysema and edema are also present.

Immunohistochemistry: neoplastic cells exhibit a similar staining pattern to that of alveolar-bronchiolar (AB carcinoma) with positive cytoplasmic staining with SPC and

cytokeratin 18 antibodies and negative staining with vimentin and CC10.

Contributor's Morphologic Diagnosis:
Lung: Alveolar-bronchiolar carcinoma (bronchiolo-alveolar carcinoma)

Contributor's Comment: Alveolar-bronchiolar carcinomas (AB carcinomas), are the most commonly induced pulmonary malignancies in rodent studies conducted by the National Toxicology Program (NTP).² However, the current literature now refers to these types of tumors as bronchiolo-alveolar carcinomas.² Although the cell of origin for bronchiolo-alveolar tumors remains somewhat controversial, the International

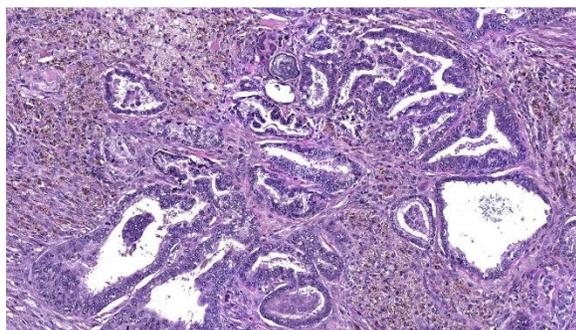
Lung, NCTR Sprague Dawley (CD) rat. Areas of abundant fibrous connective tissue (schirrous response) with large numbers of hemosiderin-laden macrophages are also observed. (HE, 200X) (Photo courtesy of: EPL, Inc., P.O. Box 12766, Research Triangle Park, NC 27709 <http://epl-inc.com/>)

Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidance document on rodent respiratory lesions indicates that bronchiolo-alveolar carcinoma may originate from club cells (formerly known as Clara cells) or type II alveolar cells, but more commonly arise from type II alveolar cells.^{2,5} These types of tumors typically invade the lung parenchyma as poorly circumscribed, nodular masses that may occupy an entire lung lobe and infiltrate adjacent tissues.¹ However, extra-pulmonary AB carcinomas that are largely mediastinal have been reported.⁴

Several growth patterns for AB carcinoma have been described in the rat, these include: Alveolar (glandular) patterns - consisting of cuboidal to columnar cells forming glandular structures, papillary growth - cuboidal to columnar cells forming papillary structures supported by a connective tissue core, tubular patterns - prominent elongated

tubules, solid- composed of tightly arranged round cells with no visible space in-between or mixed patterns where multiple patterns are apparent. Marked pleomorphism, areas of squamous metaplasia or abundant fibrosis (schirrous response) are additional features that have been described in rats and mice as well as large influxes of macrophages into the tumor and adjacent alveoli, as observed in this case.^{1,5}

This case was presented to a pathology working group (PWG) because the study and reviewing pathologists were torn between a diagnosis of AB carcinoma or malignant mesothelioma. Immunohistochemistry was applied to help determine the cell of origin. The neoplasm exhibited IHC staining characteristics similar to a positive AB Carcinoma control that was pulled from the NCTR archives. The majority of neoplastic cells demonstrated positive cytoplasmic staining for SPC (surfactant protein C, alveolar type II cells) and CK18 (cytokeratin 18, epithelial cells) markers, confirming that the neoplasm was most likely an epithelial tumor arising from type II alveolar cells. The cells lining the pleural surface of the neoplasm appeared to represent a layer of reactive mesothelium, as these cells stained positive with both CK18 and vimentin stains, similar to a malignant mesothelioma control also pulled from the NCTR archives; most of the neoplasm was negative for vimentin. Based on the IHC profile, the tumor was diagnosed



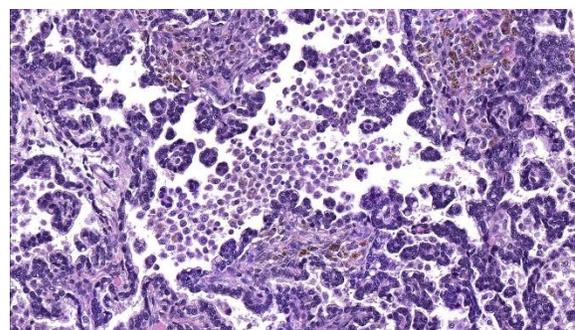
Lung, NCTR Sprague Dawley (CD) rat. Lung, NCTR Sprague Dawley (CD) rat. Large influxes of foamy alveolar macrophages are frequently found within neoplastic structures. (HE, 400X) (Photo courtesy of: EPL, Inc., P.O. Box 12766, Research Triangle Park, NC 27709 <http://epl-inc.com/>)

an AB carcinoma. However, it should be noted, that not every pathologist that reviewed these findings were convinced of the diagnosis.

Contributing Institution:

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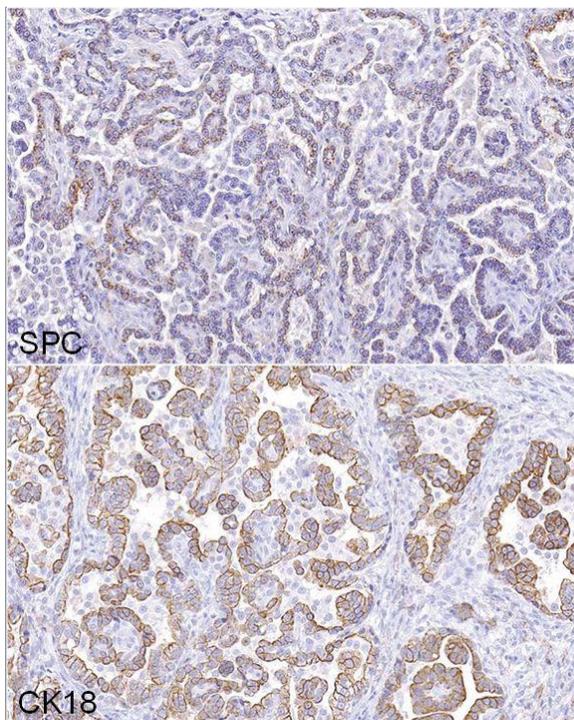
JPC Diagnosis: Lung: Alveolar-bronchiolar carcinoma, papillary type.



JPC Comment: A review of the archives of the National Toxicology Program (NTP) almost a decade ago identified the lung as the organ that is third most common target site of chemical carcinogens in the female rat (with liver and mammary gland preceding it) and as an uncommon site in male rats.² Inhalation and gavage studies account for 70% of tumors at approximately 35% each, with water-dosing, IP, topical, and *in utero* exposure accounting for the remainder. Spontaneous A/B neoplasms of the lung occur at incidences of less than 4% in both genders of F344 rats, but over 90% of chemically induced pulmonary neoplasm. The most common site of pulmonary metastases in these rats, interestingly, the skin.²

Alveolar-bronchiolar adenocarcinoma is considered one of the best models for non-small cell lung cancer (NSCLC) in humans. K-RAS, EGFR, and TP53 are the three most

commonly altered genes “driver mutations” in NSCLC, with TP53 genes exhibit the highest frequency of mutations in lung tumors (both squamous cell and NSCLC) in smokers.



Differentials for alveolar-bronchiolar adenocarcinoma in the rat should include AB adenomas, acinar carcinomas, adenosquamous carcinoma, mesothelioma, and neoplasms metastatic to the lung.⁵ AB adenomas, which may represent a continuum with AB carcinoma, are often diagnose based on size, as well as a lack of cellular pleomorphism or atypia as well as a lack of invasive growth.⁵ Acinar carcinoma grow in a glandular pattern utilizing pre-existent alveolar walls, and may contained

Lung, NCTR Sprague Dawley (CD) rat. Neoplastic cells exhibit positive cytoplasmic staining with SPC (surfactant protein C) and CK18 (cytokeratin 18) consistent with AB carcinoma. (HE, 200X) (Photo courtesy of: EPL, Inc., P.O. Box 12766, Research Triangle Park, NC 27709 <http://epl-inc.com/>)

mixed populations of pleomorphic cuboidal cells, mucous cells, or ciliated cells.

Adenosquamous carcinoma has areas of malignant squamous cells or is composed entirely of squamous cell carcinoma. Metastatic foci of primary adenocarcinoma from other organs may be multifocal and often present within vessels or in perivascular locations. Finally, mesothelioma may be the most difficult of these tumors to differentiate from A/B carcinoma due to its similar predilection to grow along pleural surfaces or within the mediastinum, as well as the variability of cellular morphology.⁵

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