Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 1

19 August 2020

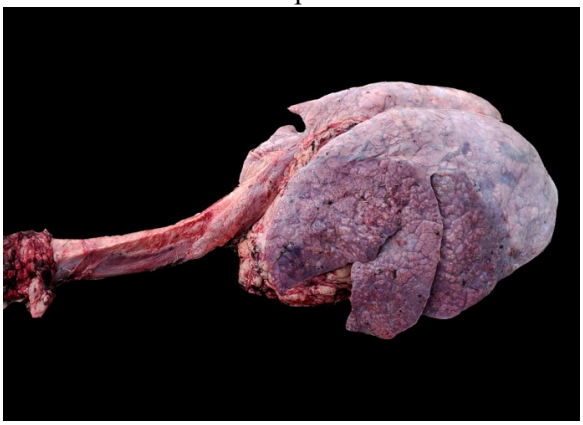
Joint Pathology Center
Silver Spring, Maryland

CASE 1: M19-03419 (4141690-00)

Signalment: Adult, gender unspecified, Red Angus (*Bos taurus*), bovine.

History: Feedlot cattle with acute onset respiratory signs with bilateral hemorrhagic nasal discharge. Two dead and five showing clinical signs out of a herd of 200. A few others had also died with similar signs in the past few months. Has been vaccinated against *Mannheimia haemolytica*.

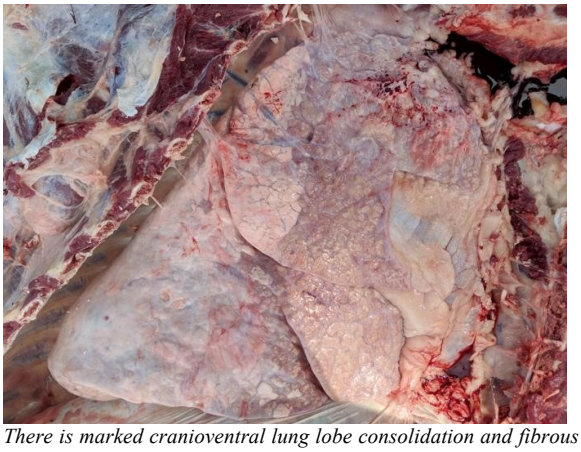
Gross Pathology: Cranioventral lung lobe consolidation with multifocal to coalescing, well-demarcated, pale yellow, slightly raised, variably sized nodules over the pleural surface. On cut surface, the lesions extend into the parenchyma. There are multifocal emphysema and multiple fibrous adhesions to thoracic cavity wall.



The lung has numerous yellow raised nodules over its surface. (Photo courtesy of: EMAI, Woodbridge Road, Menangle, NSW, 2568, Australia)

Laboratory results:

Sample	Test	Results
Ear notch	Pestivirus antigen capture ELISA (PACE)	Negative
Fresh lung	Bovine Herpesvirus-1	**Positive**
Fresh lung	Bovine parainfluenza virus-3	Negative
Fresh lung	Bovine respiratory syncytial virus	Negative
Fresh lung	*Mycoplasma bovis* PCR	**Positive**
Fresh lung	Aerobic culture	Profuse predominant growth of *Trueperella pyogenes* and *Pseudomonas aeruginosa*



There is marked cranioventral lung lobe consolidation and fibrous adhesions (top). (Photo courtesy of: EMAI, Woodbridge Road, Menangle, NSW, 2568, Australia)

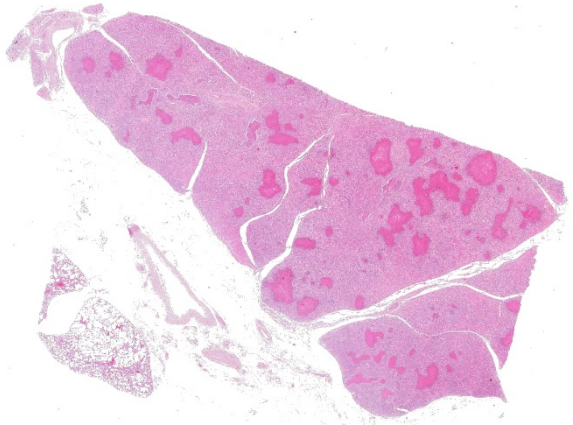
Microscopic description:
Multifocally to coalescing, mainly centered on bronchi and bronchioles are large areas of accumulation of eosinophilic debris, along with necrotic leukocytes that have lost their nuclei but retained their cellular outline (ghostlike remnants of leukocytes) (caseous necrosis) with occasional mineralization that is surrounded by variable amounts of neutrophils, a rim of macrophages with scattered lymphocytes, and in some areas, mild fibroblast proliferation. The remaining bronchi and bronchioles epithelium are attenuated, hyperplastic and lumina filled with eosinophilic and inflammatory debris, predominantly neutrophils. There are many areas with type II pneumocyte hyperplasia, and multifocally, the alveolar lumina are filled with homogenous to eosinophilic strands (fibrin) or paler eosinophilic fluid (edema), along with increased alveolar macrophages and neutrophils. The interlobular septa are moderately expanded by edema and have multiple lymphatic vessels and veins with large thrombi, some of which are attached to the vessel wall and have undergone reorganization. The pleura is mildly thickened with edema.

Lesions are similar in submitted slides, although with some variation among the sections.

Contributor's morphologic diagnosis:
Lungs: Bronchopneumonia, caseonecrotizing, subacute, multifocal to coalescing, marked; with type II pneumocyte hyperplasia, and pleural and interlobular edema and thrombosis.

Contributor's comment:
Mycoplasma bovis has significant economic and welfare consequences as it frequently results in chronic disease that fails to respond to antibiotics.[10] Some animals can be asymptomatic and shed for months to years, sometimes intermittently, with increased shedding associated with stressful events such as comingling, transportation and entry into feedlot.[1,10] Asymptomatic shedders are likely the major source of infection and transmission is via nasal secretions, aerosols, ingestion of contaminated milk, and less importantly via fomites (e.g. contaminated water, feed, housing, so forth).[3,9,10] The pathogen is capable of causing various diseases, which have been collectively termed *Mycoplasma bovis*-associated diseases.[9] These include pneumonia, mastitis, otitis media and arthritis/tenosynovitis (see table 1).[1,3,9,10] Although *M. bovis* is known to cause chronic caseonecrotic bronchopneumonia, its role in other forms of acute and chronic pneumonia remains incompletely understood.[1,2,10] Other less common or less established clinical syndromes include decubital abscesses, keratoconjunctivitis sicca, meningitis, myocarditis/endocarditis, genital infections and abortions.[1,3,9,10] *M. bovis* infection can concurrently result in one or more of these syndromes.[3,9,10]

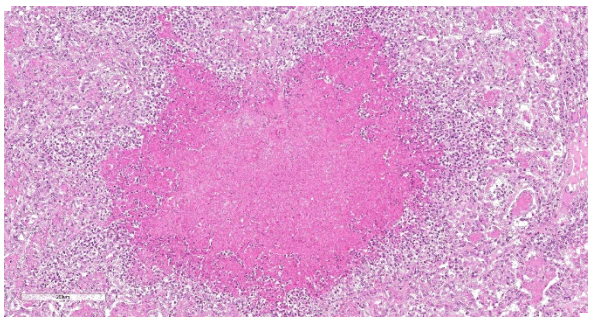
In this case, a diagnosis of *M. bovis* bronchopneumonia was made based on characteristic histological changes and a positive PCR. Diagnosis of *M. bovis* cannot be made by



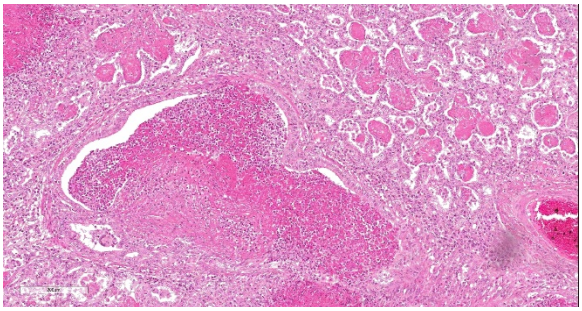
Lung, ox. A section of lung with brightly eosinophilic, well-demarcated areas of parenchymal necrosis is presented. The remainder of the alveolar parenchyma appears atelectatic and the interlobular space is expanded. (HE, 5X)

cranioventral bronchopneumonia with red discoloration and consolidation and multiple well-demarcated, white to yellow caseonecrotic foci that ranges from several millimeters to centimeters in diameter.[2,3,9] Classical histological lesions include suppurative bronchopneumonia with characteristic well-demarcated foci of caseous necrosis centered around bronchi and bronchioles, with amorphous eosinophilic material, along with ghostlike remnants of leukocytes, that are surrounded by neutrophils, followed by a rim of lymphocytes, plasma cells, macrophages and fibroblasts, which were features seen in this case.[2,3,8,9] In this case *Trueperella pyogenes* and *Pseudomonas aeruginosa* were also cultured from the lung. These two agents were considered secondary opportunistic agents and may have resulted in the histological changes that are not normally associated with *M. bovis* pneumonia, such as the large thrombus within the interlobular septa.

M. bovis is able to colonize mucosal surfaces, persist at sites of disease and evade host clearance despite eliciting strong immune responses, resulting in chronic disease.[2,10] The exact pathogenesis is not well understood, but the direct and indirect immunomodulatory effects on inflammatory cells, as well as the large family of variable surface lipoproteins (Vsps) are thought to be the major contributors.[2,9,10] Vsps undergo high frequency size and phase variation, allowing for strain variation and large antigenic variation, which have been suggested to assist in humoral immune response evasion.[1,2,3] Like other *Mycoplasma* species, *M. bovis* is able to adhere to mucosal surfaces, however unlike other *Mycoplasma* species, colonization does not result in ciliostasis.[2] Despite extensive experimental research, there is still incomplete understanding behind the exact pathogenesis of *M. bovis* bronchopneumonia, including how they elicit lung damage, and the type of immune response that contributes to disease, as opposed to protection and clearance of *M. bovis*.[2,10]



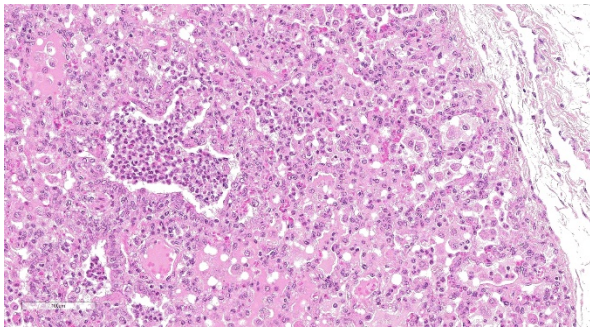
Lung, ox. Higher magnification of an area of lytic necrosis, surrounded by infiltrating neutrophils and exudate-filled alveoli. (HE, 100X)



Lung, ox. Exudate-filled bronchioles are precursors to foci of necrosis. In this bronchiole, the exudate has effaced part of the airway epithelium. Adjacent septa are markedly thickened and alveoli are filled with polymerized fibrin. (HE, 80X).

M. bovis is thought to form synergism with other respiratory pathogens, including *Mannheimia haemolytica*, *Pasteurella multocida* and BoHV-1.[1,9] Synergism with BVDV has also been suggested, but remains controversial with inconsistent results across different studies.[3,12] It is also frequently associated with other infectious agents, including viral agents such as Bovine Respiratory Syncytial Virus, Bovine Parainfluenza Virus-3, Bovine Viral Diarrhea Virus (BVDV) and bacterial agents, most commonly *Mycoplasma arginini* and *Trueperella pyogenes*.[1,2]

In this case, BoHV-1 was also detected from the lung tissue by PCR. BoHV-1 can result in bronchointerstitial pneumonia with bronchiolar erosion, type II pneumocyte proliferation, and occasionally epithelial syncytia in alveoli, as well as eosinophilic intranuclear inclusion bodies.[3] Apart from type II pneumocyte proliferation, these were not a feature in our case. The significance and role played by BoHV-1 in this case is unclear. BoHV-1 is considered to infect animals for life, and stress may cause reactivation of latent infections (e.g. from the *M. bovis* infection in this case).[3] However, an alternative theory is BoHV-1 was a predisposing factor to the development of *M. bovis* bronchopneumonia.



Lung, ox. Early bronchiolar lesion with neutrophilic exudate in the lumen. Alveolar septa are markedly thickened by hypertrophic intraseptal macrophages, circulating neutrophils, and type II pneumocyte hyperplasia.

Respiratory viral agents such as BoHV-1 can result in lysis of ciliated epithelium, impairing the *mucociliary apparatus, as well as reducing the* function of alveolar macrophages, and has immunosuppressive effects including downregulation of type 1 interferon, leukocyte apoptosis and decreases MHC 1 and MHCII expression.[3,4] Furthermore, experimentally, co-infection with BoHV-1 followed by *M. bovis* resulted in more severe clinical signs and lung lesions compared to *M. bovis* alone.[12]

Contributing Institution:
EMAI, Woodbridge road
Menangle, NSW, 2568, Australia

https://www.dpi.nsw.gov.au
https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary
https://www.dpi.nsw.gov.au/about-us/science-and-research/centres/emai

JPC diagnosis:
Lung: Bronchopneumonia, caseonecrotic, multifocal, severe, with diffuse interstitial pneumonia, and type II pneumocyte hyperplasia.

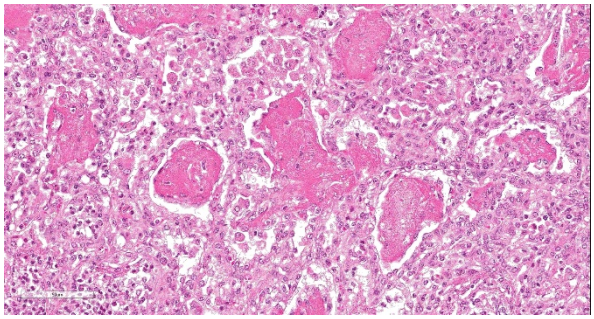
JPC comment:
The contributor describes *Mycoplasma bovis* well and summarizes the forms of disease in cattle. Recent research has increased our understanding of this bacteria, though much remains unknown.

While this disease may not have economic importance on the scale of BSE or FMD, bovine respiratory disease in U.S. feedlot cattle is responsible for approximately $55 million annually. Despite many outbreaks being associated with new exposure to infected cattle, there are no travel restrictions on movement related to *M. bovis*.[11]

Recent research has elucidated several mechanisms and attributes of *M. bovis* that contributes to its pathogenicity, including four adhesins (α-enolase, VpmaX protein, NADH oxidase, TrmFO protein), two nucleases (MBOVPG45_0215, MnuA), and a secretory nuclease (MBOV_RS02825). In vitro studies have also identified fructose 1,6-biphosphate aldolase and methylenetetrahydrofolate-tRNA-(uracil-5-)-methyltransferase as molecules that augment adhesion by binding plasminogen and fibronectin, respectively.[11]

Neutrophils stimulated by *M. bovis* do not produce NETs, and this effect is due to a reduction in reactive oxygen species production in neutrophils.[5] However, the nucleases previously mentioned are associated with cytotoxicity and the degradation of neutrophil extracellular traps (NETs), when present.[6,7]

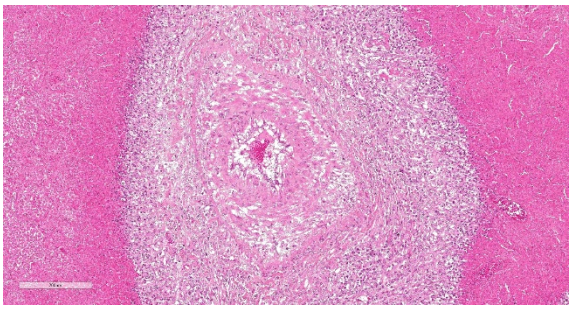
Extracellular DNA (eDNA) has been shown (*in vitro*) to be a limiting nutrient for the growth of *M. bovis*. When exposed to sufficient amounts of eDNA, the bacterium produces H_2O_2, which appears to be cytopathic for actively dividing cells. eDNA, an important component of biofilms, is often found in abundance in tissue due to necrosis, apoptosis, autophagy, pyroptosis, and extracellular release in vesicles. Importantly, this research has been performed *in vitro* only,



In some areas of the section, the alveoli also contain abundant polymerized fibrin and numerous alveolar macrophages, ingesting fibrin and cellular debris. (HE, 125X)

Table 1: The common *M. bovis* associated diseases.[3,8,9]

Clinical syndromes	Typical age affected	Clinical signs	Typical gross lesions	Typical histological lesions	Other syndromes commonly associated with this syndrome
Pneumonia	Any	Fever, tachypnoea, dyspnea, inappetence, poor weight gain, +/- nasal discharge, +/- coughing	Cranioventral lung consolidation and reddening with multifocal, well-demarcated, dry, yellow to white caseonecrotic foci ranging from few millimeters to centimeters in diameter.	Subacute to chronic caseonecrotic bronchopneumonia.	Otitis media, arthritis
Mastitis	Any age and stage of lactation	Subclinical (common), increased somatic cell count, reduced milk production, swollen mammary glands (typically >1 quarter affected).	Swollen mammary glands, mildly abnormal to gritty to purulent mammary discharge.	Mild to severe fibrinosuppurative to caseonecrotic mastitis	Arthritis, synovitis, respiratory disease
Otitis media	Calves	Fever, decreased appetite, ear droop, ptosis, head shaking/scratching/rubbing, epiphora, exposure keratitis, purulent aural discharge. Head tilt, nystagmus, circling/falling/drifting to one side if otitis interna involved.	Fibrinous to suppurative to fibrinosuppurative to caseous exudate	Suppurative to caseous otitis media with osteolysis.	Pneumonia, arthritis
Arthritis/ tenosynovitis	Any age	Acute non-weight bearing lameness, joint swelling/heat/pain, +/- fever, +/- inappetence. Commonly involves tendon sheaths and periarticular soft tissues. Most commonly involves large rotator joints (hip, stifle, hock, shoulder, elbow, carpus). Often poor response to treatment	Non-odorous serofibrinous to suppurative to fibrinosuppurative to caseous exudate and fibrosis	Caseonecrotising arthritis with articular cartilage erosion and synovial hyperplasia	Pneumonia, mastitis



Lung, ox. Arterioles within this section of consolidated lungs have markedly expanded muscular walls. (HE, 100X).

testing against embryonal bovine lung cells. *In vivo* results may not correlate exactly.[13]

North American bison (*Bison bison*) develop infections from *M. bovis* as a primary pathogen, causing polyarthritis and/or pneumonia. However, the isolate is genetically different from the *M. bovis* isolated from cattle, and to date, the bison strain has not caused natural or experimental disease in cattle. The bison variant of *M. bovis* may represent a new host-adapted variant.[11,12]

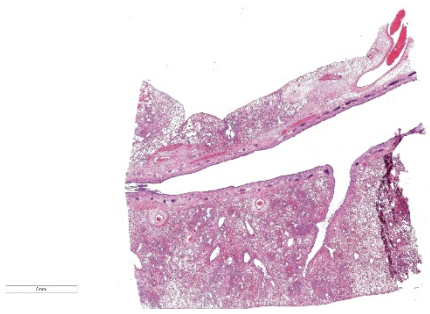
References:

1. Caswell JL, Archambault M. *Mycoplasma bovis* pneumonia in cattle. Anim Health Res Rev. 2007; 8; 161-186.
2. Caswell JL, Bateman KG, Cai HY, Castillo-Alcala F. *Mycoplasma bovis* in respiratory disease of feedlot cattle. Vet Clin Food Anim. 2010; 26; 365-379.
3. Caswell JL, Williams KJ. Respiratory system. In Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* 6th ed., vol. 2. Philadelphia, PA: Saunders Elsevier; 2016: 552-554, 537-538.
4. Ellis JA. Update on viral pathogenesis in BRD. Anim Health Res Rev. 2009; 10; 149-153.
5. Gondaira S, Higuchi H, Nishi K, Iwano H, Nagahata H. *Mycoplasma bovis* escapes bovine neutrophil extracellular traps. *Veterinary Microbiology*. 2017;199:68-73.
6. Huang J, Zhu H, Wang J, et al. Fructose-1,6-bisphosphate aldolase is involved in *Mycoplasma bovis* colonization as a fibronectin-binding adhesin. *Research in Veterinary Science*. 2019;124:70-78.
7. Josi C, Burki S, Vidal S, et al. Large-scale analysis of the *Mycoplasma bovis* genome identified non-essential, adhesion- and virulent-related genes. *Frontiers in Microbiology*. 2019;10:2085.
8. López A, Martinson SA. Respiratory system, mediastinum, and pleurae. In Zachary JF, ed. *Pathologic Basis of Veterinary Disease.* 6th ed., St. Louis, MO: Elsevier Mosby; 2017: pp.531-532.
9. Maunsell FP, Donovan GA. *Mycoplasma bovis* infections in young calves. Vet Clin Food Anim. 2009; 25; 139-177.
10. Maunsell FP, Woolums AR, Francoz D, et al. *Mycoplasma bovis* infections in cattle. J Vet Intern Med. 2011; 25: 772-783.
11. Perez-Casal J. Pathogenesis and virulence of *Mycoplasma bovis*. *Vet Clin Food Anim*. 2020.
12. Prysliak T, van der Merwe J, Lawman Z, et al. Respiratory disease caused by *Mycoplasma bovis* is enhanced by exposure to bovine herpes virus 1 (BHV-1) but not to bovine viral diarrhea virus (BVDV) type 2. Can Vet J. 2011; 52; 1195-1202.
13. Register KB, Olsen SC, Sacco RE, et al. Relative virulence in bison and cattle of bison-associated genotypes of *Mycoplasma bovis*. *Veterinary Microbiology*. 2018;222:55-63.
14. Zhu X, Dordet-Frisoni E, Gillard L, et al. Extracellular DNA: A nutritional trigger of *Mycoplasma bovis* cytotoxicity. *Frontiers in Microbiology*. 2019;10:2753.

CASE 2: 1235813-009 (4135955-00)

Signalment: 5.5-month-old, intact male, Beagle (*Canis familiaris*), canine.

History: This purpose-bred research dog was found laterally recumbent, pale, tachycardic, and dyspneic with blood coming from the mouth during a routine room check. A small amount of vomitus was noted under the cage. Humane euthanasia was elected after veterinary consultation. The dog had arrived at the facility two weeks prior to presentation and had not yet been placed on a study.



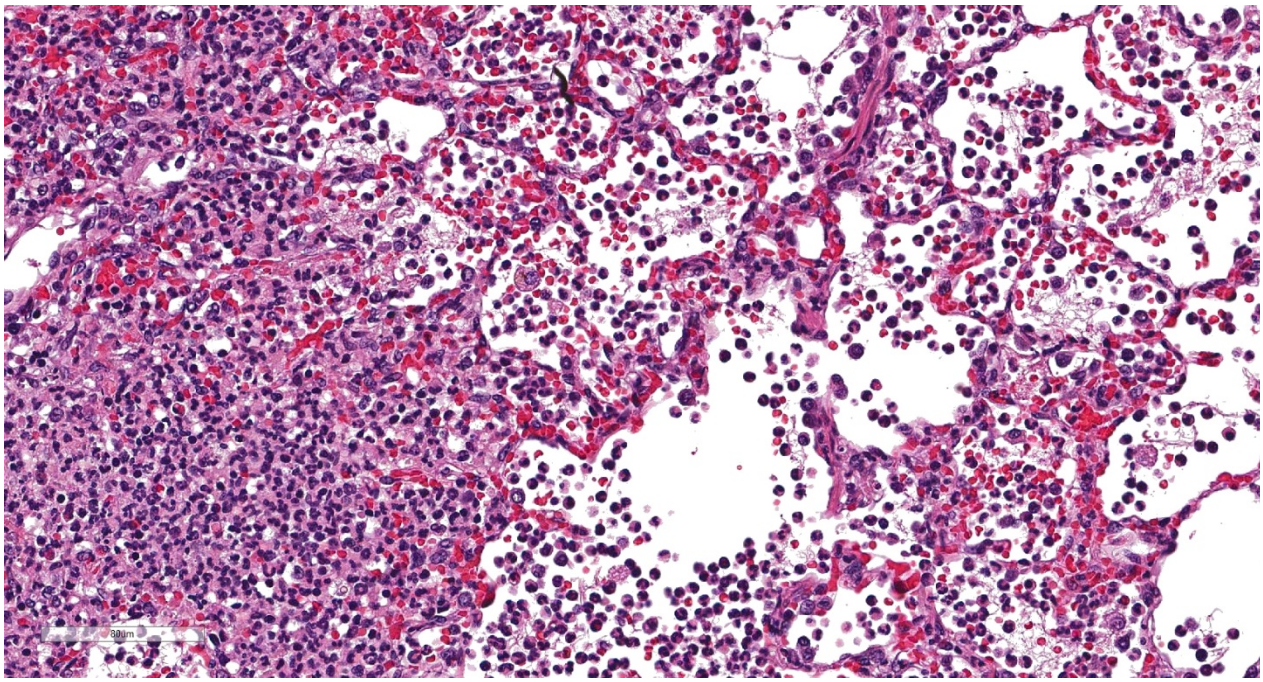
Lung, dog. A single section of lung with a large airway is presented for examination. There is consolidation of approximately 33% of the alveolar parenchyma at this

Gross Pathology: All lung lobes were diffusely mottled red to black and palpated as wet and heavy. The remainder of the postmortem examination was unremarkable.

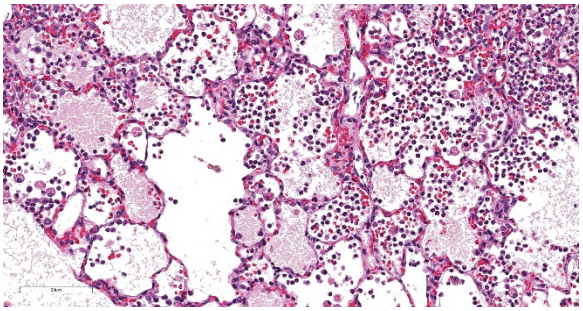
Laboratory results:
Lung bacterial culture: *Escherichia coli* 3+
E. coli virulence factor PCR: Positive for cytotoxic necrotizing factor 1 (CNF-1)
Gram stain: Gram negative bacilli present

Microscopic description:
In sections of lung, approximately 75% of the pulmonary parenchyma was effaced by multifocal to coalescing areas of hemorrhage and necrosis. Within these regions, alveoli contained dense accumulations of erythrocytes and neutrophils admixed with aggregates of fibrin, eosinophilic proteinaceous edema fluid, plump vacuolated macrophages, and scattered colonies of bacterial bacilli. Inflammatory infiltrates regionally obliterated alveolar septal architecture. The pleura, interlobular septa, and collagenous connective tissue surrounding bronchovascular bundles were expanded by pale eosinophilic edema fluid and scattered erythrocytes and neutrophils. Vessels frequently contained increased numbers of circulating and marginating leukocytes. Occasional vessels had smudgy, indistinct walls that were segmentally obliterated by neutrophils. Airway lumens multifocally eosinophilic amorphous material, and necrotic debris.

Contributor's morphologic diagnosis:
Lung: Severe multifocal necrohemorrhagic pneumonia with intralesional bacterial bacilli



Lung dog. Alveoli are filled with variable combinations and concentrations of viable and necrotic neutrophils, fewer macrophages, cellular debris, fibrin, and edema. (HE, 340X)

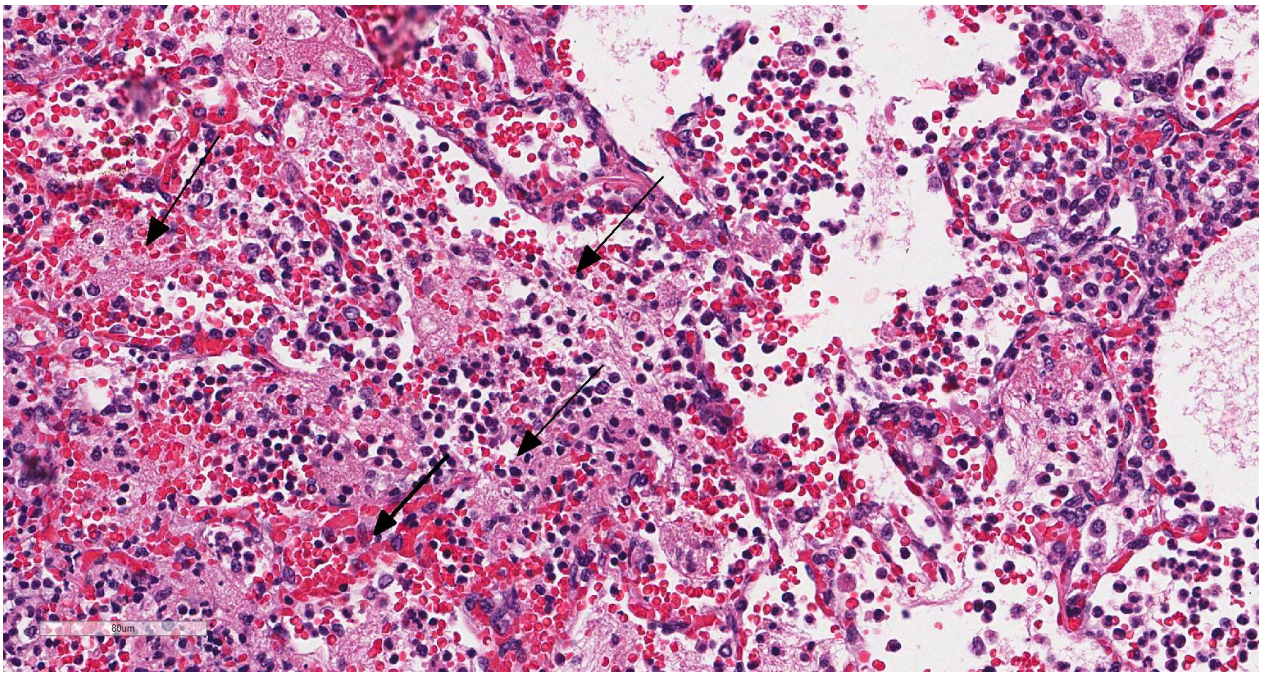


Lung dog. Less affected areas of lungs with a more dense concentration of alveolar edema, (HE, 340X)

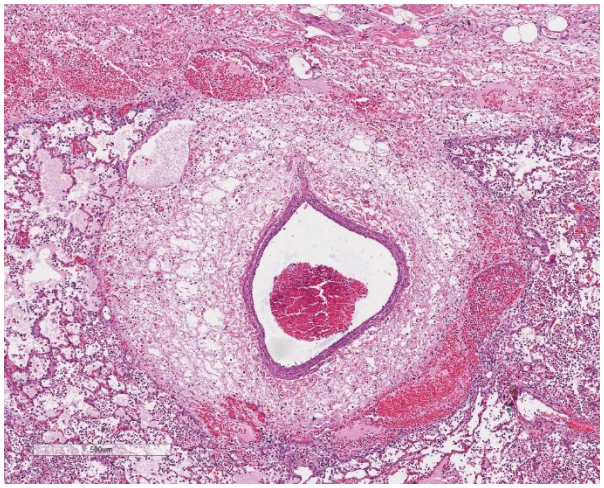
Contributor's comment: Extraintestinal pathogenic *Escherichia coli* (ExPEC) is an infrequently reported cause of acute fatal necrohemorrhagic pneumonia in canines.[2] While the majority of *Escherichia coli* strains are commensals or primary enteric pathogens, ExPEC strains are capable of causing disease in multiple organs systems outside of the gastrointestinal tract. Specifically, ExPEC-associated genitourinary infections, meningitis, pneumonia, and septicemia have been reported in multiple mammalian species including humans.[12] ExPEC strains are characterized by the production of certain virulence factors, namely cytotoxic necrotizing factor (CNF) 1 and 2.[3] The genes encoding these virulence factors are clustered within a pathogenicity island that has been linked to serogroups O4 and O6.[5] The pathogenesis of infection is unknown, but may be related to transmission of bacteria from subclinical carriers via feces or milk.[14]

Other potential infectious causes of acute hemorrhagic pneumonia in dogs that can have similar clinical presentations and lesions to ExPEC include canine influenza, leptospirosis, and *Streptococcus equi spp zoopeidemicus*.[4,6,11] In canine influenza, the lesions are primarily centered upon the airways with lesser involvement of the alveolar airspaces. [4] *Leptospiral pulmonary hemorrhagic syndrome* is characterized by severe widespread acute pulmonary hemorrhage with inconspicuous vascular changes.[6] *Streptococcus equi spp zooepidemicus* causes fibrinosuppurative and hemorrhagic pneumonia with prominent colonies of gram-positive cocci.[11]

Specifically, in purpose-bred beagle research dogs, pneumonia is uncommon and often associated with pulmonary misdosing or potential test article effects rather than infectious etiologies.[9] In this case, there were multiple



Lung, dog. Areas of septal necrosis (arrows) can be easily identified in these lesions by release of erythrocytes into the alveoli. (HE, 340X).



Lung, dog. There is marked edema and expansion of the arteriolar adventitia. (HE, 49X).

sporadic cases of severe hemorrhagic pneumonia at this facility in recent years, which prompted concern for an underlying infectious cause. Affected dogs were either found dead or presented with fulminant clinical symptoms and were euthanized *in extremis*. In most cases, dogs were affected immediately post-transport to the facility. CNF 1+ *E. coli* was isolated from lung tissue for the majority of the cases at this facility. There are rare previous reports of ExPEC-associated disease in other research facilities.[5] Given these findings, ExPEC should be considered as an emerging, important cause of acute fatal necrohemorrhagic pneumonia in purpose-bred research dogs and may often be associated with a recent history of transport.

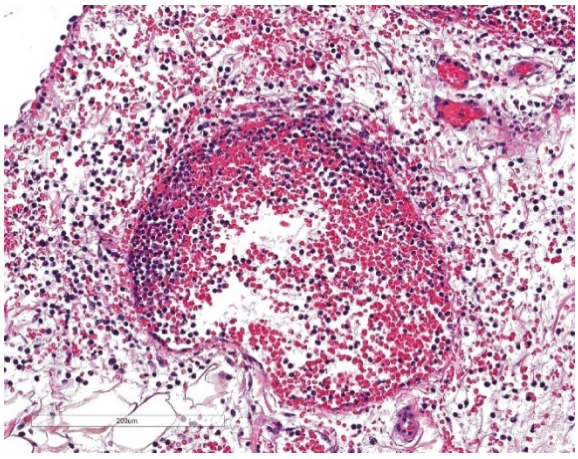
Contributing Institution:
Pathology Department
Charles River Laboratories – Mattawan
www.crl.com

JPC diagnosis:
Lung: Pneumonia, fibrinosuppurative and necrotizing, diffuse, severe, with necrotizing vasculitis and Gram-negative bacilli.

JPC comment:
The contributor describes ExPEC well, and touches upon the variety of pathogenic effects it may have on different organ systems. Recent efforts to perform sequence analysis and whole genome sequencing has identified one sequence type as most prevalent across all regions of the world. This strain of *E. coli* is known as sequence type 131 (ST131) and possesses a greater number of virulence genes than other identified sequence types. One virulence factor strongly associated with ST131 is the *espC* gene, which encodes SPATE (serine protease autotransporters of *Enterobacteriaceae*), while antimicrobial resistance does not appear to be a fitness factor for this bacterium.[7] Proteases, and SPATE in particular, allow ExPEC to hydrolyze the peptide bonds present in proteins and increases its virulence. There are more than 20 SPATEs currently identified, with most secreted from members of *Enterobacteriaceae*.[13]

Since ExPEC is an important cause of infections in humans (often urinary tract), research has attempted to determine sources of infection. A study of dog parks in Washington, DC in 2015 found that approximately 88% of dog stool samples, and 88% of swabs from shoes of dog park visitors were positive for E. coli. While the majority of these isolates were not drug resistant, a higher percentage of dog park isolates were resistant to more antibiotics than isolates from control shoes.[1]

Isolates of multiple drug resistant (MDR) ExPEC (ST73, ST127) have been isolated from the feces of endangered southern killer whales (*Orcinus orca*), though it has not been determined whether these isolates cause disease or contribute to their population decline.[8] Comparisons of ExPEC isolates from humans and avians show they have



Lung, dog. There is multifocal necrotizing phlebitis with neutrophil exocytosis and hemorrhage, fibrin and edema in the perivenular connective tissue. (HE, 400X).

significant genetic overlap and there is evidence for pathogenicity of these avian isolates in mammalian models *in vivo*. Avian strains may represent an origin of some infections in humans.[10]

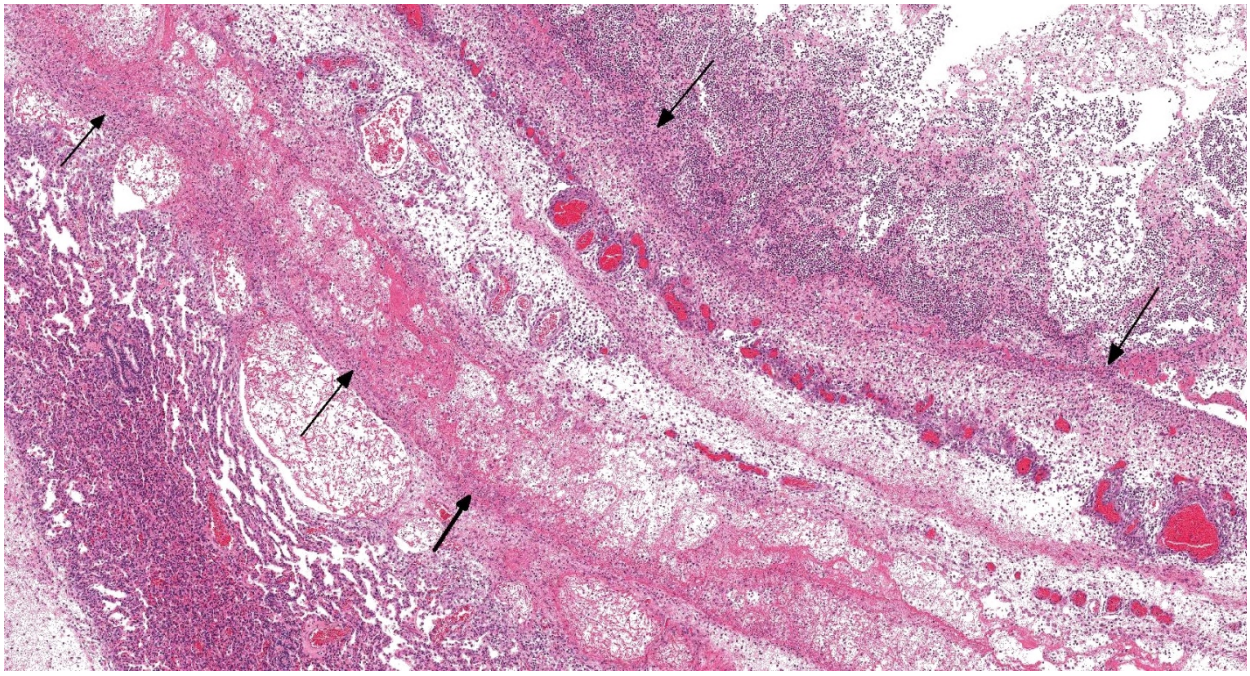
There was debate about the origin of ExPEC in this animal, and whether it most supported an interstitial pneumonia. Many features are consistent with an interstitial pneumonia and hematogenous infection, but further elucidation of pathogenesis would help determine primary changes versus secondary.

References:

1. Ahmed L, Price LB, Graham JP. An exploratory study of dog park visits as a risk factor for exposure to drug-resistant extra-intestinal pathogenic *E. coli* (ExPEC). *BMC Research Notes.* 2015;8(137).
2. Breitschwerdt EB, DebRoy C, Mexas AM, Brown TT, Remick AK. 2005. Isolation of necrotoxigenic *Escherichia coli* from a dog with hemorrhagic pneumonia. JAVMA 226(12): 2016-2019.
3. Carvallo FR, DebRoy C, Baeza E, Hinckley L, Gilbert K, Choi SJ, Risatti G, Smyth JA. 2010. Necrotizing pneumonia and pleuritis associated with extraintestinal pathogenic *Escherichia coli* in a tiger (*Panthera tigris*) cub. J Vet Diagn Invest 22: 136-140.
4. Castleman WL, Powe JR, Crawford PC, Gibbs EP, Dubovi EJ, Doris RO, Hanshaw D. 2010. Canine H3N8 influenza virus infection in dogs and mice. Vet Pathol 47(3): 507-17.
5. Handt LK, Stoffregen DA, Prescott JS, Pouch WJ, Ngai D, Anderson CA, Gatto NT, DebRoy C, Fairbrother JM, Motzel SL, Klein HJ. 2003. Clinical and microbiological characterization of hemorrhagic pneumonia due to extraintestinal pathogenic *Escherichia coli* in four young dogs. Comparative Medicine 53(6): 663-670.
6. Kohn B, Steinicke K, Arndt G, Gruber AD, Guerra B, Jansen A, Kaser-Hotz B, Klopfleisch R, Lotz F, Luge E, Nockler K. 2010. Pulmonary abnormalities in dogs with Leptospirosis. J Vet Intern Med 24: 1277-1282.
7. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. *Clinical Microbiology Reviews*. 2019;32(3):e00135-18.
8. Melendez D, Roberts MC, Greniger AL, et al. Whole-genome analysis of extraintestinal pathogenic *Escherichia coli* (ExPEC) MDR ST73 and ST127 isolated from endangered southern resident killer whales (*Orcinus orca*). *J Antimicrob Chemother*. 2019;74:2176-2180.
9. Mukaratirwa S, Garcia B, Isobe K, Petterino C, Bradley A. 2016. Spontaneous and dosing route-related lung lesions in beagle dogs from oral gavage and inhalation toxicity studies: differentiation from compound-induced lesions. Toxicologic Pathology 44(7): 962-973.
10. Nandanwar N, Janssen T, Kuhl M, Ahmed N, Ewers C, Wieler LH. Extraintestinal pathogenic *Escherichia coli* (ExPEC) of human and avian origin belonging to sequence type complex 95 (STC95) portray indistinguishable virulence features. *International Journal of Medical Microbiology*. 2014;304:835-842.
11. Pesavento PA, Hurley KF, Bannasch MJ, Artiushin S, Timoney JF. 2008. A clonal outbreak of acute fatal hemorrhagic pneumonia in intensively housed (shelter) dogs caused by *Streptococcus equi* subsp. *zooepidemicus.* Vet Pathol 45: 51-53.
12. Sura R, Van Kruiningen HJ, DebRoyD, Hinckley LS, Greenberg KJ, Gordon Z, French RA. 2007. Extraintestinal pathogenic *Escherichia coli*-induced acute necrotizing pneumonia in cats. Zoonoses Public Health 54:307-313.
13. Tapader R, Basu S, Pal A. Secreted proteases: a new insight in the pathogenesis of extraintestinal pathogenic *Escherichia coli*. *International Journal of Medical Microbiology*. 2019;309:159-168.
14. Turchetto S, Ustulin M, Citterio CV, Zanardello C, Vascellari M, Vio D, Conedera G, Ferro NM, Milone F, Cocchi M. 2015. Phenotypic features and phylogenetic background of extraintestinal hemolytic *Escherichia coli* responsible of mortality in puppies. Veterinary Microbiology 179: 126-130.

CASE 3: T18-09330 (4135870-00)

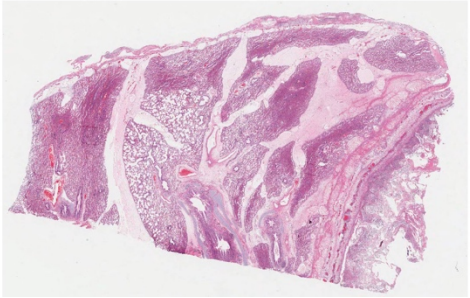
Signalment: 5-week-old pig (*Sus scrofa domesticus*)



Lung, pig. Black arrows demonstrate the thickness of the pleura. At upper right is the mat of fibrin and neutrophils within the pleural space. (HE, 44X)

History: The pig showed weight loss, and lethargy. Unthriftiness was also observed in other animals in the group. The pig was euthanized, and the carcass was received for examination.

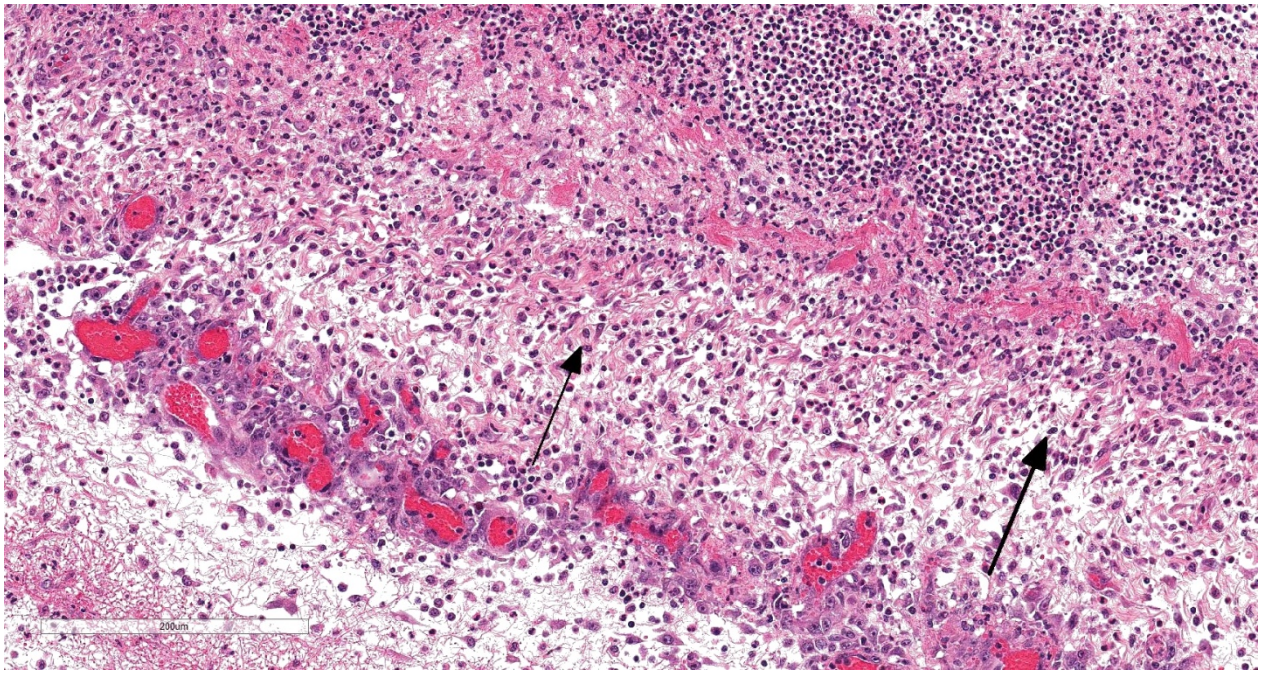
Gross Pathology:
There was no grossly visible lesion on external examination. The carcass was found in a fair nutritional condition. A focal skin abrasion was observed on the head. Upon opening the carcass, it was noted that lungs were adhered to the pericardium and the parietal pleura with threads and mats of fibrin. The pericardium also was adhered to the epicardium with thick fibrin mat. Similar thin fibrin threads were observed on the serosal surfaces of the abdominal viscera. Stomach was empty. Liver was congested.



Lung, pig. A section of lung is submitted for examination. At low magnification, there is marked expansion of both the interlobular septa and pleura by edema. A 2mm mat of fibrin and inflammatory cells covers the pleural surface. (HE, 7X)

Laboratory results: Culture on lung tissues yielded colonies of *Haemophlius parasuis.* No bacterial growth was observed on cultures from liver, spleen and kidneys. Fluorescent antibody test for Porcine Reproductive and Respiratory Syndrome was negative.

Microscopic description:
On histology, lesions vary from section to section. Abundant dead and degenerate neutrophils, necrotic debris, edema and fibrin expanded and disrupted the visceral pleura and occasionally extended to the subjacent pulmonary parenchyma. Multifocal aggregates of bacteria were observed in the necrotic areas. The remaining pulmonary tissue had diffusely collapsed alveoli (atelectasis). Similar dead and degenerate neutrophils, fibrin and necrotic debris expanded the pericardium, the epicardium, and partly extended to myocardium, and were also observed on the serosa of small intestines (slides not included). Large numbers of dead and



Lung, pig. Developing granulation tissue at the outermost edge of the pleura. (HE, 204X).

degenerate neutrophils infiltrated multiple sections of reactive lymph nodes with moderate follicular hyperplasia. The findings in the other examined tissues were within normal limits.

Contributor's morphologic diagnosis:

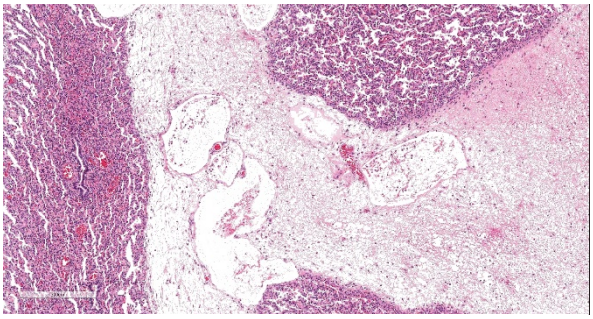
1. Severe, fibrinonecrotic and suppurative pleuritis/pleuropneumonia with bacteria

2. Severe, fibrinonecrotic and suppurative epicarditis, pericarditis and myocarditis (slide not submitted)

3. Moderate multifocal abdominal visceral serositis/peritonitis (slide not submitted).

Contributor's comment:

The grossly and microscopically observed lesions including severe, diffuse, fibrinous pleuritis, fibrinous pericarditis, and mild fibrinous peritonitis are consistent with polyserositis. Polyserositis is inflammation of serous membranes of the pleura, pericardium, and peritoneum[7] and in pigs is often associated with infection due to *Haemophlius parasuis*, the cause of Glässer's disease. The disease is characterized by polyserositis, polyarthritis, and fibrinous meningitis. It is a major bacterial infection with worldwide distribution that has caused considerable economic losses even in high health status farms in the pig industry due to increase in mortality and morbidity. The bacterium, *H. parasuis* is a gram-negative, small, rod-shaped, pleomorphic, non-hemolytic, non-motile, nicotinamide adenine dinucleotide (NAD)-dependent and microaerophile bacterium that belongs to the genus *Haemophilus* of the family *Pasteurellaceae*.[6,9,12] The bacterium colonizes the upper respiratory tract of healthy pigs and, under certain circumstances, some strains are able to invade the host and cause severe lesions. Transmission of infection occurs by aerosols and suspended particles in the air and in susceptible animals it causes Glässer's disease, pneumonia and sudden death in pigs.[9] Fifteen serotypes of *H. parasuis* have been identified[4] and individual serovars differ in virulence ranging from highly virulent to nonvirulent, and considerable differences in virulence also exist within each serovar.[4,6] Virulent strains can be microorganisms secondary to pneumonia, cause septicemia without polyserositis or Glässer's disease characterized by polyserositis, pericarditis, arthritis and meningitis.[6]



Lung, pig. Marked intralobular edema resulting in atelectasis of adjacent parenchyma. Lymphatics are massively dilated and contain edema, polymerized fibrin and neutrophils (HE, 74X)

In pathogenic bacteria, virulence factors play a critical role in pathogenesis. Factors involved in the virulence and pathogenicity of *H. parasuis* that enable some strains to cause a clinical disease remain largely unclear. Acute infections are occasional, and the clinical disease particularly affects young animals exposed to stress. The immune status of the host is a determinant factor in the pathogenic potential of *H. parasuis* infection[4,6,12] and as other members of *Pasteurellaceae* family, *H. parasuis* can avoid phagocytosis. Nowadays, the increase in the occurrence of Glässer's disease is being more associated to the current practices in animal production and with the emergence of immunosuppressive viruses.[9]

Clinical signs of acute and chronic disease are highly variable likely due to variation in the virulence of different strains and the immune status of the host.[6,12] Glässer's disease occurs sporadically in young pigs due to stress factors. Three clinical forms of infection due to *H. parasuis* are recognized. The lesions observed in the first form are fibrinopurulent exudate on serosal surface (polyserositis) of synovium, pericardium, peritoneum, pleura and meninges; and in the second form septicemia without polyserositis, sub-capsular kidney bleeding and sudden death are observed; while in the third form, *H. parasuis* can cause pneumonia and be isolated as primary or secondary agent in infections with Porcine Circovirosis (PCV2) and Porcine Reproductive and Respiratory Syndrome (PRRS).[9]

Diagnosis of Glässer's disease is based on clinical signs, presence of lesions at necropsy, and bacteriologic culture. Serological diagnosis of *H. parasuis* is inconsistent and inaccurate. Nowadays, the development of PCR with more sensitivity and specificity has improved laboratory diagnosis of infection with *H. parasuis*. The development of molecular techniques has improved identification of virulence factors, differentiated and genotyped strains, defined the true prevalence of systemic infection, and helped to better understand infection and diseases mechanisms.[9] Various bacteria may cause similar lesions and should be considered in the differential diagnoses. A significant association between the detection of *H. parasuis* and *M. hyorhinis* in serosal swabs taken from pigs with polyserositis has been documented. Therefore, infection with *Mycoplasma hyorhinis* must be considered as a differential diagnosis since it can cause polyserositis, arthritis, and pneumonia, clinically indistinguishable from Glässer's disease.[8] Polyserositis and meningitis were also reported associated with *Escherichia coli* infection in piglets. Although not a common cause of mortality in piglets, septicemic colibacillosis could cause high mortality in piglets and should be considered as a differential diagnosis in cases of polyserositis and meningitis.[11]

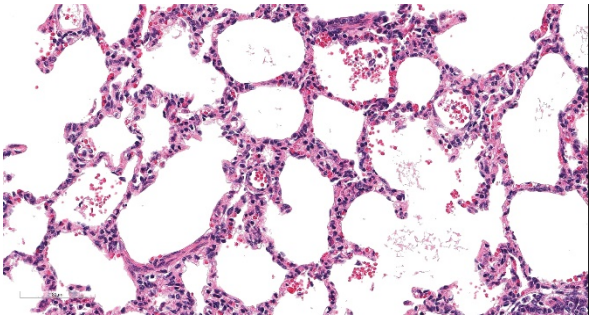
Effective prevention and control of *H. parasuis* have to be done following an epidemiological study in each herd.[9] The disease caused by *H. parasuis* including polyserositis can be treated with antibiotics; however, oral or parenteral administration of very high doses of antibiotics is necessary as soon as possible after the manifestation of clinical signs.[6,7] However, pigs with clinical signs such as growth retardation and a rough coat have a poor prognosis despite antibiotic therapy; and antibiotic treatment is usually unsuccessful because of the presence of the causative agent in the serous membranes.[7]
The ideal method to prevent *H. parasuis* infection is vaccination of sows and piglets combined with prophylactic antibiotic treatment of newborn piglets.[4] Commercial or autogenous vaccines can be used in the immunoprophylaxis of pre-parturient sows and piglets after weaning.[6] To prevent the herd from dissemination and infection, even in small farms, some practices such as early weaning and segregated production

are recommended.[9] The level of animal hygiene and animal husbandry are important factors for prevention of this disease.[6] Some stressful practices such as weaning, transportation, and numerous sites of production may influence the epidemiology of *H. parasuis* within herds. Use of uniform age to weaning and minimizing stress factors that cause immunosuppression will help to prevent and control clinical disease.[9]

Contributing Institution:
The University of Georgia, College of Veterinary Medicine, Department of Pathology, Tifton Veterinary Diagnostic & Investigational Laboratory, Tifton, GA 31793 http://www.vet.uga.edu/dlab/tifton/index.php

JPC diagnosis:
Lung: Pleuropneumonia, fibrinosuppurative, diffuse, moderate to severe, with marked intralobular edema and fibrin.

JPC comment:
Karl Glässer first described a condition in pigs in Die Krankheiten des Schweines around 1906, which included a serofibrinous pleuritis, pericarditis, peritonitis, arthritis, and meningitis. However, the bacterium was not isolated until 1922 by Schermer and Ehrlich.[6] Around the year 1912, there was a great deal of confusion about whether Hog Cholera was caused by a pair of bacteria (bacillus suipestifer and bacillus suisepticus) or a virus, there was increasing evidence of a viral etiology. However, at the time, there was a "Pectoral Form" of Hog Cholera that captured many features of what would become known as Glässer's disease. In Special Pathology and Therapeutics of the Diseases of Domestic Animals (1912), the pectoral form of Hog Cholera included a possibility of fibrinous or sero-fibrinous pleurisy and sometimes a similar pericarditis. Glässer had isolated a potentially novel bacillus from two spontaneously affected pigs which he felt might independently cause primary disease. The considerable overlap between disease presentations, the difficulty in identifying bacteria, and the shift to a viral etiology for Hog Cholera presented numerous challenges to researchers at the time.[3]



Lung, pig. Alveolar septa are expanded by a combination of congestion, edema, fibrin, increased numbers of circulating neutrophils, and hypertrophic intraseptal macrophages. (HE, 274X)

Significant resources have been allocated to research of *Haemophilus parauis* in the last decade, resulting in refinement of our understanding of the disease. More than 15 serotypes have been described around the world, with a number of identified genes encoding virulence factors such as sialyltransferase (*lsgB*), polysaccharide biosynthesis protein (*capD*), polysaccharide export proteins (*wza*), various glycosyltransferases (*HPM-1370*, *HPM-1371*, *HPM-1372*, *HPM-1373*), and virulence-associated trimeric autotransporters (*vta1*, *vta2*, *vta3*). A recent study correlated these genes encoding proposed virulence factors with the different serotypes found in Central Vietnam, showing a statistically significant correlation between the presence of the *HPM-1371*, *HPM-1372*, *capD*, and *vta1* genes and the serotype being highly virulent. Conversely, there was a decreased probability of the *HPM-1371* and *vta1* genes being present in the moderately and non-virulent serotypes.[10]

Virulence-associated trimeric autotransporters (VtaA) are exposed surface proteins expressed by virulent serotypes and strains of *H. parasuis* and use a Type V secretion system. These autotransporters have an inner membrane domain, translocator domain, and a passenger domain. The passenger domains of all VtaAs have repeats and motifs characteristic of adhesins, hemagglutinins, invasins, and variable numbers of triple helix collagen-like repeats. In the case of the *vta2* product, it was determined that this specific autotransporter likely allowed the bacteria to attach to proteins like collagen, mucin, and fibronectin, but did not contribute to cell adhesion or invasion.[1]

However, it has been shown that *H. parasuis* infection increases expression of TGF-B1, and that pre-treatment of porcine kidney cells (PK-15) in vitro inhibits invasion by *H. parasuis*. There was a correlation between TGF-B1 expression and expression of fibronectin (Fn) and a5 integrin (a5), suggesting that these are critical molecules that inhibit cellular invasion by *H. parasuis*. Interestingly, extracellular matrix molecules (e.g. fibronectin, a5 integrin) have been associated with cellular invasion in *Streptococcus pneumoniae*, group A *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Haemophilus influenza*.[5]

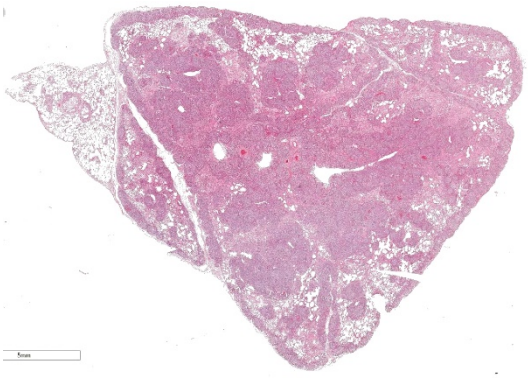
Recent phylogenetic analysis of 70 genomes showed *H. parasuis* divided into two tightly grouped clades that more recently diverged than their closest relatives, *Bibersteinia*. The two clades have so recently diverged that there are few new mutations to distinguish the groups, and classification cannot be performed confidently. Through combined genetic analysis and morphologic differences from *Haemophilus*, this bacterium has been recently taxonomically classified and renamed as *Glaesserella parasuis*.[2]

References:

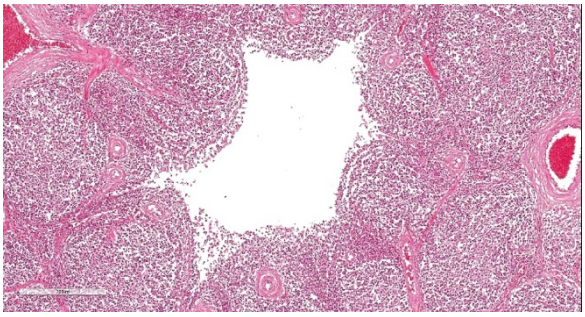
1. Costa-Hurtado M, Garcia-Rodriguez L, Lopez-Serrano S, Aragon V. *Haemophlus parasuis* VtaA2 is involved in adhesion to extracellular proteins. *Veterinary Research*. 2019;50(1):69.
2. Dickerman A, Bandara AB, Inzana TJ. Phylogenomic analysis of *Haemophilus parasuis* and proposed reclassification to *Glaesserella parasuis*, gen. nov., comb. nov. *Int. J. Syst. Evol. Microbiol*. 2020;70:180-186.
3. Hutyra F, Marek J, eds. *Special Pathology and Therapeutics of the Diseases of Domestic Animals*, Vol. 1. Alexander Eger: Chicago, IL; 1912:257-285.
4. Kielstein P, Rapp-Gabrielson VJ. Designation of 15 serovars of Haemophilus parasuis on the basis of immunodiffusion using heat-stable antigen extracts. J Clin Microbiol. 1992; 30(4): 862–865.
5. Li Y, Zhang Y, Xia Y, Shen Y, Zhang J. *Haemophilus parasuis* modulates cellular invasion via TGF-B1 signaling. *Veterinary Microbiology*. 2016;196:18-22.
6. Nedbalcova K, Satran P, Jaglic Z, et al. Haemophilus parasuis and Glässer's disease in pigs: a review. Veterinarni Medicina, 51, 2006 (5): 168–179.
7. Oh Y, Han K, Seo HW, et al. Program of vaccination and antibiotic treatment to control polyserositis caused by Haemophilus parasuis under field conditions. Can J Vet Res. 2013; 77(3):183-90.
8. Palzer A, Haedke K, Heinritzi K, et al. Associations among Haemophilus parasuis, Mycoplasma hyorhinis, and porcine reproductive and respiratory syndrome virus infections in pigs with polyserositis. Can Vet J. 2015; 56 (3):285-7.
9. Pereira D, Dalla Costa F, Ferroni L, et al. The challenges with Glässer's disease in technified pig production. Aust J Vet Scien. 2017; 49(2), 63-69.
10. Van CN, Thanh TVT, Zou G, et al. Characterization of serotypes and virulence genes of *Haemophilus parasuis* isolates from Central Vietnam. *Veterinary Microbiology*. 2019;230:117-122.
11. Wilkie W. Polyserositis and Meningitis Associated with Escherichia coli Infection of Piglets Can Vet J. 1981; 22 (5): 171–173.
12. Zhang B, Tang C, Liao M, et al. Update on the pathogenesis of Haemophilus parasuis infection and virulence factors. Vet Microbiol. 2014; 168 (1):1-7.

CASE 4: T2813/19 11 (4140288-00)

Signalment: Finishing pig, male, 7-8 month, SKS line, *Sus scrofa*



Lung, pig. There is diffuse and marked hypercellularity highlighting the airways and pleura in a lobular fashion. (HE, 7X)



Lung, pig: Large nodules composed of lymphocytes and histiocytes surround the airways, efface the submucosa, and impinge on the airway lumen. (HE, 70X)

History:
This pig was part of an "in field"-vaccination study against *Mycoplasma hyopneumoniae* and porcine circovirus type 2 (PCV2). The clinical examination revealed respiratory symptoms typical for *M. hyopneumoniae*, like dry and non-productive coughing. To confirm this suspected diagnosis the lungs were examined grossly at the abattoir. The determined lung score, using the scheme of Madec and Kobisch (5), with values up to 20 confirmed the clinical diagnosis.

Gross Pathology:
The tissue collected at the abattoir showed cranioventral consolidated lungs with lesions consistent with catarrhal and suppurative bronchopneumonia.

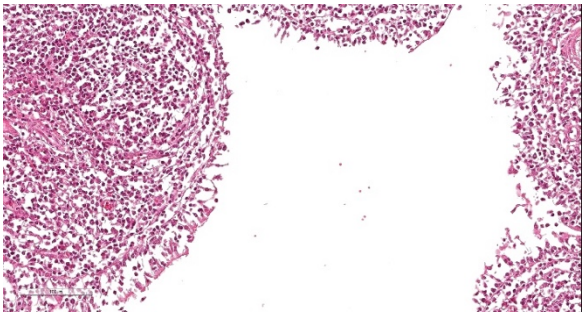
Laboratory results (*PCR/RT-PCR):*
PCV2 (DNA): negative
PRRSV (RNA) (EU+US): negative
M. hyopneumoniae (DNA): positive
A. pleuropneumoniae (DNA): negative
H. parasuis (DNA): negative
Swine influenza Virus (RNA): negative

Microscopic description:
Lung: Affecting 80 % of the tissue, there is a marked follicular hyperplasia of peribronchiolar, peribronchial, and perivascular lymphoid tissue compressing, infiltrating and effacing the structure of the adjacent alveoli. Often follicles line up beneath the pleura. The follicles are characterized by a central paler zone of lymphocytes (germinal center) rimmed by a zone of more densely packed lymphocytes (mantle zone). Adjacent alveoli are either compressed (atelectasis) or expanded by a mixture of homogenous eosinophilic fluid (edema) admixed with neutrophils, macrophages, lymphocytes and plasma cells, and few erythrocytes. Alveolar septa are expanded by lymphocytes and plasma cells. Bronchi and bronchioles often have attenuated (sometimes lost), cuboidal epithelium with loss of cilia. Lumina frequently contain sloughed epithelial cells admixed with degenerate inflammatory cells. There is mild edema of the interlobular septa with infiltration of low numbers of neutrophils, macrophages, lymphocytes and plasma cells.

Immunohistochemistry
CD3/CD20 - for lymphocyte characterization

Contributor's morphologic diagnosis:
Lung: Pneumonia, bronchointerstitial, lymphohistiocytic, multifocal to coalescing, chronic, severe with marked lymphoid hyperplasia; Bronchopneumonia, catarrhalic, multifocal to coalescing, chronic, moderate. Fattening pig. *Sus scrofa.*

Contributor's comment:
Enzootic pneumonia (EP) caused by *Mycoplasma hyopneumoniae* is a common chronic lung disease of pigs. EP is the most important lung disease in grower-finisher pigs worldwide and causes major economic losses to the pig industry. The course of the disease progresses over weeks with low mortality, but high morbidity (up to 100%). Coughing, reduced weight gain and reduced feed conversion are the main clinical

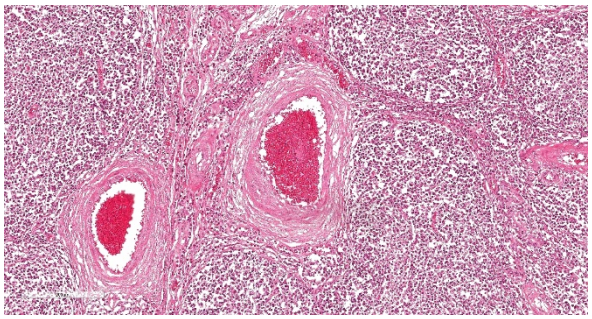


Lung, pig: The lymphoid nodules efface the submucosa and the overlying mucosa is multifocally eroded. (HE, 300X)

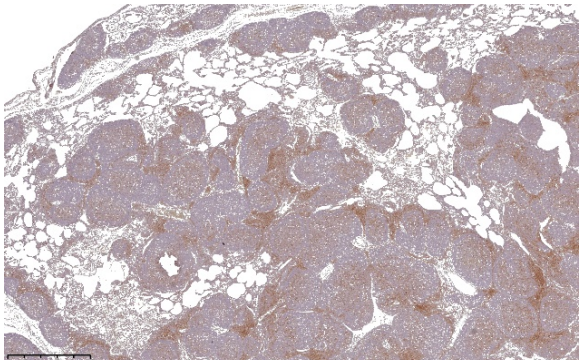
signs.[1,5] Furthermore, the pathogen is a primary agent of the porcine respiratory disease complex (PRDC) interacting with other porcine pathogens (e.g. PRRSV, PCV2, Swine influenza virus, *Pasteurella multocida, Actinobacillus pleuropneumoniae, Haemophilus parasuis, Bordetella bronchiseptica*). Other influencing factors are housing conditions and management practices (like crowding, poor air quality, stress). The result is a more severe multifactorial disease.[10]

Mycoplasma hyopneumoniae, like other mycoplasma species, is pleomorphic, lacks a cell membrane and has a small genome. Dams and piglets often serve as reservoirs. The most important way of transmission is horizontal. Vectors do not play an important role, but airborne transmission has been suspected.[1,5,7] The incubation period is variable (1-6 weeks post infection) and the ciliated epithelium of the respiratory tract is the first target using different adhesins (e.g. P97, P102, P159). Mycoplasmas lack classical virulence factors like toxins, but they can use glycerol as a carbon source and the production of hydrogen peroxide can be triggered.[7]

Lungs are affected cranioventrally along the bronchioles, showing purple to gray consolidated areas with atelectasis and a rubbery or thymus like texture of the parenchyma. Histopathologically, inflammatory infiltrates are centered around the airways and expand into the interstitium, with extensive lymphoid hyperplasia and increased number of alveolar macrophages. Additionally, goblet cell hyperplasia, bronchial gland hyperplasia, alveolar and interstitial edema,



Lung pig: There is adventitial fibrosis of arteriolar walls. (HE, 200X).



Lung, pig. T-cells populate the periphery of the lymphoid nodules. (anti CD-3, 40X). Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet Giessen, Frankfurter Str. 96, 35392 Giessen, Germany http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie

loss of ciliated cells, and epithelial hyperplasia can be detected.[1,5]

Cell membrane proteins of *Mycoplasma sp.* are prone to serve as superantigens, leading to polyclonal lymphocyte proliferation.[1] Immune evasion of the mycoplasmas and the immune response of the host are considered to be the main driver of pulmonary lesions in EP. Inflammation is triggered by different cytokines like IL-1, TNF, and IL-6 as well as by interactions of Mycoplasma adhesin molecules with the host's plasminogen.[7]

Immunohistochemically, lymphocytes in the affected tissue are composed of numerous follicle forming-B cells (CD20) and lower numbers of perifollicular T cells (CD3) (see figs.). This finding highlights the importance of the pathogen/immune system interaction for the pathogenesis of EP.

In the presented case lymphoid hyperplasia is remarkable with extensive loss of respiratory epithelium and loss of normal tissue architecture, characterizing the chronicity of the infection. Hyperplasia is so marked, that it may even resemble lymphoid neoplasia histologically. It can be suspected that the animals were exposed to virulent strains of *Mycoplasma hyopneumoniae* for longer periods and that the vaccination protocol in this group (a significant number of animals showed similar lesions) did not sufficiently control the infection.

Detection of *Mycoplasma hyopneumoniae* is possible by different established methods, including immunohistochemistry, in-situ hybridization or PCR. Due to the special growth requirements of the pathogen bacterial culture is not a useful method.[6,7,10] In the presented case other relevant pathogens were excluded by PCR.

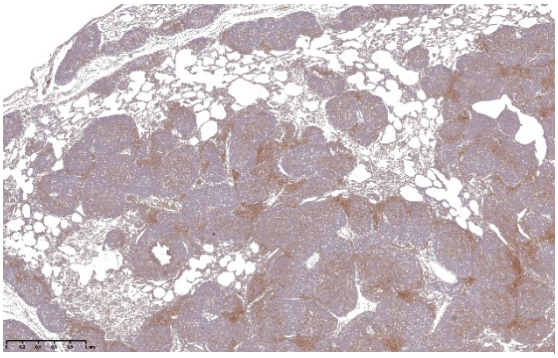
Vaccination is able to reduce clinical signs and lung lesions, and thereby improves performance. A reduction of the number of infectious organisms on the respiratory epithelium can be observed.[6,7] Different vaccination protocols exist depending on the type of herd, management and production system, and infection pattern. The most common strategy is single or two-dose application. The antigens of different pathogens often need to be combined to achieve the best beneficial effect of vaccinations.

Nevertheless, there are still knowledge gaps, especially with regard to the interaction of *Mycoplasma hyopneumoniae* with the respiratory tract. Therefore, further research is necessary, for a better understanding, control and elimination of the pathogen.

Contributing Institution:
Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet Giessen
Frankfurter Str. 96, 35392 Giessen, Germany
http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie

JPC diagnosis:
Lung: Pneumonia, bronchointerstitial, lympho-histiocytic, diffuse, severe, with marked lymphoid hyperplasia.

JPC comment:
The contributor provides a good summary of enzootic pneumonia of swine. There are numerous adjuvanted, inactivated, whole-cell preparation vaccines used in swine-producing countries, and live-attenuated vaccines available in China and Mexico. Unfortunately, these vaccines do not provide complete protection, and further elucidation of *M. hyopneumoniae*'s pathogenesis and virulence factors may improve the effectiveness of new vaccines. Virulence



Lung, pig. B-cells dominate the lymphoid nodules. (anti CD-20, 40X). Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet Giessen, Frankfurter Str. 96, 35392 Giessen, Germany http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie)

factors such as surface lipoproteins, surface aminopeptidases, and hydrogen peroxide production have been investigated. More recently, the ability to evade host defenses has received increased attention. Gentamicin survival experiments performed on immortalized porcine alveolar macrophages (cell line PAM 3D4/21) which showed that very few *M. hyopneumonia* organisms are phagocytosed by alveolar macrophages as compared to a control *E. coli* strain, suggesting the ability to evade phagocytosis. Opsonization with anti-*M. hyopneumoniae* antibody did not promote increased phagocytosis, which may help explain the lack of efficacy of current vaccines.[2]

While vaccines are used, the other arm of treatment of *M. hyopneumoniae* infection is antibiotic therapy. As with testing of any kind across multiple labs in different locations, worldwide antibiotic susceptibility testing is problematic. When testing is performed on different equipment (let alone different brand equipment), under different conditions, by different personnel, for different populations, the results are not necessarily *transportable*. In each testing in the distributed model, there may be *effector modifiers*, known or unknown, that can lead to variation in test results. When trying to apply a result to a new population, it is often not possible to know whether conditions are sufficiently similar as for the result to be valid in the new population.[3] However, the MycoPath program in Europe aims to improve some aspects of transportability by performing antibiotic

susceptibility testing of numerous strains of *M. hyopneumonia* and *M. bovis* in a single lab under controlled settings. In this testing program, strains of *M. hyopneumoniae* from Belgium, Spain, and the United Kingdom were collected from national laboratories and forwarded to the MycoPath central laboratory in Brussels, Belgium for testing. Appropriate caution should be used in the attempt to apply these results to new populations. However, one clear benefit of the testing highlighted the differences in MIC between *M. hyopneumonia* and *M. bovis* strains.[4]

Similar to *M. bovis*, further elucidation of virulence factors will aid our understanding of these pathogens. Currently, it has been shown that *M. hyopneumoniae* are capable of entering and surviving within vesicle-like structures in endothelial cells of the respiratory tract. They make contact with fibronectin and integrin B1 receptors on the epithelial cell surface and are engulfed and enter the cell through clathrin-coated pits and caveolae-mediated endocytosis. Within the cell, this diminutive bacterium then induces cytoskeleton rearrangements within epithelial cells, causing significant pathology to the respiratory tract.[9]

References:

1. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, ed. Jubb, Kennedy and Palmers *Pathology of Domestic Animals*. 6th ed. Vol . Elsevier, St. Louis, Missouri; 2015:465-891.
2. Deeney AS, Maglennon GA, Chapat L, Crussard S, Jolivet E, Rycroft AN. *Mycoplasma hyopneumoniae* evads phagocytic uptake by porcine alveolar macrophages in vitro. *Veterinary Research*. 2019;50:51.
3. Institute of Medicine. Ethical and Scientific Issues in Studying the Safety of Approved Drugs. 2019. Washington, DC: *The National Academies Press*. https://doi.org/10.17226/13219.
4. Klein U, de Jong A, Moyaert H, et al. Antimicrobial susceptibility monitoring of *Mycoplasma hyopneumonia* and *Mycoplasma bovis* isolated in Europe. *Veterinary Microbiology*. 2017;204:188-193.
5. Lopez A, Martinson SA. Respiratory system, mediastinum, and pleurae. In: Zachary JF, ed. *Pathologic basis of veterinary disease*. 6th ed. Elsevier, St. Louis, Missouri; 2017:471-560.
6. Maes D, Segales J, Meyns T, Sibila M, Pieters M, Haesebrouck F. Control of *Mycoplasma hyopneumoniae* infections in pigs. *Vet Microbiol*. 2008;**126**(4):297-309.
7. Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M. Update on *Mycoplasma hyopneumoniae* infections in pigs: Knowledge gaps for improved disease control. *Transbound Emerg Dis*. 2018;**65**(Suppl.1):110-124.
8. Ostanello F, Dottori M, Gusmara C, Leotti G, Sala V. Pneumonia disease assessment using a slaughterhouse lung-scoring method. *J Vet Med A Physiol Pathol Clin Med*. 2007;**54**(2):70-75.
9. Raymond BBA, Turnbull L, Jenkins C, et al. *Mycoplasma hyopneumonia* resides intracellularly within porcine epithelial cells. *Scientific Reports*. 2018; 8(1):17697.
10. Sibila M, Pieters M, Molitor T, Maes D, Haesebrouck F, Segalés J. Current perspectives on the diagnosis and epidemiology of *Mycoplasma hyopneumoniae* infection. *Vet J*. 2009;**181**(3):221-31.