Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 3

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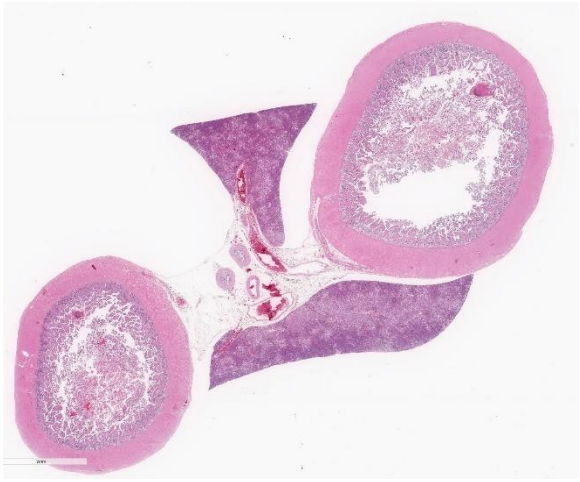
CASE I: W343-12 (JPC 4020435).

Signalment: Pigeon (*Columba livia*), age and sex unknown

History: An onset of high mortality affecting pigeons occurred in Victoria (Australia) in 2011. Clinical presentations in affected birds were lethargy, sudden death or neurological signs not further specified. Hundreds of birds were subsequently investigated, including racing pigeons, feral pigeons, domestic and commercial poultry and native birds of many species.

Gross Pathology: All the affected pigeons had diffuse pale and enlarged kidneys. The majority of birds also had enlarged spleens and grey mottled discoloration of the pancreas.

Laboratory results: Initial PCR testing for Newcastle Disease (APMV-1) was positive. Virus isolation and sequencing showed the virus to be the virulent strain of APMV-1. lmmunohistochemistry for paramyxovirus showed widespread immunoreactivity in the kidneys and in many cases pancreatic and splenic positivity were also observed.



Pancreas and intestine, pigeon. Up to 50% of the pancreatic tissue is replaced by multifocal to coalescing areas of pallor (necrosis). (HE, 9X)

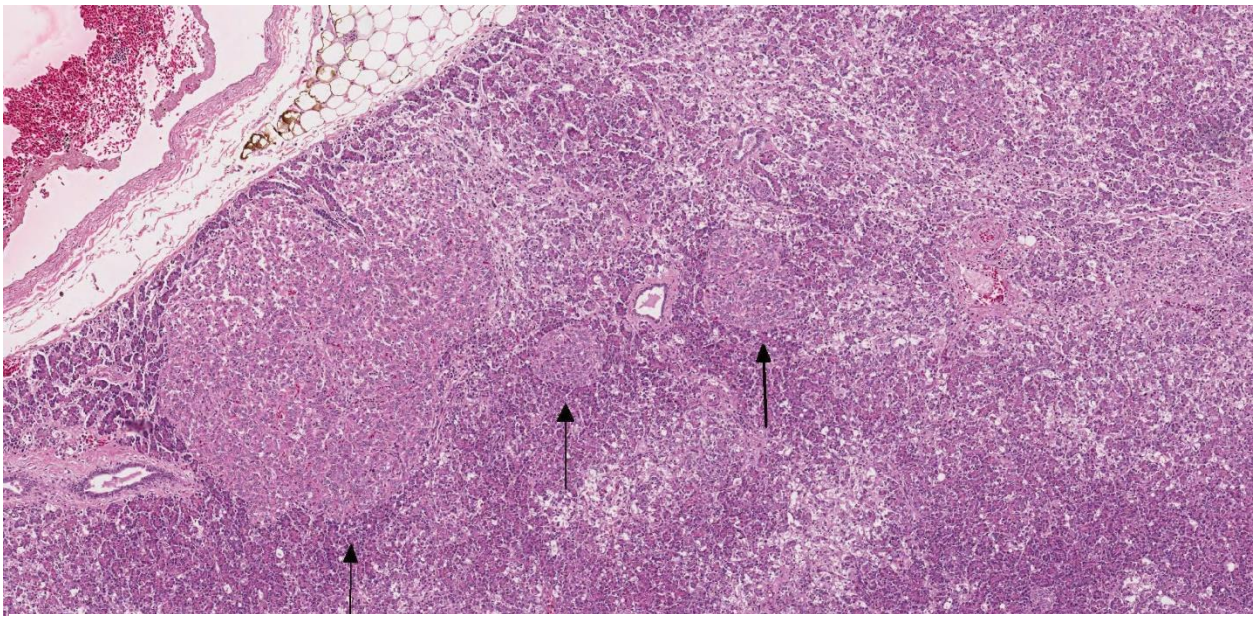
Microscopic Description: Pancreas: Multifocally and randomly distributed throughout the parenchyma there are numerous variably sized necrotic areas, characterized by disruption of normal architecture and stromal collapse, loss of cellular detail and accumulation of amorphous necrotic material, cellular debris and fibrin. There are scattered lymphocytes and plasma cells within the necrotic areas.

Small intestine: in the lamina propria of the mucosa there are small numbers of inflammatory cells, predominantly composed of lymphocytes and plasma cells. Not all slides: in the intestinal lumen there are few cross and longitudinal sections of nematode parasites, characterized by external cuticle, digestive tract, reproductive tract containing eggs with bipolar plugs, and hypodermal bacillary bands (consistent with *Capillaria* sp.).

Contributor's Morphologic Diagnoses:
1. Pancreatic necrosis, multifocal, acute, severe, pancreas, pigeon (*Columba livia*)
2. Mild, chronic, diffuse lymphoplasmacytic enteritis with intraluminal nematode parasites, consistent with *Capillaria* sp., small intestine, pigeon (*Columba livia*)

Contributor's Comment: The clinical, pathological and laboratory findings in this case are consistent with infection with avian paramyxovirus 1 (APMV-1). APVM-1 or Newcastle Disease Virus (NDV) belongs to the family of Paramyxoviridae, genus Avulavirus [1]
Different strains and isolates of NOV cause quite distinct clinical signs and severity of disease, even in the same host species. Based on the disease produced in chickens under laboratory conditions, NOV isolates have been placed in five pathotypes:

- viscerotropic velogenic, NOV strains that cause a highly virulent form of disease in which hemorrhagic lesions are characteristically present in the intestinal tract;
- neurotropic velogenic, NOV strains that cause high mortality following respiratory and nervous signs;
- mesogenic, NOV strains that cause respiratory and sometimes nervous signs with low mortality;



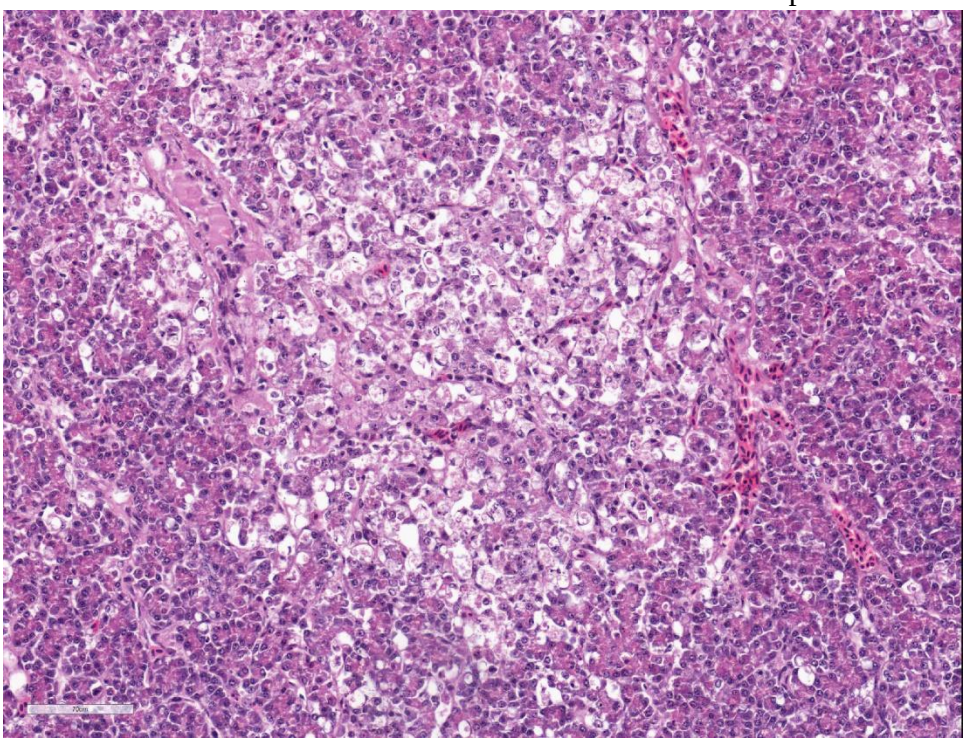
Pancreas, pigeon. Areas of necrosis are largely confined to acinar tissue, largely sparing islets (arrows). (HE, 95X)

- lentogenic, NOV strains that cause mild or unapparent respiratory infections;
- asymptomatic enteric, NOV strains that cause unapparent enteric infections.

However, such groups should be regarded only as a guide because there is always some degree of overlap and some viruses are not easily placed in a specific pathotype.[8]

Newcastle Disease (ND) is mainly a disease of poultry but NDV infections have been established in at least 241 species from 27 of the 50 orders of birds. All birds are probably susceptible to the infection, but the disease observed with any given virus may vary enormously from one species to another. Infection with NDV has been reported to infect animals other than birds, ranging from reptiles to humans.[1] APVM-1 has been responsible for outbreaks in pigeons in Africa, Middle East, Europe, Japan Canada and United States.[1] The variant nature of the virus enabled unequivocal demonstration of infection with this strain and for pragmatic purposes it became known as PPMV-1, although if this virus infects poultry, including pigeons reared for food, it fulfills the definitions of notifiable ND currently in use by the OIE.[2] Infection of feral and domestic pigeons with APMV-1 has been reported for the first time in Australia in August 2011.

In pigeons, neurological signs, such as torticollis, disturbed equilibrium, pecking aside seeds, paresis of wings or feet, and digestive symptoms (watery to hemorrhagic diarrhea) are frequently observed but respiratory signs are usually absent. Atypical digestive forms are being seen increasingly frequently and consist of persistent diarrhea without neurological signs.[1] The major clinical presentation in pigeons from the Australian outbreak were sudden death or neurological signs not further specified. Some of the birds submitted alive for euthanasia and autopsy had head tilt and/or ataxia.



Pancreas, pigeon. In areas of necrosis, there is loss of acinar architecture with areas vacuolation and fragmentation of acinar cells and pyknosis. (HE, 313X)

Histologically, the main lesions were in the pancreas and kidney. Pancreatic necrosis was invariably present in all the pigeons examined, occasionally associated with a lymphoplasmacytic infiltrate. Renal lesions were

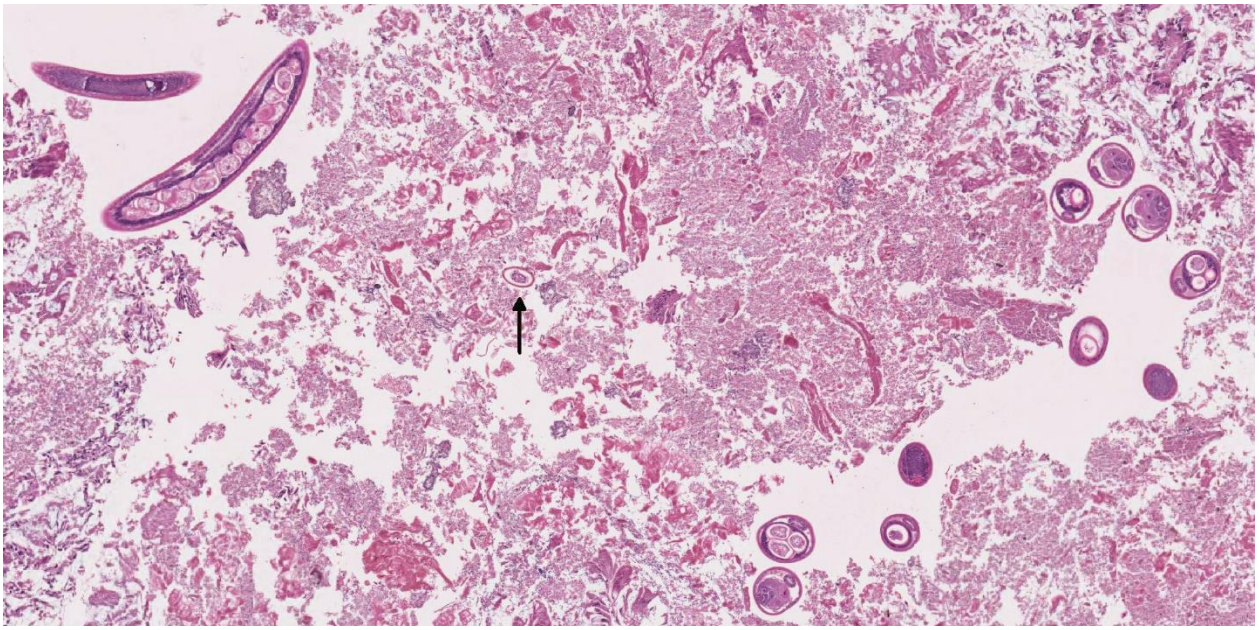
characterized by acute tubular necrosis, sometimes associated with a lymphoplasmacytic inflammation. Although behavioral and neurological disease was usually the presenting sign where sudden death had not intervened, histological lesions in the brain were rare and, where present, mild, limited to a mild lymphocytic infiltrate in the meninges. Similar lesions were described in the outbreaks previously reported in pigeons in Canada, United States and Europe; the lesions most commonly encountered were focal non-suppurative meningoencephalitis, lymphoplasmacytic infiltration in liver, pancreas and lung, multifocal hepatic necrosis, pulmonary congestion, piecemeal pancreatic necrosis, interstitial lymphoplasmacytic nephritis and tubular necrosis.[3,6]

In pigeons, experimental inoculation with the pigeon variant of PPMV-1 produced histological changes consistent with those we found in naturally infected pigeons, including pancreatic necrosis as well as necrotizing enterocolitis, necrotizing hepatitis with periportal hepatitis, pulmonary hemorrhage, tracheitis, and perivascular cuffing in the brain.[8]

The cases of PMV-1 infection in pigeons documented in the present report were not linked to any known Newcastle disease outbreaks in commercial poultry. The source of infection is not known, but in the previous European and Californian outbreaks the racing activities were the main means of transmission.[2,3]

Contributing Institution:
Faculty of Veterinary Science, University of Melbourne, Australia
www.vet.unimelb.edu.au

JPC Diagnosis: 1. Pancreas: Pancreatitis, necrotizing, random, multifocal to coalescing, moderate.
2. Small intestine: Enteritis, ulcerative, multifocal, mild.
2. Small intestine, lumen: Few adult adenopharsid (aphasmid) nematodes.

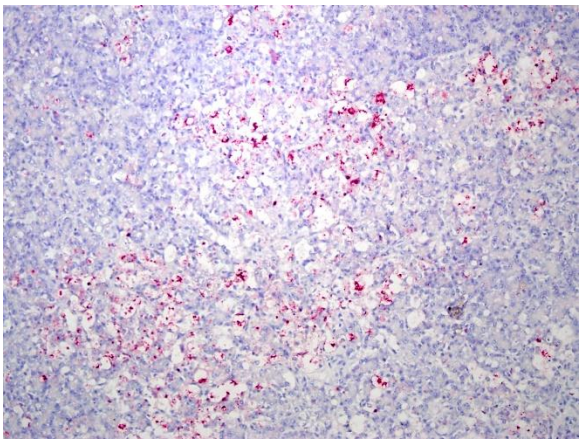


Intestine, pigeon. The lumen of the intestine contains cross sections of adult nematodes and rare eggs with bipolar plugs.

JPC Comment: Newcastle disease is a constant threat to the poultry industry worldwide, and the importance of not only recognizing its clinical signs, but also the pathogenesis and infection kinetics of this virus cannot be overestimated. This Tier 1 USDA Select agent has been reclassified from its previous genus Rubulavirus to a new genus of Avulavirus (which includes the previous rubulaviruses which infect birds). OIE reporting is required for velogenic and mesogenic viruses, and these strains are endemic in Asia, Africa, the Middle East and parts of Central and South America. An ongoing outbreak continues in California arising from backyard flocks (a similar outbreak spilled over into commercial poultry in California in 2003, resulting in losses of over three million birds and a cost of over $120 million).[4]

In addition to the infectious threat posed by illegal importation of backyard fowl in the US, several wild bird species pose a reservoir threat for virulent strain, including pigeons and doves, and waterfowl such as double-crested cormorants and swans, and upland game birds (pheasants and partridges)[5]. Most cases of Newcastle disease in pigeons are the result of pigeon-specific strains, denoted as pigeon paramyxovirus-1 (in order to differentiate them from the rest of the avulaviruses that infect birds.[5] Pet psittacines, including parrots, conures, budgerigars, and conures also develop neurologic disease after NDV infection, and a psittacine isolate was responsible for an outbreak of NDV in California in 1971.[5]

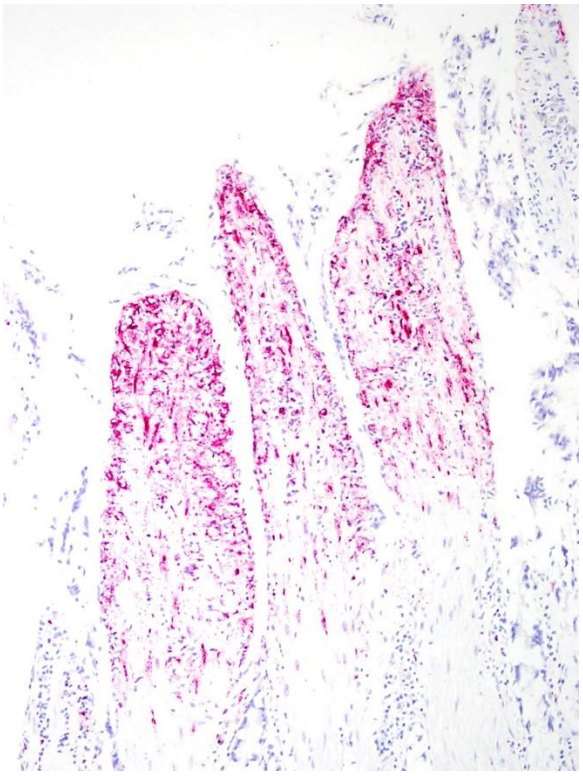
Strains of NDV virus have also been identified in wild anseriformes, shorebirds, and gulls, but not virulent fusion protein sequences have been identified.[4] Interestingly, bird surveys in many countries have found vaccine strain virus (lentogenic forms of the virus are often used in vaccination programs), which leads to concern that passaging through wild bird species may result in reversion to a more virulent strain. This particular event has occurred in poultry resulting in a 1998 outbreak in Australia, but not yet in wild birds.[4]



Small intestine, pigeon. Villi show extensive immunopositivity for avian paramyxovirus antigen within remnant epithelium as well as mesenchymal and inflammatory cells within affected villi. (anti-NDV, 400X) (Photo courtesy of: Dr. Corrie Brown, UGA Department of Veterinary Pathology).

Backyard fowl are a special problem to the biosecurity of our nation's poultry as a result of poor vaccination practices as well as their mobility. While all most all birds produced by the commercial industry receive one or more NDV vaccines during their lifetime (more long-lived birds like broiler-breeders may receive 3 or more vaccinations), estimates are that less than 10% of backyard birds are vaccinated,[4] and their exposure to other unvaccinated birds and potential wild reservoir birds makes the potential for an outbreak of mesogenic or velogenic NDV highly likely in this country.

Another area of concern is the minimal mutation required for transformation of an avirulent NDV into one of high virulence. The NDV genome encodes for 6 structural proteins – nucleocapsid protein,



Pancreas, pigeon. Necrotic pancreatic cells and infiltrating inflammatory cells demonstrate strong immunopositivity for avian paramyxovirus. (anti-NDV, 400X) (Photo courtesy of: Dr. Corrie Brown, UGA Department of Veterinary Pathology).

phosphoprotein, a matrix protein, a fusion protein, a hemagglutinin-neuroaminidase protein, and an RNA polymerase. Virulence has been attributed to amino acids at the cleavage site on the fusion protein, with three or more lysine or arginine residues starting at position 133 and a phenylalanine residue at 117 are common to all virulent strains of the virus. Hence, mutations resulting in this particular lineup of amino acid residues in the fusion protein may potentially give rise to new and virulent strains of the virus.[4]

On a somewhat lighter note, Newcastle disease received its name from the second documented outbreak of Newcastle disease which occurred in Newcastle-on-Tyne, England in 1927. A previous outbreak in Java (the Dutch East Indies at the time) did not result in the disease being called "Dutch East Indies chicken fever" or some other appellation, presumably as public relations experts were not in great numbers in Indonesia at the time. It has been theorized that an even earlier outbreak of Newcastle Disease may have occurred in South Uist on the Outer Hebrides islands (where cormorants are occasionally shot and consumed by the inhabitants – with the offal presumably tossed out on the ground). An outbreak of this type was memorialized in the Gaelic poem "Call nan Cearc" (The Loss of the Hens) written by John Campbell in 1898.[7]

The moderator reviewed the disease causes by pigeon paramyxovirus, an avuloviral disease first identified in the 1980s which has spread around the world, largely as a result of racing pigeon enthusiasts and the mobility of their charges. The virus, belonging to the same family as NDV is not particularly pathogenic for poultry, although if it has a high intracerebral pathogeneicity index (a measurement of pathogenicity for NDV), it can halt trade.

The moderator also perfomed an immunohistochemical study on the stlide, using an anti-NDV antibody. The amount of staining within small intestinal villi was surprising, as attendees obviously misinterpreted much of the necrosis in this tissue as autolysis.

As a final note, the class Aphasmidia (to which *Capillaria* belongs), has recently been renamed Adenophorasda.

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CASE II: 14-102 (JPC 4048789).

Signalment: 8-month-old purpose-bred Dorset cross ewe (*Ovis aries)*

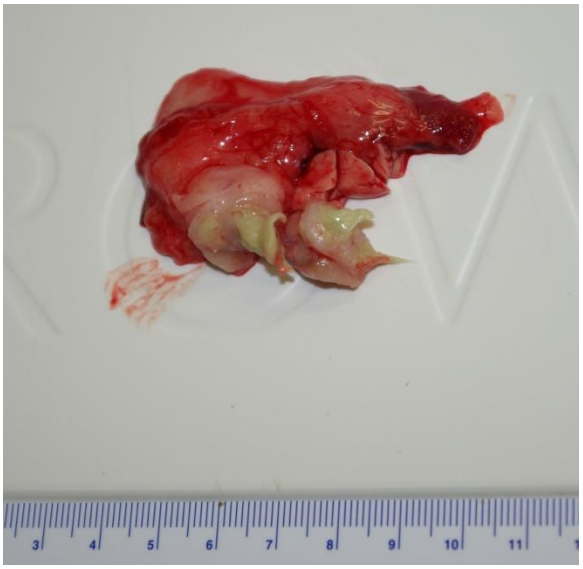
History: Received from supplier 17 days before euthanasia. Vaccinated for CD&T, pasteurellosis and orf 6 months previously. Negative Q fever serology. Dewormed with an avermectin at time of shipping. Eleven days after arrival she developed a 5 cm diameter subcutaneous abscess of the cranioventral neck near the angle of the mandible. FNA was performed and a sample was submitted for culture. The next day the abscess had ruptured and incompletely drained. When culture results were received, the decision was made to euthanatize.

Lymph node, sheep: There is a fibrous subcutaneous swelling at the angle of the jaw. (Photo courtesy of: Department of Comparative Medicine, Penn State Hershey Medical Center, http://www.hmc.psu.edu/comparativemedicine/)

Gross Pathology: At necropsy there was a firm fibrous subcutaneous swelling at the angle of the right mandible with a cutaneous scab. The left submandibular lymph node was firm (fibrosis) and exuded thick green material on cut section. There were several small (2-5 mm) tan nodules in the lungs and liver.

Laboratory results: Gram Stain of FNA: Large numbers of gram-positive rods.
Aerobic culture of abscess (PADLS PVL): Heavy growth of *Corynebacterium pseudotuberculosis*.

Microscopic Description: The lymph node is partially effaced by one large or several coalescing discrete, incompletely encapsulated pyogranulomas with a large central core of abundant necrotic cellular debris and degenerate neutrophils with numerous coarse basophilic refractile



Lymph node, sheep: When incised, the swelling exuded a green tenacious exudate. (Photo courtesy of: Department of Comparative Medicine, Penn State Hershey Medical Center, http://www.hmc.psu.edu/comparativemedicine/)

mineralized concretions. This is surrounded by a layer of moderate numbers of epithelioid and foamy macrophages with occasional multinucleate giant cells, mostly of the Langhans type. Peripheral to this are moderate to large numbers of plasma cells with fewer lymphocytes and macrophages, and rare Mott cells. There is abundant nascent (immature) and mature fibrosis circumscribing the lesion. The lymph node is mildly reactive with pale germinal centers, paracortical lymphoid hyperplasia, and medullary sinus plasmacytosis.

Similar pyogranulomas were present in the liver and lungs (tissue not submitted), often with prominent eosinophil infiltration.

Contributor's Morphologic Diagnosis:
Lymph node, left submandibular, pyogranuloma, focally extensive, chronic, moderate with mineralization

Contributor's Comment: Caseous lymphadenitis is a disease of small ruminants caused by infection with *Corynebacterium pseudotuberculosis* (*C. ovis*).[1,5] The agent is so named for the gross and histologic similarity of the pyogranulomas to those of tuberculosis, including mineralization and caseation. The organism generally gains entry through cutaneous wounds (often related to shearing or castration), although none were present in this case. Given the lesion location, infection via a wound in the oral cavity is likely. Organisms localize in the local draining lymph node. Infections can also spread internally, commonly to the lungs (as in this case), making this animal unsuitable for research purposes. The organism can survive intracellularly within macrophages due to a leukotoxic surface lipid, and infections tend to be persistent but subclinical. The characteristic green color of the gross exudate is imparted by the accumulation of eosinophils in the lesions. Inspissation of the exudate over time produces the classic lamellated cheesy material.

Corynebacterium pseudotuberculosis is a pleomorphic, gram-positive, non-motile, facultatively anaerobic member of the *Actinomycetaceae*.[1,3] Members of this group are notable for the mycolic acid content of the cell walls and prolonged environmental persistence. *Corynebacterium pseudotuberculosis* is closely related to *C. diptheriae and C. ulcerans.* There are two biochemically and genetically distinct biovars of *C. pseudotuberculosis,* biovar *ovis* (biotype 1) and biovar *equi* (biotype 2). The former is typically a pathogen of small ruminants and does not reduce nitrate; the latter is more commonly a pathogen of cattle and horses and does reduce nitrate. Infections in horses include pigeon fever (skeletal muscle abscesses) and ulcerative lymphangitis.[4] Virulence factors for this

organism include phospholipase D, a sphingomyelin-specific phospholipase, as well as mycolic acids within the cell wall.

Differential diagnosis for this case would include *Trueperella (Arcanobacterium) pyogenes*, *Staphylococcus aureus* (botryomycosis), *Actinobacillus lignieresii*, and *Mycobacterium bovis*. Gram positive organisms of veterinary importance can be remembered by the acronym SCRAMBLED SCENT, encompassing the genera:

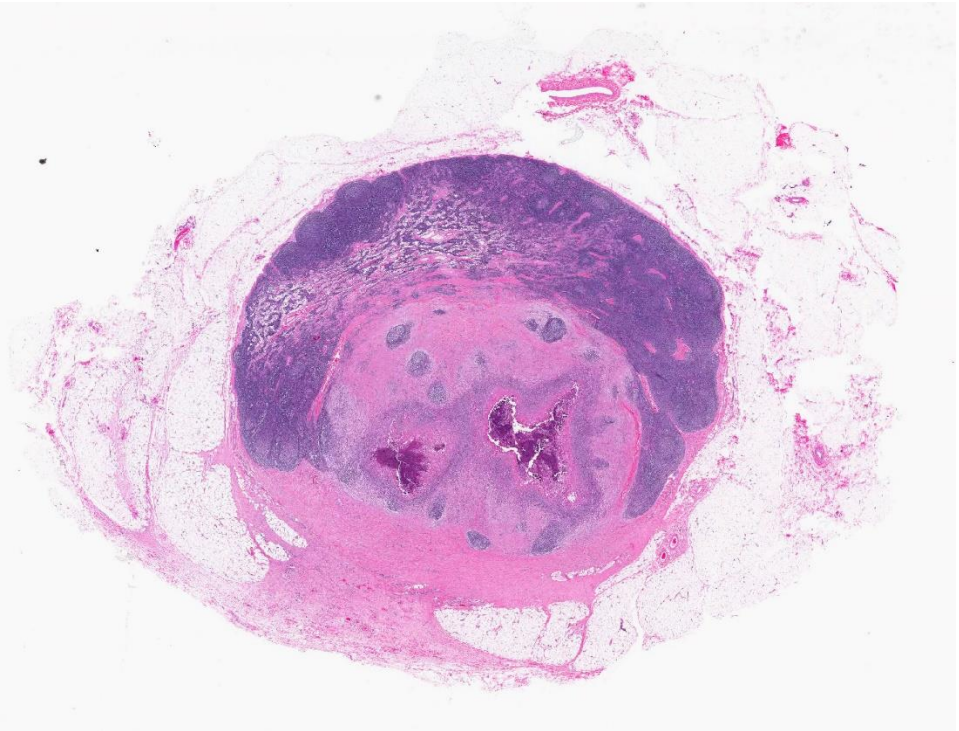
- *Staphylococcus*
- *Clostridium*
- *Rhodococcus*
- *Actinomyces*
- *Mycobacterium*
- *Bacillus*
- *Listeria*
- *Erysipelothrix*
- *Dermatophilus*
- *Streptococcus*
- *Corynebacterium*
- *Enterococcus*
- *Nocardia*
- *Trueperella*

Subcutaneous reactions tocClostridial vaccines, particularly around the neck or scapula, can also be mistaken for CLA.

Contributing Institution:
Department of Comparative Medicine
Penn State Hershey Medical Center
http://www.hmc.psu.edu/comparativemedicine/

JPC Diagnosis: Lymph node: Pyogranuloma, focal.

JPC Comment: The contributor gives a concise review of *Corynebacterium pseudotuberculosis* infection in small ruminants. This gram-positive facultative intracellular pathogen, which may exhibit pleomorphism in tissue, such as coccoids and filamentous rods,[2] is best known for abscess formation in small ruminants, also affects a wide range of other species, including horses (as previously mentioned), cattle, camelids, deer, and humans. The bacterium was first identified by the French bacteriologist Edward Nocard from a cow, and three years later, by the Bulgarian Hugo von Priesz from a ewe.

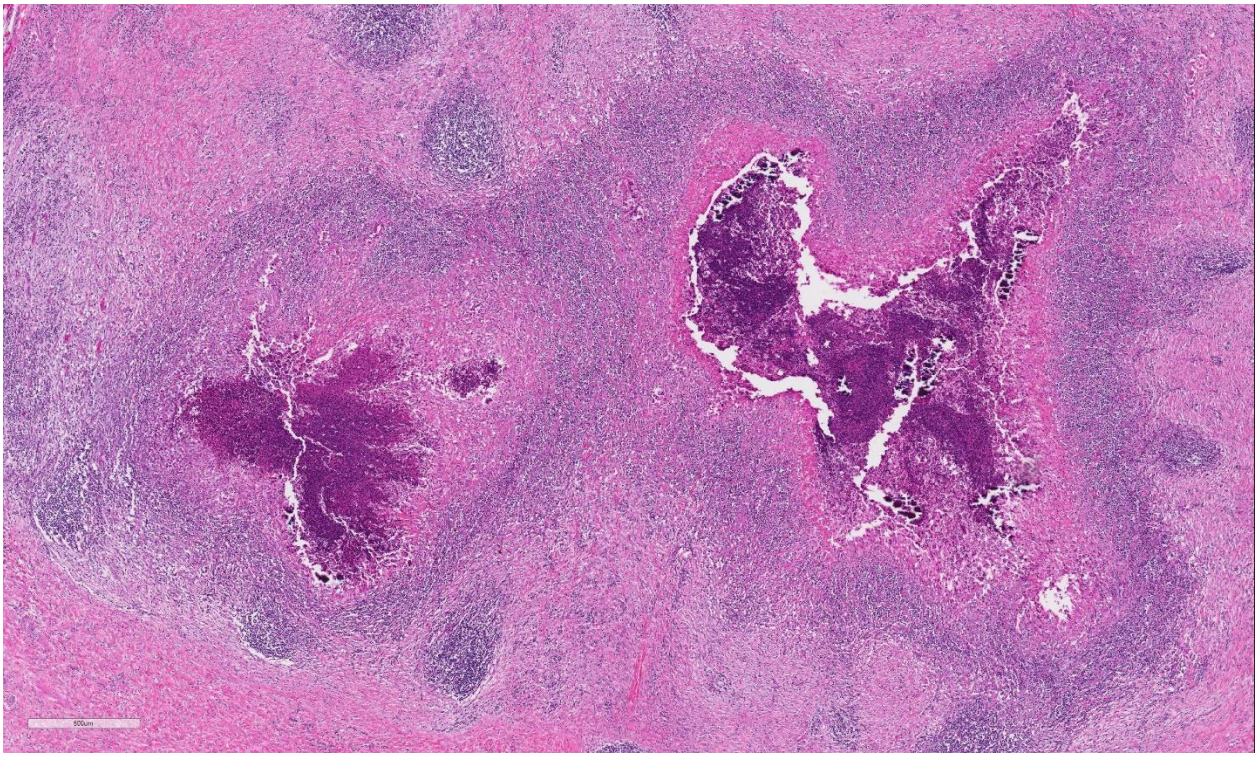


Lymph node, sheep: Approximately 50% of the node is effaced by a large abscess with a core of lytic material and a thick capsule. (HE, 5X)

For the next thirteen, it was referred to as the "Priesz-Nocard" bacterium, whereupon it was renamed *Bacillus pseudotuberculosis* in the atlas by prominent German bacteriologists Lehman and Neuman. In the 1923 first edition of Bergey's *Manual of Determinative Bacteriology* it was placed in the genus of *Corynebacterium,* where it remains today. It was at that time called *Corynebacterium ovis*, but after discovered to cause infection in a number of species, reverted back to *C. pseudotuberculosis* in 1948, by the sixth edition of that manual.[1]

In sheep, infection usually follows wound infection, and at shearing time, infected abscesses may be punctured, bacterial liberated in a common dip tank, and the bacterium may invade shearing wounds on other sheep or even penetrate intact skin. In addition to direct contact, the disease may also be spread by sheep with established respiratory infections coughing on the open wounds of penmates.[2] If the bacteria are not confined to and eliminated from the skin, the infection may progress to draining nodes.[6] Mature abscesses in the lymph nodes of sheep may achieve a greenish lamellated "onion-skin appearance" due to recurrent and alternating episodes of suppuration and encapsulation; in goats, the abscesses tend to demonstrate a more liquefied appearance. When the infection reaches the lymph nodes, the condition is considered persistent and lifelong.[1]

In sheep, especially older sheep, the infection may progress from peripheral lymph nodes to internal nodes or organs, especially the lungs, resulting in chronic systemic disease referred to as the "thin ewe syndrome" (apparently more common in the US than in other countries)[1]. The presence of abscesses within



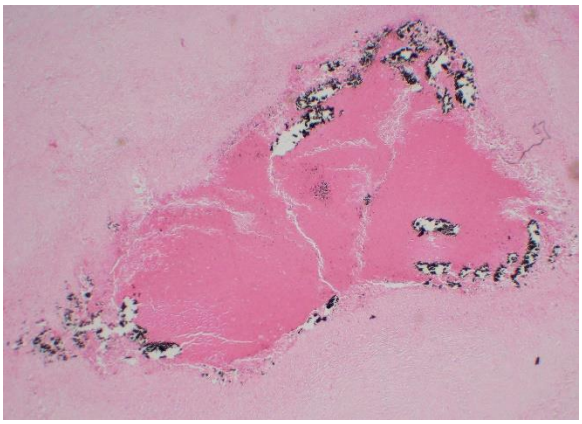
Lymph node, sheep: Higher magnification of the necrotic core of the abscess. Lymphoid aggregates are scattered throughout the abscess wall. (HE, 45X)

nodes and carcass meat generally results in condemnation, which, in countries which utilize lamb for religious celebration, may result in a loss of $200 per animal to the purveyor. Many countries have strict importation guidelines regarding contamination of small ruminants with this bacterium. The importance of CLA vaccination is exemplified by the decrease in CLA in Australia alone - in 1973, CLA among sheep in Western Australia was estimated at 58%; following introduction of a CLA vaccine in 1983, similar studies recorded a prevalence of 45%, which in turn dropped to 20% in 2002.[1]

In goats, lesions are more often severe, and abscesses tend to cluster in the nodes of the face and neck. The liquid nature of abscessed nodes is reminiscent of melioidosis (*Burkholderia pseudomallei* infection) in this species.[6] Other lesions associated with *C. pseudotuberculosis* in sheep and goats include mastitis (presumably resulting from local spread from abscessed supramammary nodes)[1] and polyarthritis in young lambs, but overall, the disease is rarely fatal, even in prolonged infection.[6]

Another disease caused by *C. pseudotuberculosis* in the horse is equine folliculitis and furunculosis (also referred to as equine contagious acne, , equine contagious pustular dermatitis and Canadian horsepox. It is most often seen at points of contact with tack in animals with pre-existent seborrheic dermatitis, and likely represents secondary invasion by bacteria spread on contaminated tack.[1]

The moderator, who did her PhD studying this agent, reviewed the various pathogenic factors which allow it to cause disease in a range of ruminants and horses. Like other higher bacteria, the presence of mycolic acid in the wall of the bacterium, which allows it



Lymph node, sheep: A Von Kossa stain highlights the mineral within the lesion. (von Kossa, 20X)

to resist digestion in phagocytes also lends a unique property to colonies in culture – the ability to slide easily across the plate, known as "shuffleboard colonies". Another virulence factor, phospholipase D, is important for tissue invasion. In sheep and goats, phospholipase D allows the bacteria to invade through sphingomyelin-containing endothelium, allowing for extensive intravascular spread of the bacterium. In the biovar infecting horses, the bacterium do not possess sufficient phospholipase D for intravascular spread, and must be introduced in a wound, resulting in a slow progressive lymphangitis.

The moderator also described the synergistic hemolysis-inhibition titers (with a rather rude acronym) which were used in the 1980s for diagnosis of occult infection of *C. pseudotuberculosis* in small ruminants in Brazil. The synergistic hemolysis-inhibition test detects antibodies to an exotoxin of *C. pseudotuberculosis* by the inhibition of a synergistic hemolysis between the toxins of *C. pseudotuberculosis* and *R. equi.*

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CASE III: 2019B (JPC 4134828).

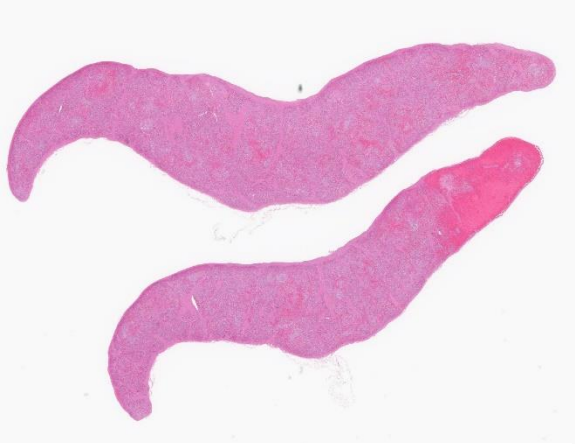
Signalment: Pig, *Sus scrofa domesticus*, LWD, 8-weeks-old, female

History: This is one of two experimental cases of pigs infected with classical swine fever virus (CSFV). This case was intraorally inoculated with 1mL of $10^{6.5}TCID_{50}$/mL of a strain of CSFV. Clinical signs of the animal were observed and total leukocytes in the whole blood of the pig were counted daily for 2 weeks. Clinical samples of the heparinized hole blood, sera, saliva, nasal swab and feces were collected for the detection of the virus gene by RT-PCR analysis.

The pigs inoculated with CSFV showed fever and abolition of appetite from 4 days post infection (dpi) and developed reddened skin lesion in hindlegs and conjunctivitis at 14 dpi. The pig was euthanized at 14 dpi.

Gross Pathology: In the pig infected with CSFV, multifocal infarction of the margin of the spleen was observed. Mild hyperemia on the brain was seen in the pig. Hemorrhagic lymph nodes in the pig were appeared. Button ulcers in the colon was find in the pig. There were no pathological findings in the respiratory organs in the pig.

Laboratory results: The body temperature of this CSFV inoculated pig was over 40^{o}C from 4 to 14 dpi. The decrease of leukocyte counts in blood (under 10,000 cells/µl) were also confirmed from 3 to 14 dpi. The viral RNA was detected from the blood samples from 4 dpi and from saliva, nasal swab and feces from 5 dpi in the pig.



Spleen, pig. Two sections of spleen are presented for examination. A large area of congestion and hemorrhage (peripheral infarct) is present in the bottom section. (HE, 7X)

Microscopic Description: Spleen: There were multifocal necrosis with hemorrhage, karyopyknosis and karyorrhexis in the red pulp. The lumen of some blood vessels was dilated and was occluded by a fibrin thrombus. Necrosis of arterial walls were occasionally observed; however, vasculitis was not prominent. The stromal and parenchymal cells in the wedge-shaped margin of the spleen was replaced by extravascular erythrocyte accumulation. The remaining stromal cells in that area showed necrosis with karyopyknosis and karyorrhexis (hemorrhagic infarction).

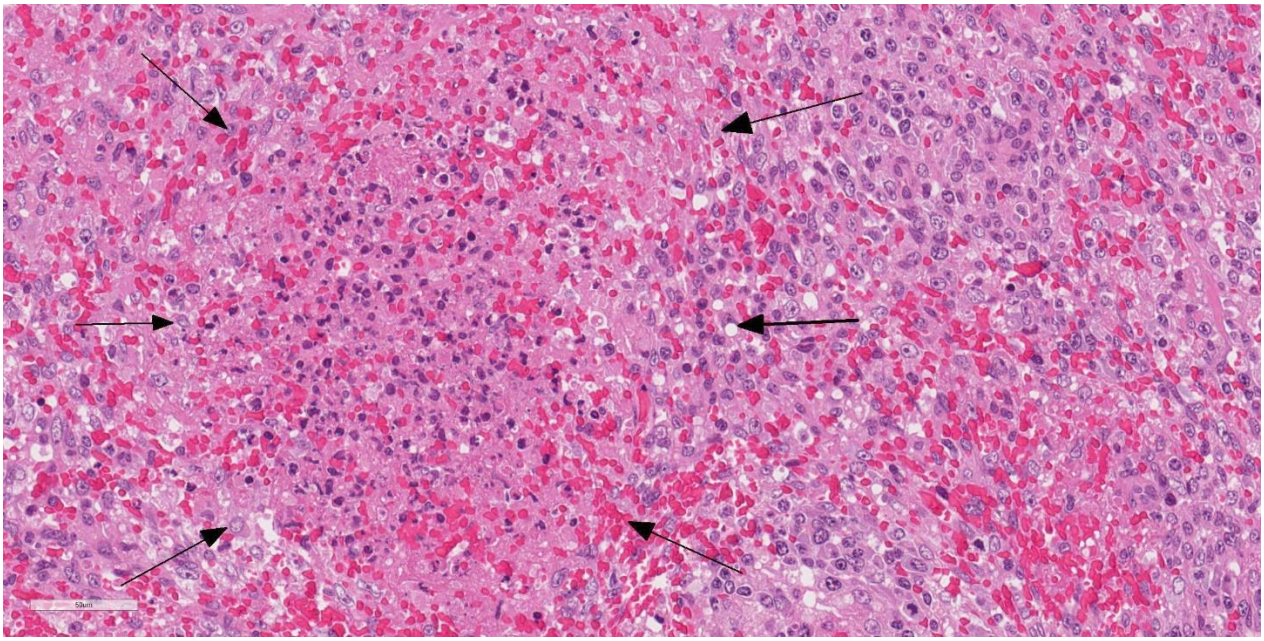
The white pulp in the adjacent parenchyma is of markedly reduced cellularity (lymphoid depletion) and almost of lymph follicles were atrophic.

Contributor's Morphologic Diagnosis:

1. Spleen, red pulp: Multiple fibrin thrombosis with hemorrhagic infarction, multifocal necrosis, moderate.
2. Spleen, white pulp: Lymphocyte depression, diffuse, severe.

Contributor's Comment: We confirmed that the characteristic lesion of multifocal infarction of the margin of the spleen arises following inoculation with CSFV by intraoral inoculation. The histological lesion of hemorrhagic infarction was reproduced experimentally; however, vasculitis was not prominent in this case, suggesting this case was in an early stage of an infection of CSFV.

Classical swine fever (CSF) is a highly contagious viral disease induced by CSFV of the genus *Pestivirus* in the family *Flaviviridae*.[3] This disease is widely distributed in the world including Asian countries. CSFV is classified into three genotypes (1, 2 and 3) and several subgenotypes (1.1–1.4, 2.1–2.3, and 3.1–3.4).[4,7] In East and Southeast Asia, several genotypes or subgenotypes of CSFV isolates including 1.1, 2.1-2.3 and 3.4 have been identified.[1,5] The virulence of the CSFV ranges from highly virulent with almost 100% mortality, to avirulent. The severity also depends on the condition of host animals including age, breeds and health status.[2]

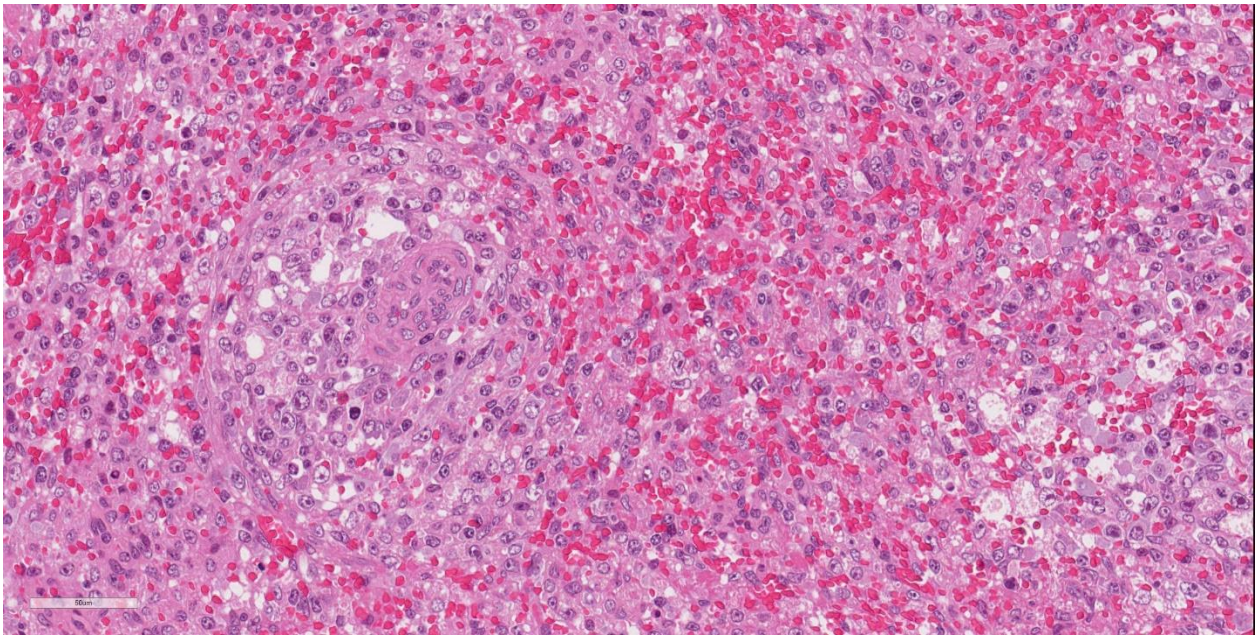


Spleen, pig. Within the red pulp, there are multifocal areas of lytic necrosis and fibrin deposition. (HE, 400X)

In the present experiment, the infected pigs developed clinical signs of lethargy, anorexia, reddish dermal macula and conjunctivitis. These clinical symptoms were quite mild, and the pig did not die within 2 weeks of the experimental period. These results suggest that the virulence of the CSFV used in this study is considerably lower than a high virulent strain of CSFV.[2]

Splenic infarction is considered pathognomonic for CSF.[2] Macrophage-driven cytokine storms following infection of CSFV play an important role of the pathogenesis of splenic infarctions in CSF. [2] A marked increase in macrophage/monocyte-derived pro-inflammatory cytokines such as TNFα, IL-1, and IL-6 is suggested to be the main mediator of systemic endotheliotoxicity. These cytokines induce endothelial swelling, followed by degeneration and necrosis of endothelial cells and fibrinonecrotizing vasculitis.[13] The formation of arterial infarcts at the multifocal sites of vasculitis within the spleen leads to vascular occlusion followed by ischemic necrotic cell death of the dependent parenchyma. A single splenic arterial supply with minimal anastomoses is an important predisposing factor for infarction in the spleen.[6] This infarction area is rapidly followed by hemorrhage from damaged blood vessels and inflow of erythrocytes from the surrounding parenchyma with intact perfusion leading to the commonly illustrated hemorrhagic infarcts.[6]

Severe lymphocyte depression is also common in the splenic white pulp of the CFSV-infected pigs. In acute stages of infection, CSF is accompanied by severe lymphopenia and resulting immunosuppression as well as granulocytopenia.[2,8,11] The infection of dendritic cells induces secretion of large amount of IFN-α which is a key element in the pathogenesis of CSF. The high serum level of IFN-α is suggested to be the central inducer of dysregulation of the immune system, which manifests as lymphocyte apoptosis, depletion and immunosuppression.[10]



Spleen, pig. Splenic follicles (one at left) are devoid of lymphocytes but replete with macrophages. (HE, 400X)

The acute form of the disease is characterized by atypical clinical signs such as high fever, anorexia, gastrointestinal symptoms, general weakness and conjunctivitis during the first two weeks upon infection of CSFV and then skin lesions including hemorrhages, cyanosis or reddish macula appeared in different areas of the body such as the ear, limbs and ventral abdomen around two to four weeks after infection.[2,9] In this study, this typical skin lesion could find from 14 dpi in the inoculated pig and the clinical appearance was quite difficult to diagnose as CSF in this experimental study. The results in this study showed that the pigs infected with this strain of CSFV excrete viruses into the clinical samples and viral RNAs were detected from the blood samples, especially whole blood samples, from 4 or 5 dpi until at least 2 weeks after infection. Because it is difficult to detect clinical sigms in pigs infected with this strain, the results in this study are significantly valuable to establish countermeasures and diagnostic strategy for CSFVs that has similar characteristics with mild pathogenesis.

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JPC Diagnosis: 1. Spleen, margins: Necrosis, multifocal,l moderate, with thrombosis, hemorrhage and fibrin deposition (infarcts).
2. Spleen, white pulp: Lymphoid depletion, diffuse, severe.

JPC Comment: The contributor has done a nice job describing an investigation of the effects of intraoral inoculation of a mildly virulent strain of classical swine fever virus (CSFV). CSFV, a pestivirus in the same family as bovine viral diarrhea virus and border disease virus, is one of the most economically important viruses in the world. While a number of countries have eliminated the virus (United States, Canada, Mexico and many Western European countries), the virus remains endemic in many others, including China, India and many countries in Central and South America. A number of countries that had eliminated CSF have recently seen recurrences in light of a large current outbreak in China.[15]

The disease of classical swine fever is divided into acute and chronic forms. The acute form is also known as the "lethal" form (although both forms end with death of infected pigs) or the "transient" form (another odd choice of name.) In the acute form, clinical signs appear after 4-7 days, and non-specific (referred to as "atypical" signs) clinical signs of fever, pyrexia and gastrointestinal distress are the rule.[2] From 2-4 weeks, more diagnostic signs of including neurologic deficits, paralysis and convulsions appear, and are often accompanied by cutaneous hemorrhage and cyanosis of the extremities and abdomen (the so-called "typical" of classic signs of CSF. Due to the immunosuppression which accompanies CSF (such as seen in the section of spleen submitted in this case), respiratory and gastrointestinal disease may also be present. Infection of pregnant sows may result in abortions, stillbirths, and mummification.[2]

Chronic infections are usually associated with immunotolerant pigs who were infected during gestation. These infections, while prolonged and mostly associated with "atypical" signs, ultimately also result in death of infected animals; albeit with a longer course of several months in which they continually shed high levels of virus in the saliva, urine, feces and other secretions.
Death is often the result of immunosuppression, and affected animals

exhibit marked depletion of lymphoid organs and ulceration of the gastrointestinal tract.[2]

CSF control is based largely on two strategies – comprehensive culling (which is effective in countries which have eliminated the virus), and vaccination, which is practiced in countries in which the CSFV is endemic.[15] While many of these countries now mandate vaccination against CSV, small and medium pig farms (in which help irregular vaccination schedules or the maintenance of immune-tolerant animals) may help to maintain the virus in an endemic state. Vaccination failure may be further impacted by the presence of multiple genotypes in a given area or immunosuppressive coinfections by other viruses include PRRS virus and PCV-2. In some countries, the virus is maintained in the population of wild boars from which it may spill over into the domestic pig population, especially on small farms.[15]

Another potential weapon in the fight to eradicate CSV in endemic areas is the development of virus-resistant animals. Recently, Chinese scientists reported the development of genetically modified pigs using CRISPR-Caspase 9 mediated knock-in technology. Antiviral small haripin RNAS were inserted into porcine DNA at the *Rosa26* locus and transgenic pigs were produced by somatic nuclear transfer. F1-generation pigs were challenged with virulent virus and and developed only minor clinical symptoms and markedly decreased blood levels of virus as compared to non-transgenic controls.[12]

One of the more interesting aspects of this particular slide is the relatively mild changes noted in the splenic arterioles, which rather than a significant vasculitis (which would be expected given the endotheliotropism often exhibited by this virus) exhibited at best a mild vasculopathy with no mural inflammation and rare pyknosis of mural smooth muscle cells and a hint of protein within the wall. A spirited discussion of the usage of the terms vasculitis and vasculopathy for this change in the absence of any significant inflammation failed to yield a clear victor.

To further illustrate the marked lymphoid depletion in this specimen, a JPC-run CD20 highlighted the almost absolute lack of B-cells (a favorite target of CSFV), and a CD3 showed marked depletion of T-cells. An IBA-1 stain showed numerous macrophages, many of which appeared activated.

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CASE IV: P17-969 (JPC 4135861).

Signalment: 7 months old, female, Holstein, *Bos taurus*, bovine.

History: The animal was experimentally infected with 10,000 ID_{50} foot-and-mouth disease virus (FMDV A IRN/22/2015) into the tongue. After 24 hours, the animal was euthanized and submitted for necropsy.

Gross Pathology:
Tongue: Multiple oval to round vesicles (aphthae), up to 3 cm diameter were multifocally present at the dorsum of the tongue.
Hoof: Multiple vesicles and pustules were also found on the left hoof within the interdigital space and bulb of the heel.

Laboratory results: None.

Microscopic Description:
Tongue. Expanding within the lingual mucosa, there is a 6.5 mm large, well-demarcated vesicle (vesiculopustule) which contains variable numbers of viable and degenerate neutrophils, erythrocytes, fibrillar beaded eosinophilic material (fibrin) and a low amount of proteinaceous fluid. The

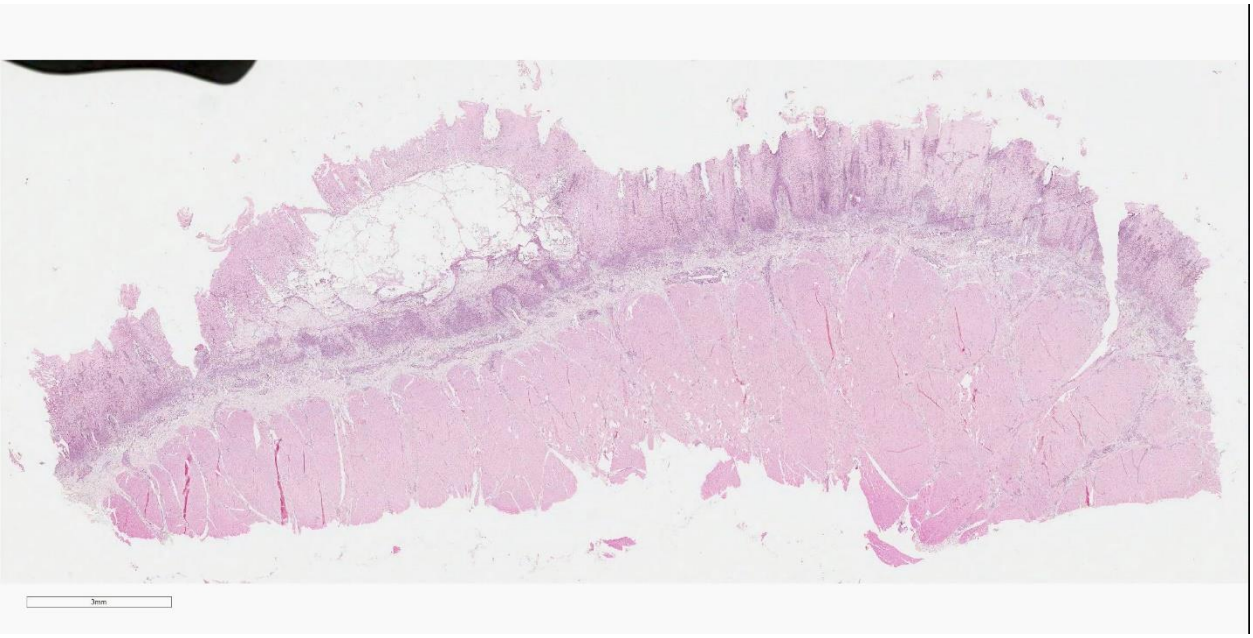
Tongue, ox. The surface of the tongue contains severe discrete vesicles within the mucosa. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de)

adjacent epithelial cells are polygonal to round, swollen with hypereosinophilic cytoplasm, basophilic karrhyorectic and pyknotic nuclei (lytic necrosis), and are separated by intercellular edema (spongiosis). Diffusely, the epithelium is infiltrated by a large number of neutrophils, focally obscuring the basal epithelial layer. Predominantly, perivascular, moderate numbers of neutrophils and few macrophages are present within the submucosa and adjacent connective tissue. Endothelial cells are hypertrophic (endothelial activation).

Contributor's Morphologic Diagnosis:
Tongue. Glossitis, vesiculopustular, focal, suppurative, moderate, acute with intramucosal edema, Holstein (*Bos taurus*), bovine.

Contributor's Comment: Foot-and-mouth disease (FMD) is a highly contagious, viral disease of cloven-hoofed animals. The etiologic agent is the FMD virus (FMDV), a picornavirus, of which there are seven known FMDV serotypes: O, A, C, Asia 1, SAT1, SAT2, and SAT3[4,9]. Although FMD outbreaks are often of low mortality rates, morbidity is often high due to the characteristic vesicles within the mouth and on the feet, resulting in profound agricultural production and economic losses[2,9]. Furthermore, the high level of contagiousness of FMD and high tenacity of the FMDV pose a considerable challenge in controlling a disease outbreak.

In the United Kingdom in 2001, an outbreak of FMD resulted in the mass culling of over 6 million cattle and sheep, costing nearly $12 billion.[9] From 2010-2011, three outbreaks occurred in Japan and South Korea, with 3 million pigs, and 100,000 cattle being culled.[9,10] Throughout the FMD endemic regions, outbreaks are frequently reported and ongoing, including (as of June 17, 2019) 6,370 cases in Algeria, 968 in China, 386 in Malawi, 239 in Morocco, 6,391 in Mozambique, 1,200 in Sierra Leone, 1,220 in Zambia, and 2,108 in Zimbabwe[5]. Globally,

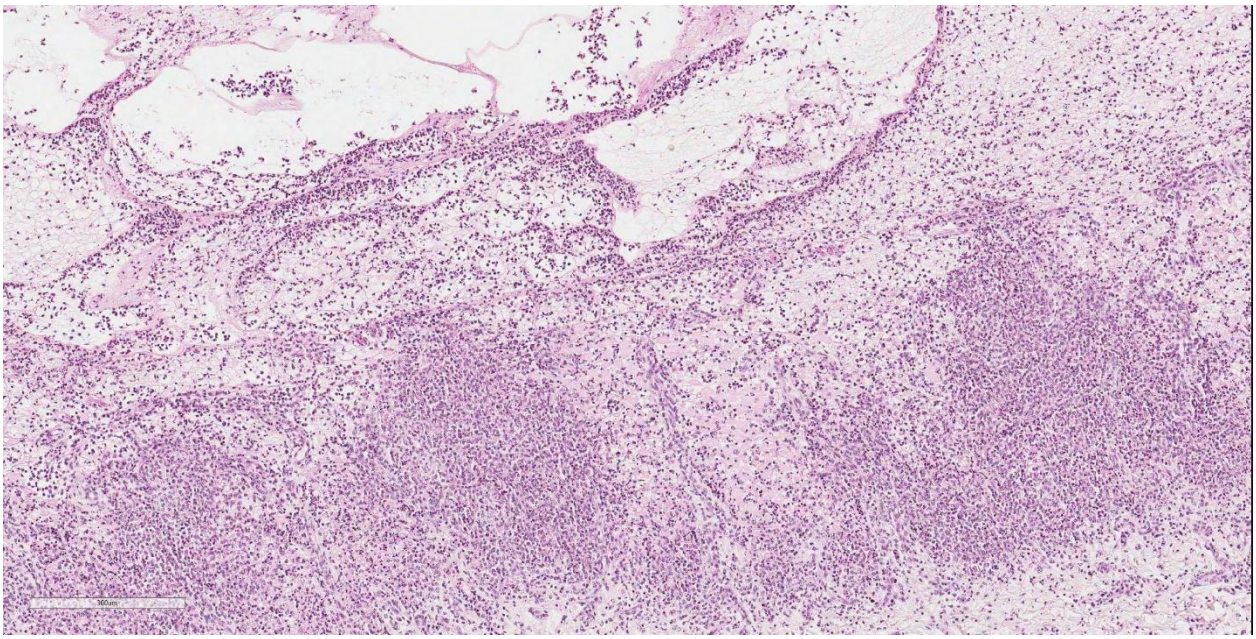


Tongue, ox. A large vesicle undermines approximately one-third of the mucosa. There is diffuse marked infiltration of the mucosa by innumerable neutrophils, largely concentrated at the edges of the vesicle as well as in the basal layers of the mucosa. (HE, 7X)

in the past 5 years (as of June 17, 2019), FMD outbreaks resulted in 376,367 cases, 12,201 deaths, 314,063 culls, with an estimated 3,650,220 animals identified as susceptible[5].

Although FMD constitutes a major global health threat, research into understanding the fundamental mechanisms of disease pathogenesis remains limited due to, in part, the necessary biosecurity and infrastructure to work with FMDV, as well as the diversity of viral serotypes and strains[2]. In general, FMD is designated into three stages: pre-viremia, viremia, and post-viremia[2]. The definitive location of viral entry remains to be elucidated, although the pharynx and larynx appear to be an important anatomical site.[2,9] Furthermore, although infected animals develop a profound viremia 1-2 days before the onset of clinical signs, the source of the viremia also remains poorly understood.[2,9] Vesicles often hold higher viral titers than the blood, however, viremia may occur prior to vesicle formation.[2] Other tissues with viral titers include the skin (whether or not lesions are present), lungs, lymph nodes, and heart.[2] After the clearance of viremia, approximately 50% of cattle may become persistent carriers, although to what degree the carriers pose a threat to naïve animals, also remains poorly understood.[1,2,4]

Clinically, FMD manifests as a fever 2-7 days post-infection, followed by the vesicle formation in the mouth and on the feet, which may rupture under mechanical stressors, thus forming large erosions[7]. Damage to the epithelium results in hypersalivation and lameness, ultimately leading to weight loss and a drop in milk production.[9,10] Characteristic histologic lesions include vacuolar degeneration of epithelial cells, leading to hydrophobic cell swelling, cellular degeneration, lysis, and intraepithelial edema.[9] Healing occurs first through fibrin exudation with neutrophilic granulocytes, to granulation tissue and re-epithelialization.[9]. In addition to classical lesions, FMDV also induces a fatal lymphocytic-histocytic myocarditis especially in young animals,

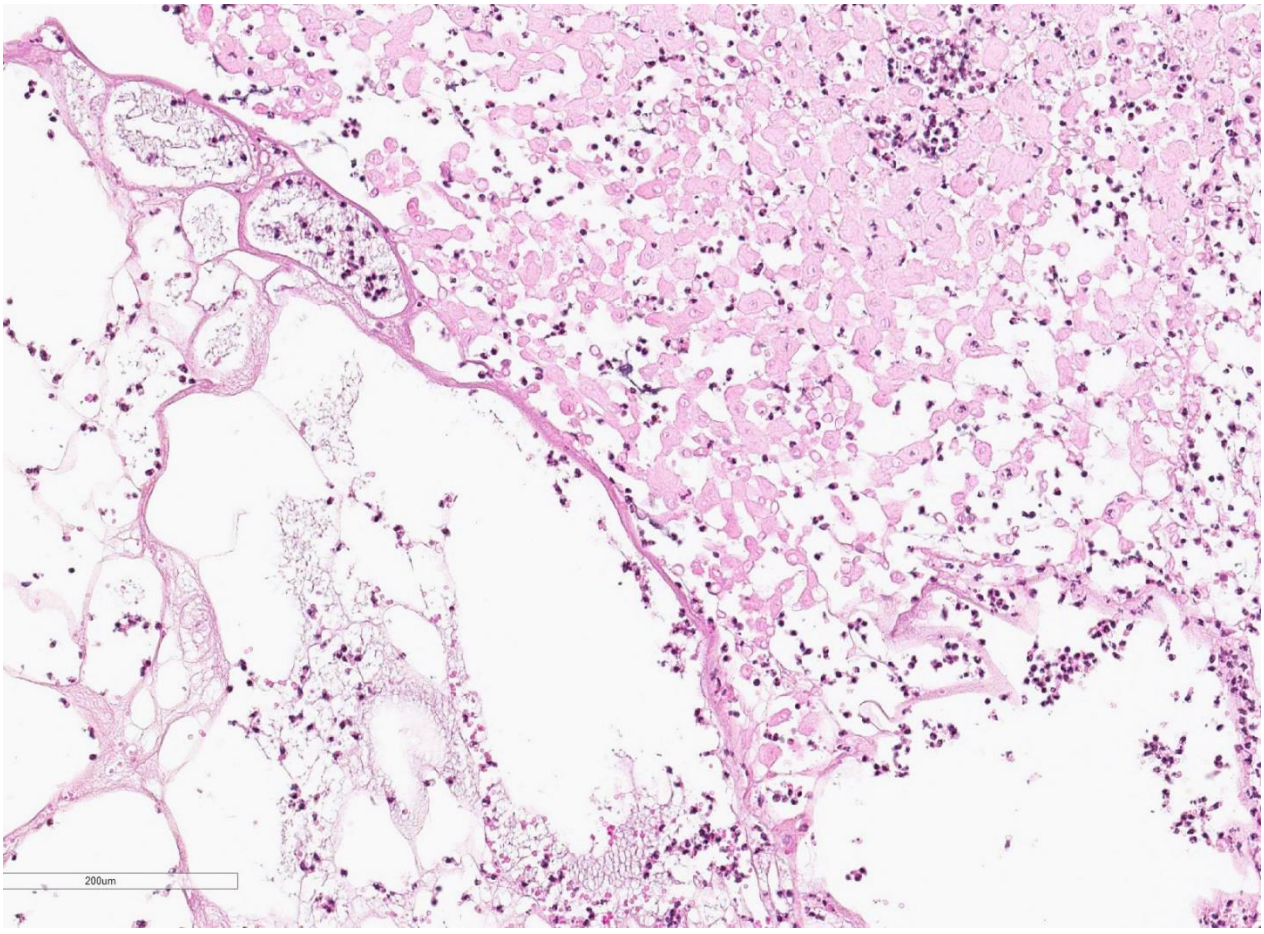


Tongue, ox. The floor of the vesicle and basal layers of the mucosa are outlined by a heavy infiltrate of neutrophils and cellular debris. (HE 97X)

characterized by small, grey-white foci within the myocardium of the left ventricle and septum.[4,9]

Important infectious differential diagnoses for ulcerative and erosive lesions include vesicular stomatitis (VS), vesicular exanthema in swine (VES), swine vesicular disease (SVD), bovine virus diarrhea (BVD), bluetongue virus (BTV), and malignant catarrhal fever (MCF).[9] Non-infectious causes include burns, trauma, and toxicants (selenium toxicity, cantharidin (blister beetle), toxic plants (buttercup, St. John's Wort, lantana, elderberry)), and photodermatitis.[3,9] Differentiation of these diseases may be made through laboratory diagnostics, including detection of the pathogen (antigen ELISA, PCR, viral isolation), histopathology, or antibodies produced by the infected animal[1,4,9] In many countries, prior to laboratory confirmation of disease, cases of vesicular lesions must be reported to official veterinarians in order to ensure prompt response and preparedness in the event of an FMD positive animal.

A primary objective in controlling FMD globally is immediate detection of disease, as well as controlling the movement of animals and animal products, and contacts with animals between countries.[9,10] It is imperative that animal health practitioners are trained to not only identify early cases of



Tongue, ox. Adjacent to the vesicle, degenerating mucosal epithelial cells are rounded up and disassociated, furthering additional vesicle formation or enlargement. (HE, 173X)

FMD, but also practice high standards of biosecurity and workplace hygiene.[9] Although FMDV is sensitive to changes in pH (below 6.0, above 9.0) and standard disinfectants (2% sodium hydroxide, 0.2% hydrochloric acid, 2% formalin, 0.5% citric acid), a challenge lies in controlling potentially contaminated materials, as all secretions and excretions of an infected animal are considered infectious.[6] Furthermore, FMDV may survive in the soil for up to 28 days, in urine for 39 days, and fecal slurries for up to 6 months.[6] In addition, cattle that become carriers may continue to harbor virus for up to 3.5 years[1]. Vaccinations are available, however, a vaccine is not protective across serotypes, and vaccination does not prevent animals from becoming carriers.[2,9]

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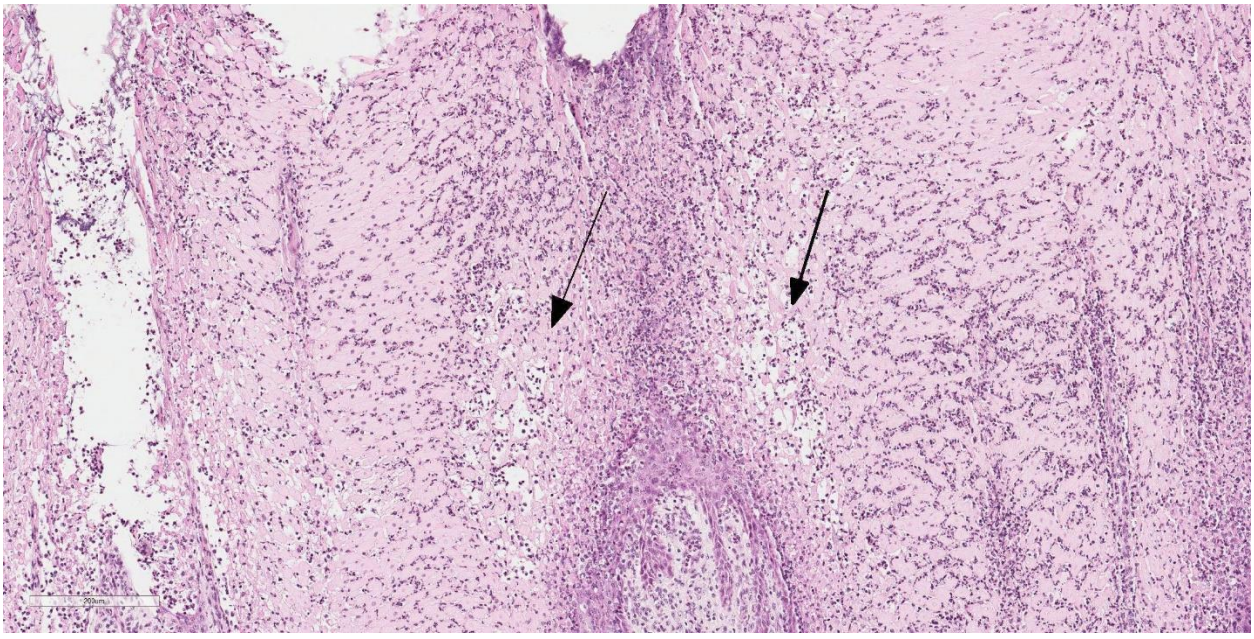
JPC Diagnosis: Tongue: Glossitis, vesicular and neutrophilic, multifocal to coalescing, severe.

JPC Comment: The contributor has done an outstanding job describing this disease of global veterinary importance with particular regard to cattle. The apthovirus that causes foot and mouth disease affects a range of other species, some of which may contribute significantly to the potentiation and duration of an outbreak. Small ruminants may facilitate the spread of outbreaks as lesions are often inapparent, and these animals are easily transported. In countries in the virus is endemic, African buffalos and impala have been documented as reservoir hosts and well as being able to pass the virus on to domestic cattle.[7]

Much of the available research has been performed on cattle; however, swine are a major component of the world's meat production as well, and the pathogenesis of the disease is significantly different in this species. Swine are considered amplifier hosts and have the ability to produced large amounts of virus, which may be transmitted



Tongue, ox. In mucosa adjacent to the vesicle, there is extensive transmigration of neutrophils and incipient vesicle development (arrows). (HE, 121X)

by aerosols to other farms. While the clinical signs of FMD in swine are similar to those in cattle, the pathogenesis of the disease in swine demonstrates considerable different.[8] Pigs are more susceptible to oral inoculation (through oropharyngeal tonsillar tissue) than via aerosol exposure which suggests that physical separation of pigs may be successful to decrease infection rates in swine, but not in cattle. During clinical disease, the highest quantities of virus is produced within vesicles in the oral cavity and feet; however, pigs exhale massive quantities of infectious FMDV, likely with tonsillar tissue as the source.[8] Convalescence occurs within 1-2 weeks, although serious injuries to the feet may resulting in secondary infection and persistent lameness. Pigs that clear the disease also apparently completely clear the virus with 17 days; carrier states apparently do not occur in this species.[8]

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