Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 17

3 February, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: F4/19 (4136413-00)

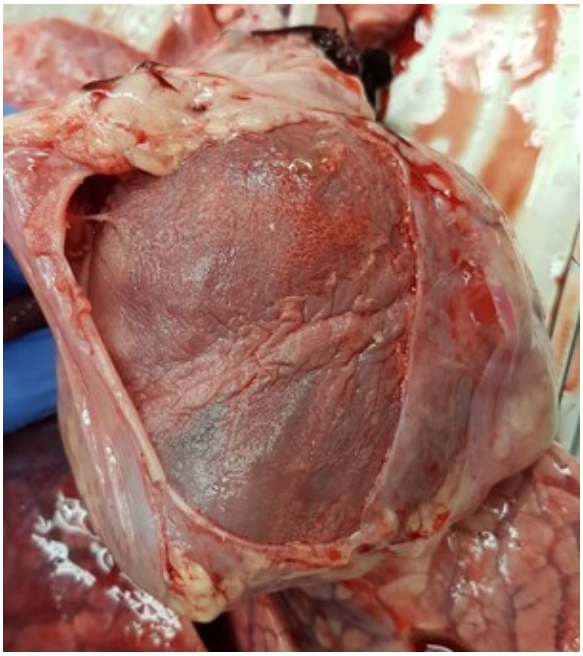
Signalment:
10-week-old female domestic pig, mixed breed, Hampshire/Yorkshire/Landrace, *Sus scrofa domesticus*

History:
Previously healthy pig that suddenly developed dyspnea and became lethargic. Its ears and snout were cyanotic. The pig was euthanized the same day as clinical signs appeared and submitted for necropsy.

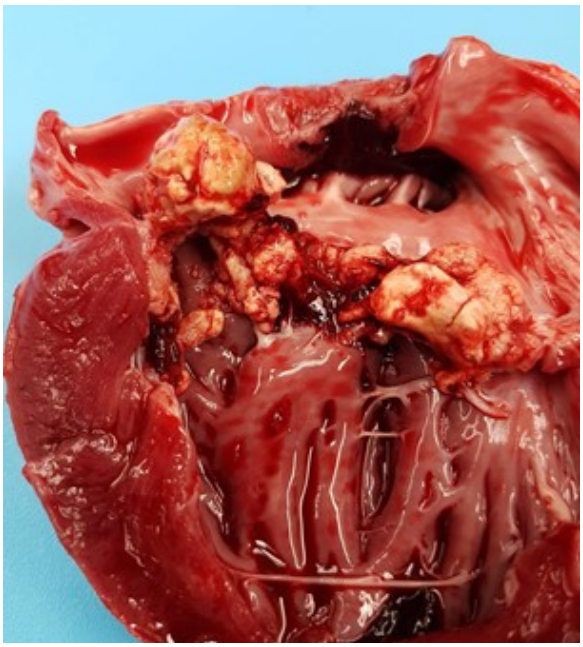
The farm at which the pig was raised was a piglet producer with approximately 360 sows. Lately they had had several pigs showing similar kind of symptoms.

Gross Pathology:
Bilaterally the pinna and the snout were cyanotic. Main findings were located to the thoracic cavity, which contained approx. 1 dl of clear yellow tinted fluid with gelatinous proteinaceous material. The visceral and parietal surfaces of the pericardium were diffusely covered by a thin layer of fibrinous exudate. The heart was moderately enlarged with a moderate hypertrophy of the left ventricular wall. The entire left atrioventricular valve was covered by multiple, raised, irregularly shaped, pale light yellow-gray, friable vegetations ranging from 1 mm in diameter to 2 x 2 x 1 cm in cross section.

In the renal cortices bilaterally, there were multifocal pale foci, approximately 2-10 mm in diameter.



Heart, pig. Pericardial sac opened, displaying parts of the right and left ventricles. The visceral and parietal surfaces of the pericardium were covered with a thin layer of fibrinous exudate. (Photo courtesy of: Swedish University of Agricultural Sciences; Department of Biomedical Science and Veterinary Public Health; Pathology Unit)



Heart, pig. Left atrioventricular valve covered by irregular friable vegetations. (Photo courtesy of: Swedish University of Agricultural Sciences; Department of Biomedical Science and Veterinary Public Health; Pathology Unit)

Laboratory results:
Growth of *Streptococcus suis* in pure culture from sample taken from the left atrioventricular valve.

Microscopic description:
The atrioventricular valve is expanded and replaced by an excessive accumulation of pleomorphic and plump spindle-shaped cells (reactive fibroblasts), loose collagen strands and small vascular structures outlined by plump endothelial cells (angiogenesis) (granulation tissue), and in multiple areas, both on the valve surface as well as in the center, there are large deposits of brightly eosinophilic amorphous material (fibrin). In the deposited fibrin there are multiple separate round foci and larger coalescing areas of coccoid bacteria. Multifocal in the border between granulation tissue and fibrin there are moderate to large aggregates of degenerative and non-degenerative neutrophils. Scattered within the granulation tissue are mild to moderate infiltrates of mixed inflammatory cells, dominated by macrophages with lesser amounts of neutrophils, lymphocytes and plasma cells.

Focally, affecting the endocardial and subendocardial tissue of the opposing left ventricular wall there is a similar inflammatory lesion as described above with addition of a minor acute hemorrhage. In this region there is also mild infiltration of mixed inflammatory cells disrupting Purkinje fibers and separating cardiomyocytes.

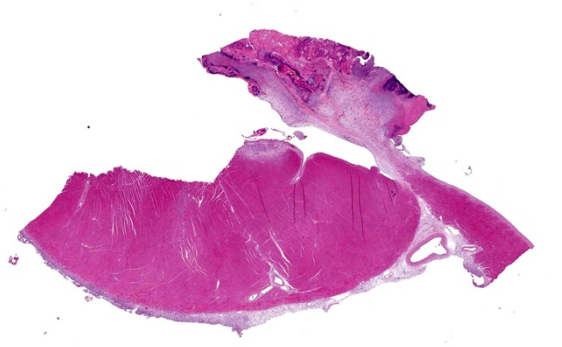
In the myocardium there is an additional focal area with reactive fibroblasts, mild infiltration with mononuclear cells and cardiomyocytes with hypereosinophilic clumped sarcoplasm, some with small hypercondensed (pyknotic) nucleus (myocardial necrosis). The subepicardial tissue is markedly thickened by a similar inflammatory lesion dominated by granulation tissue with multifocal surface fibrin deposits.

In the ventricular wall of some sections there is an intravascular aggregate of hypereosinophilic amorphous material (fibrin), focally containing coccoid bacteria, attached to the endothelium (thrombus).

Contributor's morphologic diagnosis:
Heart: Pancarditis, fibrinosuppurative, multifocal to coalescing, severe, chronic, with granulation tissue and intralesional coccoid bacteria.

Contributor's comment:
In swine bacterial endocarditis is a common lesion, and usually detected at postmortem inspection of slaughter pigs. Bacterial endocarditis in swine typically manifests as vegetations involving the cusps of the left atrioventricular valve.[6,9] In acute bacterial endocarditis *Streptococcus suis* is the most commonly found bacteria.[8] Apart from *Streptococcus suis*, *Erysipelothrix rhusiopathie* is an important etiologic agent, but the disease can also be caused by infection with other opportunistic bacteria such as *Pasturella multocida, Trueperella pyogenes,* and *Staphylococcus aureus*, as well as other *Streptococcus* spp.[8,9]

Worldwide, *Streptococcus suis* is an important swine pathogen, causing septicemia and acute death, meningitis, polyarthritis, polyserositis and valvular endocarditis, typically in 5–10-week-old



Heart, pig. A section of left ventricle and atrioventricular valve is submitted for examination. At subgross examination, the AV valve (top) is expanded and variegated in color, and the opposing endocardium is expanded and pale. The left ventricular epicardium is also expanded. (HE 6X)

pigs.[4] The bacteria have zoonotic potential and in 2014 there were estimates of over 1600 confirmed human cases of *Streptococcus suis* reported in over 30 countries.[3] *Streptococcus suis* can be found in clinically healthy pigs as a part of the normal bacterial flora in the upper respiratory tract. In diseased pigs *Streptococcus suis* isolates typically belong to serotypes 1-9, with serotype 2 being considered to be the most common and virulent subtype in Euroasian countries, and that subtype is also responsible for the majority of human cases.[4]

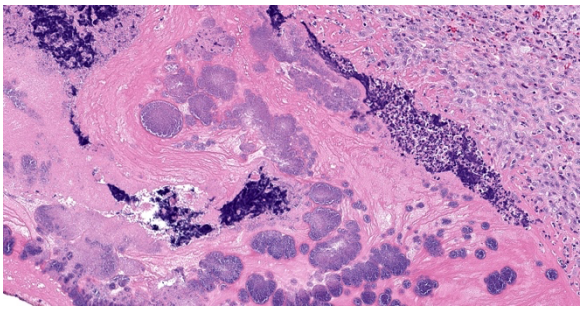
Bacterial valvular endocarditis arises in animals with a sustained or recurrent bacteremia. It is thought to occur either by adherence of blood borne bacteria to a sterile thrombus on a valve with a damaged endothelium, or by bacterial colonization beginning in the capillaries of the valve during septicemia.[7,9,11]

Emboli from valvular endocarditis can cause secondary lesions in other organs. Left sided lesions give rise to systemic infarcts, nephritis and myocarditis among several possible sequela, while emboli arising from a lesion on the right side most often leads to complications involving the lungs, such as infarcts, abscesses or pneumonia.[11]

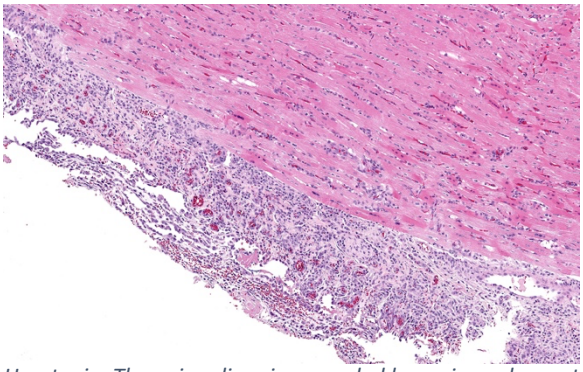
Contributing Institution:
Swedish University of Agricultural Sciences; Department of Biomedical Science and Veterinary Public Health; Pathology Unit

JPC diagnosis:
Heart, left ventricle and AV valve: Valvular and mural endocarditis and epicarditis, fibrinosuppurative, focally extensive, severe, with granulation tissue and numerous colonies of cocci.

JPC comment:
Streptococcus suis is a capable bacterium, with a wide array of virulence factors that enable infections at various sites in pigs and humans. It expresses various adhesins, such as fibronectin-binding protein, enolases, a dipeptidyl-peptidase-4, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and others, which allow for efficient attachment to host cells or extracellular components like collagen, fibrinogen, or fibronectin. Though more likely in non-encapsulated strains, *S. suis* can form a biofilm when in the presence of fibrinogen. The expression of suilysin, a cytotoxic hemolysin, likely enables destruction of epithelial cells and allowing *S. suis* to progress past the epithelial barrier. From recent research, suilysin affects the proteins of tight junctions, and alters the distribution of occludin and zona occludens-1 in tracheal epithelial cells.[2] The polysaccharide capsule is one of the most important virulence factors, as it inhibits phagocytosis my neutrophils, macrophages, dendritic cells, and monocytes, as well as allowing for intracellular survival. S. suis has the capacity to degrade chemokines such as CCL5 and IL-8, decreasing



Heart, left AV valve: The valve is remodeled by proliferating granulation tissue. Fibrin and bacterial colonies are at lower left. (HE 165X)



Heart, pig. The epicardium is expanded by an irregular mat of granulation tissue and fibrin. (HE, 99X)

signaling for phagocytes. It also has the ability to interact with the complement system by binding Factor H, potentially decreasing or removing an important regulatory component that limits the pro-inflammatory response, which can be injurious to the host. Also, in the arsenal of virulence factors include several DNases, which help *S. suis* evade neutrophil extracellular traps (NETs).[5]

The extent to which *S. suis* alters the complement pathway is only recently becoming clear, with the recent identification of an IgM degrading enzyme, designated Ide_{Ssuis}. IgM is an approximately 1000 times more potent activator of the classical complement cascade than IgG, with IgM being the first class of antibodies produced in response to antigenic stimulation. By preventing early opsonization by IgM and activation of the complement cascade, the bacterium has opportunity to replicate and cause increased host tissue damage.[10]

Often, non-encapsulated *S. suis* isolates are obtained from the heart of pigs at slaughterhouses and are considered incidental findings. These bacteria are generally considered avirulent. Most of these strains have a mutation in the capsule gene (*cps*), and while the capsule does not form, the expressed adhesins are more easily able to attach to host cells of the heart. However, inoculation of mice with an acapsular isolate with subsequent culture and passage to new mice allowed *S. suis* to regain the ability to produce a capsule. The bacteria cultured and tested in vitro did not demonstrate this ability, suggesting the host microenvironment is critical for S. suis to repair and/or alter its virulence genes.[1]

There is mild slide variation, and the scanned slide has a focus of basophilic material, posited to be mineral. While not performed in this case, additional stains could have helped determine the composition of the material. Von Kossa stains stain mineral, but not specifically calcium. Alizarin red stain would be an appropriate stain to correctly identify calcium mineralizations, if available.

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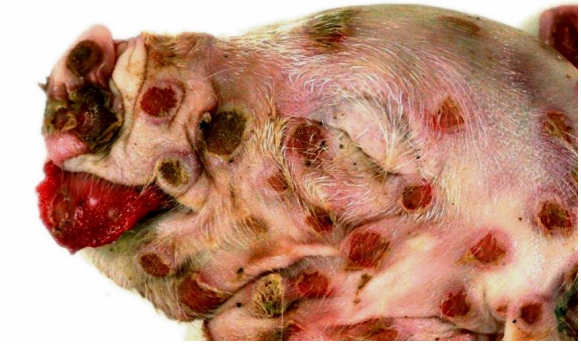
CASE 2: S 818/19 FB (4152315-00)

Signalment:
One-day-old, male piglet (*Sus scrofa* domesticus)

History:
The piglet from a clinically healthy sow was born with multifocal severe skin lesions, affecting the whole body and died spontaneously one day *post natum*.

Gross Pathology:
Macroscopically, the animal showed multifocal severe, erythematous maculae covering the whole body as well as the tongue and the cranial part of the esophagus. Lesions appeared as cutaneous and mucosal erythematous, round to coalescing papules with a depressed center and a raised, wall-like border. Partially, encrusted exudate was present on the surface of the alterations.

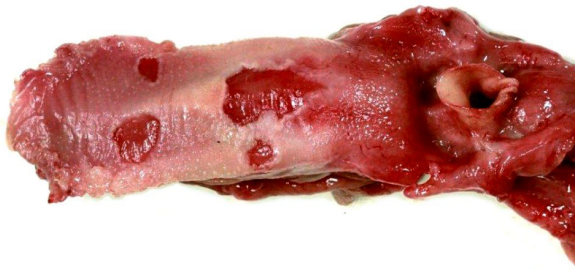
Presentation, piglet. The skin of the piglet is covered by erythematous macules with an ulcerated center. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 1, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)



Presentation, piglet. Closeup of ulcerated macules showing a wide ulcerated center and a rim of unattached epithelium. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 1, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Laboratory results:
Using PCR, swinepox virus was detected in samples from representative lesions. Microbiologically, a moderate to high amount of *Escherichia coli*, *Proteus* sp., as well as coliform germs were isolated from lung, small intestine, liver and skin.

Microscopic description:
Skin, pinna: there are multifocal complete losses of the epidermis including the basal membrane (ulceration) with adjacent parts of the epidermis detached from the dermis, including areas of keratinocytes that show multifocally cellular shrinkage with nuclei undergoing karyopyknosis, karyorrhexis and karyolysis (necrosis). Multiple areas of the remaining epidermis show a moderate increase of cellular layers (hyperplasia) and cellular swelling with ground glass appearance of the cytoplasm and nuclear enlargement (ballooning degeneration). Within these keratinocytes often eosinophilic, cytoplasmic inclusion bodies are present. In several locations, the underlying dermis is infiltrated by a moderate amount of macrophages, lymphocytes and neutrophils, focally accompanied by large-scale necrosis. In some locations, the inflammatory process involves



Presentation, piglet. Discrete focally extensive areas of lingual ulceration. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 1, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

adnexal structures, and occasionally, some of the hair follicles show similar alterations as the epidermis.

Contributor's morphologic diagnosis:

Dermatitis, multifocal, severe, subacute, proliferative and ulcerative with ballooning degeneration of keratinocytes and cytoplasmic inclusion bodies; adnexal structures: folliculitis, multifocal, moderate, lympho-histiocytic and necrotizing with ballooning degeneration of keratinocytes and cytoplasmic inclusion bodies.

Contributor's comment:

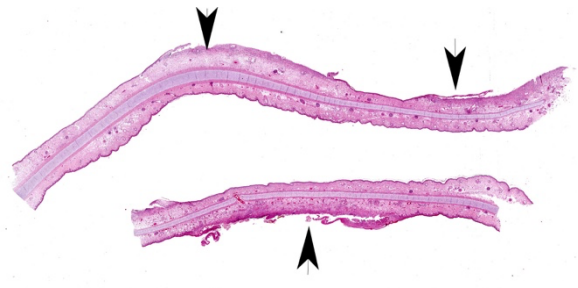
The swinepox virus is the only member of the genus Suipoxvirus in the family *Poxviridae* and the subfamily *Chordopoxvirinae*.[5,7] It occurs worldwide, is endemic in regions with intensive swine production, and is particularly associated with poor sanitary conditions.[7,8,9] Pigs are the only natural hosts, though earlier studies have shown that experimental infection in rabbits is possible.[3] The resistant virus persists in dried crusts from infected animals for up to a year.[5,7,12] Virus transition occurs either by direct contact or by mechanical vectors, such as the blood sucking swine louse (*Haematopinus suis*),[5,7] mosquitoes or biting flies,[12] assisting infection by causing skin trauma.[5,7]

In affected herds, the morbidity may reach high levels.[9] Predominantly, young growing piglets are affected but some authors also described lesions in neonates, which raises the theory of an intrauterine infection.[5,7,10] A viremic stage of the disease has not yet been reported, although transplacental infection suggests its occurrence.[12] The presented case of a one-day-old piglet supports an intrauterine infection because the histological investigation revealed reactive inflammatory changes indicative for a lesion duration of more than one day.

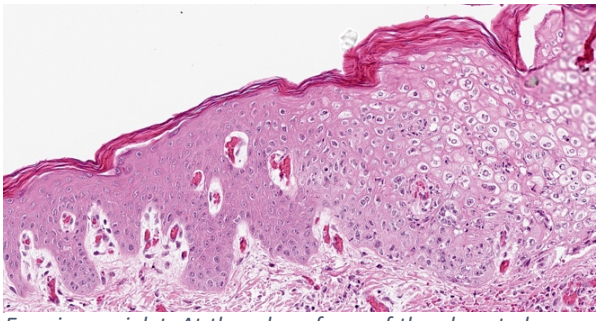
Usually, the course of disease is mild with low mortality, so the virus has caught rather little attention in the past.[5,7] The severity is often age-related, as some authors report more severe alterations in younger piglets.[9,10] After an incubation period of about seven days,[13] the macroscopic changes typically appear on the ventral and lateral abdomen, lateral thorax, as well as the medial forelegs, and the thighs. They show the typical pattern of pox infections, beginning as erythematous papules that transform into umbilicated pustules.[5,7]

In contrast to the pathogenesis of poxvirus infections in other species, a vesicular stage is insignificant, though some authors reported the occurrence of vesicles in individual animals that have experimentally been infected.[1,3,5,7] After healing, a white macule may remain.[5,7] Sometimes the alterations are more prominent on the back.[5,7] A periodic sequence of lesion development may result in multiple stages of pox lesions, that can be found simultaneously.[13]

Secondary dermatitis may accompany the virus-induced alterations.[8,9] In cases of severe infections, the lesions may turn out to be generalized and affect alongside the integument the mucosa of the gastrointestinal tract from oral cavity to the stomach as well as the trachea and



Ear pinna, piglet. Two sections of ear pinna are submitted for examination. Multifocal ulcerated areas (arrowheads) are visible at subgross magnification. (HE, 6X)



Ear pinna, piglet. At the edge of one of the ulcerated areas, keratinocytes at all level of the epidermis and extending into the adjacent follicular epithelium exhibit ballooning degeneration. (HE, 251X)

bronchi.[5,7,10] Cytoplasmic inclusion bodies may be found frequently in keratinocytes of the stratum spinosum,[1,3,9] however, even intranuclear inclusion have been reported sporadically.[1] As etiological differential diagnosis vaccinia virus infection has to be considered as the morphological changes are quite similar to swinepox induced changes.[3,7] Infection of pigs with the swinepox virus does not prevent infection with vaccinia virus.[13]

Contributing Institution:
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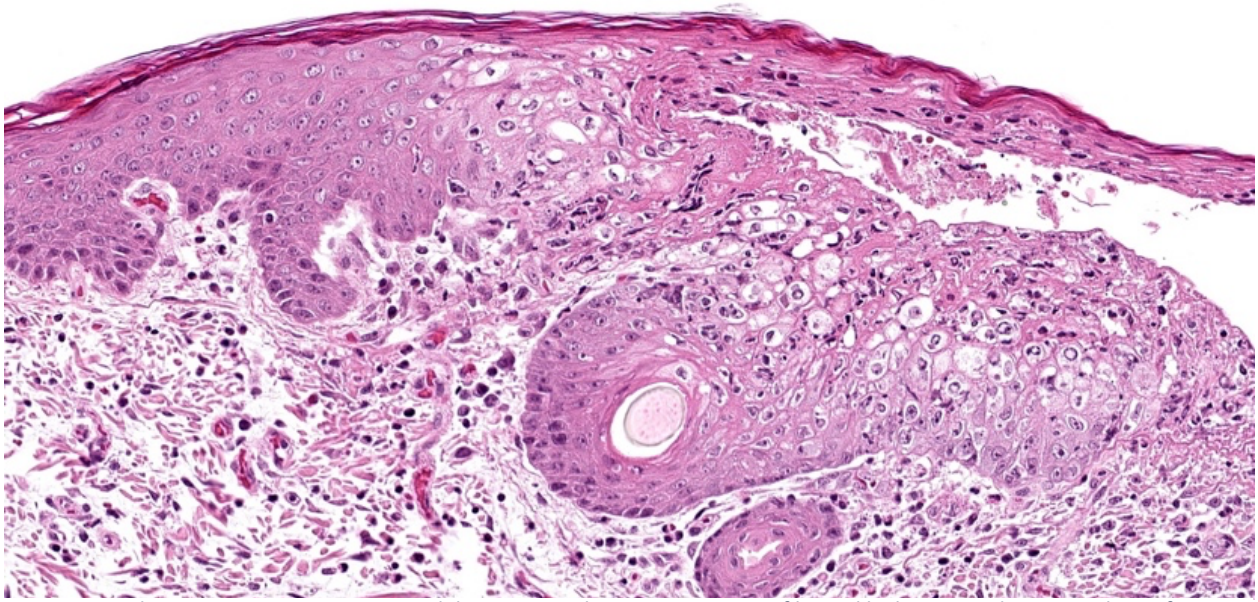
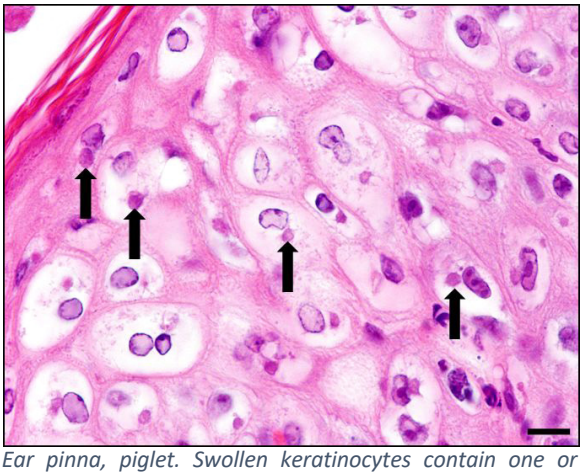
JPC diagnosis:
Ear, pinna, haired skin, epidermis and follicular epithelium: Dermatitis, proliferative and necrotizing, multifocal, moderate, with ballooning degeneration and intracytoplasmic viral inclusions.

JPC comment:
The contributor provides a good summary of swinepox. While the primary transmission routes are direct contact or through natural vectors, there are potential methods of transmission that are not often considered. When large ships travel oceans, in order to provide stability during the voyage, and during loading and unloading operations, they have ballast water. Vast quantities of ballast water are taken into tanks while the ship is in port, which is then pumped between different tanks for balance, and often discharged in a destination port. This transported water reservoir is the potential medium of delivering invasive species, as well as infectious organisms. A



Ear pinna, piglet. In some areas, necrotic and degenerating keratinocytes, are infiltrated by low to moderate numbers of necrotic neutrophils admixed with cellular debris. (HE, 251X)



Ear pinna, piglet. Swollen keratinocytes contain one or multiple brightly eosinophilic intracytoplasmic inclusion bodies (arrows). (HE, 400X). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 1, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

characterization of ballast water that had arrived in Busan, South Korea, a variety of viruses were found, including swinepox virus in ballast water from a port in Panama. There were a variety of other concerning viruses as well, including *suid herpesvirus* 1, *Raccoonpox* virus, a variety of bacteriophages, and a host of viruses that typically infect marine invertebrates, amoebas, and algae.[6]

Pox viruses have host specificity, with little spillover from the host specific viruses such as swinepox virus, sheeppox virus, myxoma virus, and yaba monkey tumor virus. Protein kinase R (PKR) is a normal and critical part of the host immune response and limits viral replication in an infected cell by phosphorylating eukaryotic initiation factor 2α (eIF2α). Pox viruses produce proteins E3 and K3 that antagonize PKR and allow viral activity within cells. E3 is a dsDNA binding protein that inhibits PKR activation, but K3 acts as an eIF2α analog substrate for PKR, limiting activation of eIF2α. Using vaccinia virus, E3 and K3 genes were deleted, which eliminated the virus's ability to replicate. Subsequently, E3 and K3 orthologs from host specific pox viruses were expressed in the modified vaccinia virus, and it was found that the expressed K3 orthologs restored the ability of the virus to replicate in host specific cells. This suggests that the K3 family proteins confer host specificity to these viruses.[2]

Because the swinepox virus genome is so large (146kb), is host specific, and usually causes a self-limiting form of the disease, it has become an ideal candidate for vaccine development. Introducing additional DNA (transgenes) into the genome is easily performed, and even multiple transgenes can be accommodated for multivalent vaccine development.[4] A variety of recombinant vaccines have been investigated, using swinepox as the delivery mechanism, such as *Streptococcus suis*, porcine circovirus, swine influenza virus, swine infectious gastritis virus, and a number of other pathogens.[11] This virus is likely to remain a useful tool for vaccine development.

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CASE 3: 17-0646 (4120045-00)

Signalment:
Female Yucatan mini pigs *(Sus scrofa),* 7-8 months of age, 20kg.

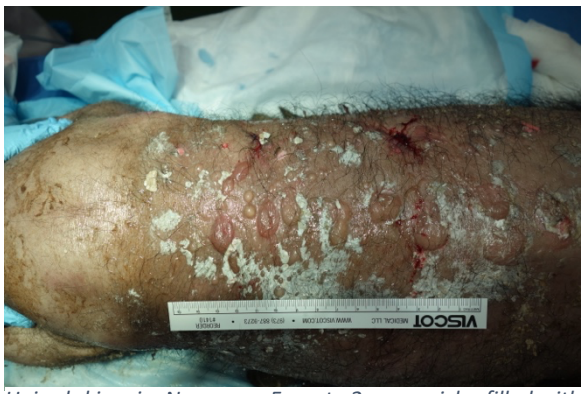
History:
Ten female, 7-8 month old, ~20kg, Yucatan mini-pigs were purchased from a single vendor. Left ulnar nerve transection was performed per protocol 26 days after arrival. All pigs recovered uneventfully from the experimental procedure. All pigs were scheduled to be housed 6 months post-surgery with no other procedures performed. Approximately two months post-procedure, the first lesions appeared in the first pig. Two other pigs also developed similar lesions, one of which recovered from the first instance and developed them a second time. The animal in this report was the third to develop the lesions and appeared to be the worst affected. All three pigs were treated with nystatin-neomycin sulfate-thiostrepton-triamcinolone acetonide ointment. In addition to the ointment, the pig in this case was initially treated with silver sulfadiazine ointment.

Gross Pathology:
Multifocal, 5 mm to 2 cm diameter, vesicles filled with thick clear viscous fluid were concentrated over the dorsal back with extension down the dorso-cranial aspect of both hind legs to within 1 cm of the coronary band. The vesicles ruptured easily resulting in round ulcerated lesions. Two 10 mm punch biopsies were taken, one from the wall of a large vesicle, the second of an entire intact small vesicle.

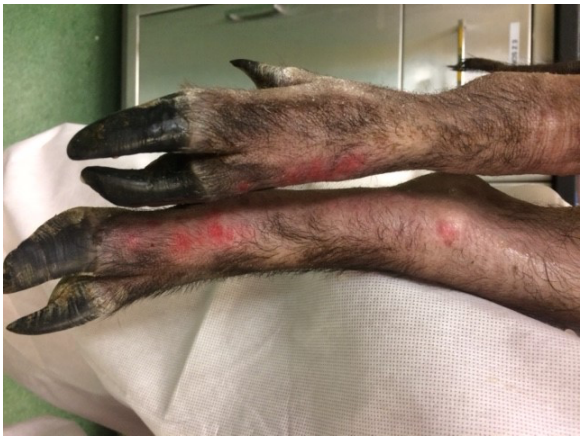
Laboratory results:
N/A

Microscopic description:
Haired skin, dorsum: Multifocally there are vesicles measuring up to 2 cm in length that elevate the sub basilar space below the epidermis up to 4 mm and contain numerous viable and degenerate eosinophils, lesser lymphocytes, few Mott cells, intact erythrocytes, fibrin, and wispy degenerated collagen, along with eosinophilic proteinaceous fluid, and increased clear space (edema). Frequently vesicular contents indent or extend into the overlying epidermis or form small 1 mm pustules within the stratum spinosum. Affected keratinocytes are shrunken, hypereosinophilic with pyknotic nuclei (necrotic) or vacuolated (degenerate). The overlying stratum corneum is intact and there is mild orthokeratotic hyperkeratosis. The underlying dermis is superficially smudged and contains few perivascular, previously described inflammatory cells.

Contributor's morphologic diagnosis:
Haired skin (dorsal back): Dermatitis, vesicular and sub basal, multifocal, with mild perivascular inflammation.



Haired skin, pig. Numerous 5mm to 2 cm. vesicles filled with thick clear viscous fluid are present over the dorsum. The vesicles are easily ruptured. (Photo courtesy of: Walter Reed Army Institute of Research/Naval Medical Research Center, www.wrairintranet.com)



Haired skin, pig. Vesicles also extend down the legs to the coronary band. (Photo courtesy of: Walter Reed Army Institute of Research/Naval Medical Research Center, www.wrairintranet.com)

Contributor's comment:
Autoimmune dermatoses occur when aberrant T or B cells attack self-antigens resulting in a variety of clinical presentation,[3] or when autoantibodies attack antigens.[4]

A recent proposal by Olivry[4] has been made to separate diseases based on one of these two mechanisms of action in order to facilitate treatment methodologies that target specific branches of the immune system. Previously, animal autoimmune disorders were separated into "vesiculous" or "bullous" (i.e., the blister-forming pemphigus and pemphigoid variants) and "non-bullous" diseases (i.e., discoid and systemic lupus erythematosus).

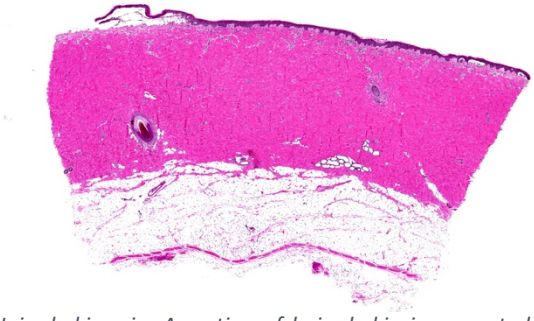
Inciting factors include but are not limited to drug therapy, paraneoplastic syndrome, tissue injury, infectious diseases, genetic makeup, and other autoimmune diseases.[3]

Numerous pathological processes are suspected, which include[3]:

- Epitope spreading: targets of autoimmune response drift to include other epitopes on the same protein or nearby proteins of the same tissue, resulting in regional variation of the same disease
- Drug induced: Haptenization of keratinocyte antigens makes them immunogenic
- Environmental influences: alters DNA methylation, which in turn, alters gene expression.
- UV-light: induces keratinocyte ICAM-1 and induction of pro-inflammatory cytokines.
- Infectious: structural proteins of agents mimic host tissue proteins
- Gonadectomy: Despite the advantages, neutering is associated with increased risk for certain autoimmune disorders including pemphigus complex[6]

In people, and potentially animals, blistering dermatoses share the same pathogenic mechanisms as multiorgan diseases. Clinicians should consider potential systemic involvement, as well as the autoimmune and inflammatory conditions that are frequently associated with it.[8]

- In paraneoplastic pemphigus/ paraneoplastic autoimmune multiorgan syndrome, the internal organs (particularly the lungs) are affected by the autoimmune injury
- Pemphigus erythematosus is associated with lupus erythematosus
- Bullous pemphigoid is associated with neurologic disease, possibly caused by cross-reactivity of the autoantibodies with isoforms of bullous pemphigoid antigens expressed in the skin and brain
- Anti-laminin 332 pemphigoid shows an increased risk for adenocarcinomas



Haired skin, pig. A section of haired skin is presented for examination. Several discrete subepidermal vesicles are present. (HE, 69X)

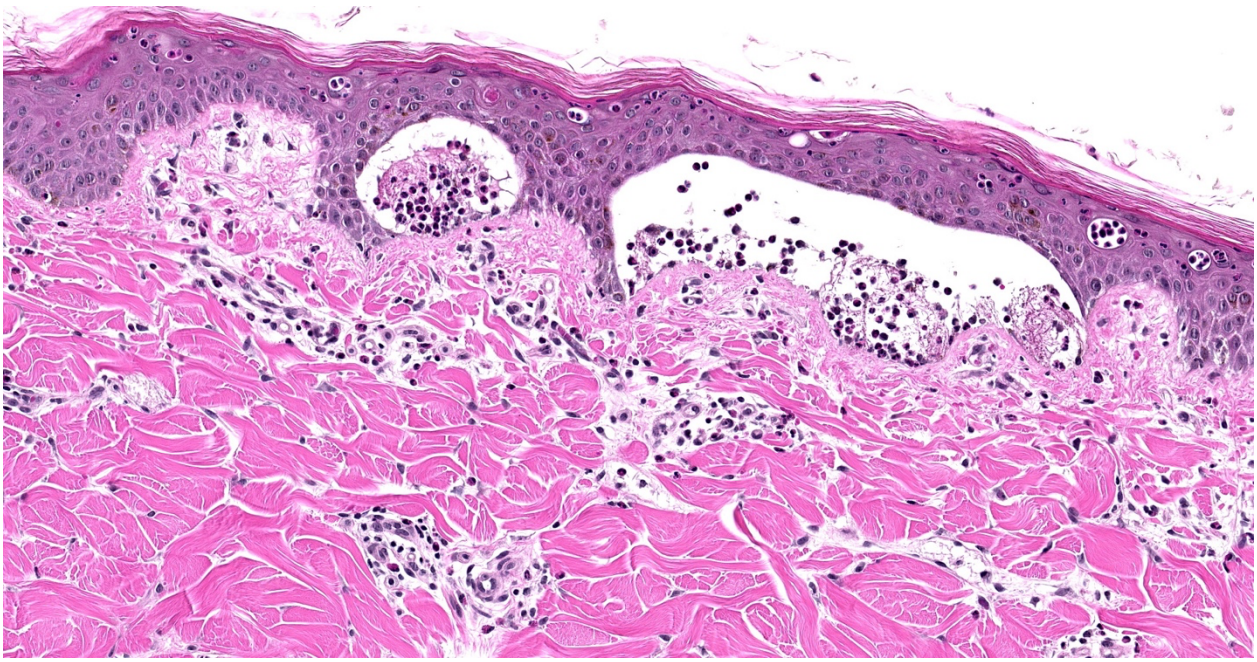
- Patients with anti-p200 pemphigoid often suffer from psoriasis
- Bullous systemic lupus erythematosus is part of the clinical spectrum of systemic lupus erythematosus, a prototypic autoimmune disease with multisystem involvement
- α5 chain of type IV collagen blistering disease is associated with nephropathy
- Pemphigoid gestationis is associated with pregnancy or trophoblastic tumors
- Linear IgA dermatosis is associated with inflammatory bowel disease
- Dermatitis herpetiformis is associated with celiac disease
- Epidermolysis bullosa acquisita is associated with inflammatory bowel disease

Contributing Institution:
Walter Reed Army Institute of Research/Naval Medical Research Center or WRAIR/NMRC
www.wrairintranet.com

JPC diagnosis:
Haired skin: Dermatitis, vesicular and eosinophilic, sub-basal, multifocal moderate to severe, with eosinophilic pustules.

JPC comment:
The contributor provides a succinct summary of bullous pemphigoid. While this disease has common features across species, the specific antigens attacked differ. In humans, antibodies attack BP antigen 1 (also called BP230) and BP antigen 2 (BP180), most often targeting the components of hemidesmosomes of the skin. In animals, only BPAG2 has been identified as a target of autoimmunity.[3]

In human epidemiological studies, a variety of factors may be risk factors for developing bullous pemphigoid. One of the most interesting risk factors with relevance to veterinary patients is medication history. More than 60 drugs have been reported to induce BP, including some antibiotics, diuretics, antihypertensives, anti-TNF-α drugs, and vaccines. However, one of the highest correlations between prior use and BP is the drug class of dipeptidylpeptidase-4 inhibitors, used for the treatment of type II diabetes mellitus. While developed for their ability to degrade glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), it also has the additional ability to cleave N-terminal dipeptides on molecules like eotaxin (CCL11), RANTES, CCL5, CXCL9, CXCL10,



Haired skin, pig. Subepidermal vesicles are filled with eosinophils, few basal keratinocytes, and polymerized fibrin. There are small eosinophilic pustules in the overlying dermis, and an eosinophilic perivascular dermatitis with edema. (HE, 216X)

and CXCL11. With veterinary medications typically lagging human development, we may someday see medications like sitagliptin, alogliptin, saxagliptin, or linagliptin.[2,6]

While a variety of inflammatory cells are found around the sites of BP, recent research has renewed focus on the role of mast cells in the development of this disease. More than 40 years ago, it was noted that an increased number of mast cells and increased degranulation of mast cells were present in the earliest stages of these lesions. Using tryptase as a marker for mast cells, research has shown increased tryptase levels in BP blister fluid, suggesting an early role for mast cells. While BPA2 IgG autoantibodies make up most of the antibody response, IgE autoantibodies with the same or similar epitope specificity are present in 70-90% of human lesions. Patients treated with omalizumab, a monoclonal antibody designed to inhibit IgE binding to FcεRI, experienced decreased disease severity.[1] Additional research with a focus on veterinary patients may reveal similar findings.

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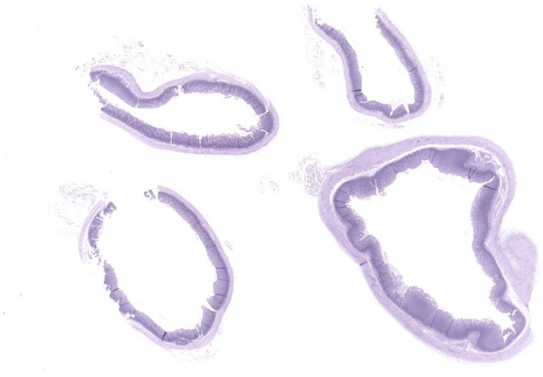
CASE 4: H15-877 (4073704-00)

Signalment:
5-day old pig, breed and gender unspecified, *Sus scrofa*, porcine

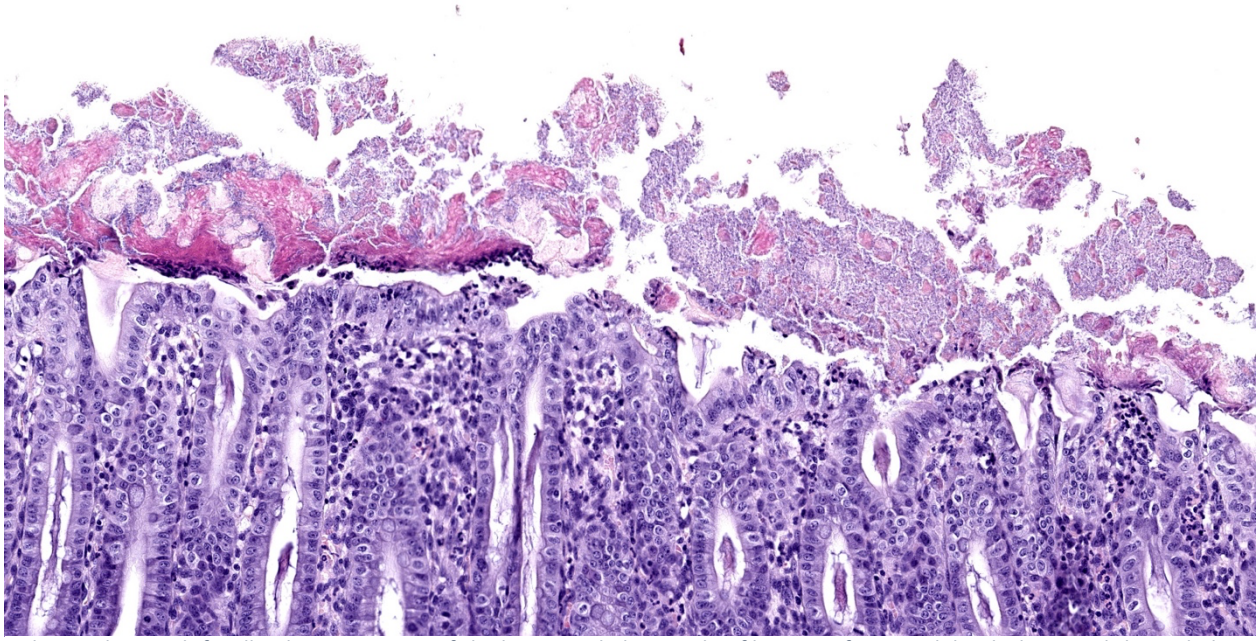
History:
Commercial, fully integrated pig farm with a neonatal diarrhoea problem ongoing for 2-3 weeks. 25% of (mainly gilt) litters affected. 100% morbidity within litters and approximately 20% mortality. Five very recently dead piglets submitted for necropsy.

Gross Pathology:
Carcasses well preserved with adequate body fat reserves. Stomachs filled with milk. Fluid yellow intestinal and colonic contents. Mild mesocolonic oedema. No gross changes noted in intestinal, cecal or colonic mucosa. Other body systems unremarkable.

Laboratory results:
ELISA for *Clostridium difficile* toxins A and B positive on colonic contents. No pathogenic



Colon, piglet. Multiple sections of colon are submitted for examination. (HE, 5X)



Colon, piglet. Multifocally, there is erosion of the lining epithelium with infiltration of neutrophils which expand the underlying edematous lamina propria. There is exudation of fibrin, neutrophils, and cellular debris along the mucosal surface. (HE, 167X)

bacteria isolated from intestinal contents. ELISA for *Clostridium perfringens* toxins negative. PCR for porcine epidemic diarrhea virus, porcine delta corona virus, transmissible gastroenteritis virus and porcine reproductive and respiratory syndrome virus negative. Polyacrylimide gel electrophoresis (PAGE) for rotaviruses negative.

Microscopic description:
Caecum and spiral colon: There is some variation in sections. Multifocally there is superficial erosion/ulceration of epithelium with exudation of neutrophils into the subjacent lamina propria and spilling of neutrophils, and in some sections fibrin, into the lumen ('volcano' lesions). Within lumens are multifocal mixed aggregates of bacteria (long bacilli and cocci). Mild, multifocal to coalescing oedema of superficial lamina propria and mild mesocolonic oedema.

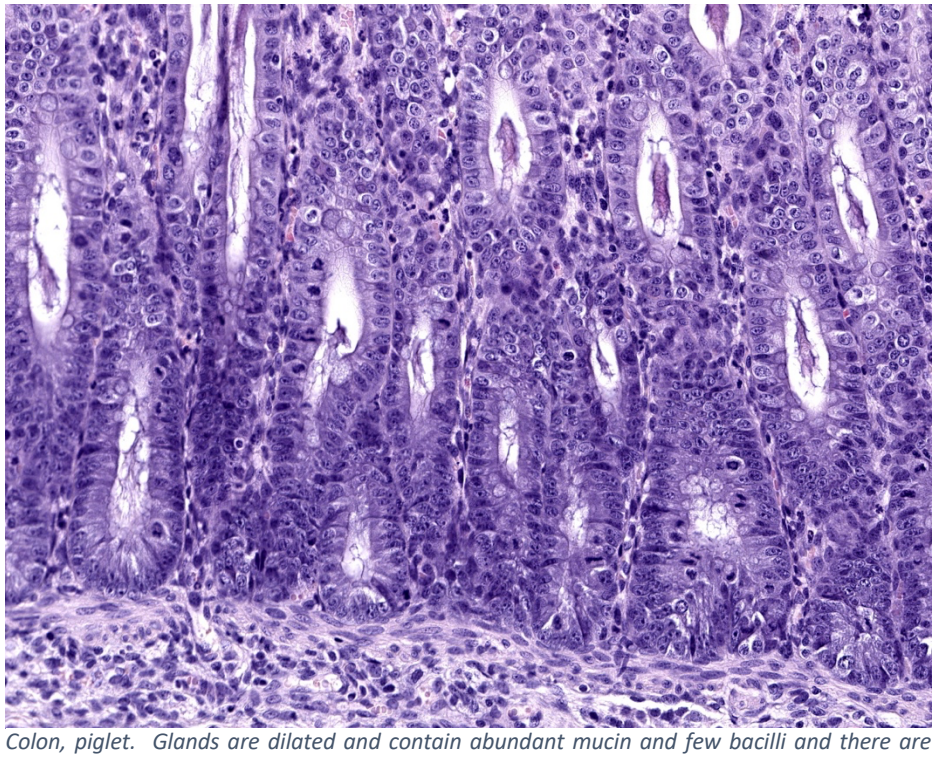
Contributor's morphologic diagnosis:
Cecum and colon: Typhlocolitis, fibrinopurulent, erosive, necrotizing, acute, superficial, multifocal, moderate.

Contributor's comment:
Clostridium difficile is a gram-positive spore-forming anaerobic enteropathogen of humans and animals.[4] Porcine neonatal *Clostridium difficile*-associated disease (CDAD) typically affects animals 1-7 days of age and has recently emerged as a major cause of diarrhoea in young piglets in North America and Europe. The clinical history typically includes early-onset diarrhoea, rarely respiratory distress, and sudden death. There may be oedema of the mesocolon and the colon may have pasty-to-watery yellowish content.[5] Hydrothorax, ascites and scrotal oedema have also been described.[8] Mucosal lesions of pigs are limited to the cecum and colon. These are typically mild, but vary from grossly inapparent, multifocal necrosis of surface epithelial cells to transmural necrosis. Pigs with spontaneous disease have microerosion/ulceration with effusion of fibrin and neutrophils into the lumen, so-called 'volcano ulcers'. Suppuration of the colonic lamina propria is the common microscopic lesion, with colonic serosal and mesenteric oedema with accompanying infiltration of mononuclear inflammatory cells and neutrophils. Segmental erosion of colonic mucosal epithelium is common, and volcano lesions may be evident.[5]

CDAD occurs opportunistically when the niche usually occupied by endogenous intestinal flora is disrupted, either by a change in diet or by antimicrobial treatment.[6] *C. difficile* produces

two major toxins. Toxin A (TcdA) and Toxin B (TcdB) that act synergistically to cause apoptosis of mucosal epithelial cells and disruption of actin filaments that results in loss of cell-to-cell contact and increased paracellular permeability of mucosae. Toxins A and B have other effects, notably initiation of an inflammatory cascade that can result in increased damage to host tissues and exudation of fluid.[4] The requirements for development of CDAD are disruption of normal intestinal or colonic flora, presence of the organism in the environment and the production of toxins.[6]

The standard diagnosis for porcine CDAD is detection of TcdA and TcdB in faeces or colonic content, generally using commercially available enzyme immunoassays. Cultivation of *C. difficile* is difficult and because it can be found in healthy pigs, its isolation may have little diagnostic relevance.[5] CDAD develops spontaneously in a variety of other species including horses, hares, non-human primates, domestic dogs, domestic cats, ostriches, and black-tailed prairie dogs.[4] *C. difficile* is also one of the most important nosocomial pathogens of humans, primarily associated with intestinal dysbiosis following antibiosis. In recent years the epidemiology of the disease in human patients is changing with more community acquired infections and the emergence of strains in humans that are common in domestic animals. The pathogen is thus now being considered a potential zoonosis.[6]



Colon, piglet. Glands are dilated and contain abundant mucin and few bacilli and there are numerous mitotic figures. (HE, 207X)

Contributing Institution:
Veterinary Sciences Centre
School of Veterinary Medicine
University College Dublin
Belfield, Dublin 2, Ireland
http://www.ucd.ie/vetmed/

JPC diagnosis:
Colon: Colitis, erosive and neutrophilic, multifocal to coalescing, moderate, with edema and crypt hyperplasia.

JPC comment:
This pathogen, now named *Clostridioides difficile*, remains a significant pathogen in human and veterinary populations. In 2011, there were approximately 453,000 human *C. difficile* infections in the United States. While the distribution of different virulent ribotypes has changed with the use of differing antibiotic classes in different parts of the world, incidence of ribotype 78 *C. difficile* infection has been increasing since 2005. This virulent strain primarily affects pigs and calves, but also infects humans, and there has been an association between pig farms and human infection documented in the Netherlands.[2]

Contamination of food destined for human consumption remains a problem around the world. A study in Japan tested commercially available foods at supermarkets and found *C. difficile*

contamination in 3.3% of free vegetables, 6.7% of chicken meat, 3.6% of chicken liver, 0.5% of pork meat, and 1.6% of beef meat sampled. Additionally, the presence of Toxin A, Toxin B, and binary toxin (CDT) was detected in samples, as well as some isolates being resistant to common antibiotics. The rates of contamination in this study were comparatively low compared to the United States and should provide ample motivation for thorough food inspections.[7]

While the most common method of contracting disease is an alteration of the gut flora, usually through antibiotic use. However, exposure and transmission has not been well investigated. A great number of spores are likely in most contaminated environments, but they may not all be perpetuated by pigs. A survey of pigs and rodents collected across two farms revealed the mice and rats were capable of carrying *C. difficile,* with a prevalence between 25-50%. Further research is necessary to determine the extent of rodent transmission or persistence of *C. difficile* infections in pigs.[1]

While there is no commercially available vaccine against this agent, an alternative treatment has been explored. Following work performed in laboratory rodents, the non-toxigenic strain of *C. difficile* Z31 was administered to 1-day old piglets, with subsequent fecal analysis 24 hours later. Administration of the Z31 strain statistically significantly reduced *C. difficile* infection, as compared to controls. In the animals that did develop disease, the intensity of diarrhea was reduced. This may represent a valid preventive for the pig industry.[3]

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