CASE I: C13-122 (JPC 4032962).

Signalment: 4-year-old, 1.4 kg castrated male ferret (Mustela putorius furo).

History: The ferret presented for an approximately 2-month history of difficulty ambulating, progressive ataxia, and proprioceptive deficits in all limbs. MRI and CT identified a non-resectable mass at the level of C1 to C2. Fine needle aspirational cytology was performed. The animal was euthanized approximately 2 months later after failing to improve neurologically after attempting definitive radiation therapy.

Gross Pathology: An approximately 2 x 1.8 x 1 cm, multi-nodular, clear and gelatinous to hard and white mass effaced the C1 vertebra, extended ventrally into the cervical musculature, and projected dorsally into the spinal canal and extended cranially into the foramen magnum along the ventral aspect of the brainstem. There was marked, segmental compression of the cervical spinal cord and brainstem.
Cytologic Description: Fine needle aspirate, cervical mass: The sample is of good quality with minimal hemodilution. There are moderate numbers of nucleated cells on a moderately thick, grainy, eosinophilic background. Nucleated cells are present individually or in small clusters, showing marked (up to 5-fold) anisocytosis. The smaller cells are approximately 10 microns in diameter with a small amount of basophilic cytoplasm. The larger cells (physaliphorous cells) are markedly distended up to approximately 80 microns by cytoplasmic vacuolation that is clear or contains pink, grainy material. Nuclei are round to oval and often eccentric, with reticular chromatin, typically lacking obvious nucleoli. Mitotic figures are not observed.

Contributor’s Morphologic Diagnosis: Cervical mass: Chordoma.

Contributor’s Comment: The chordoma was confirmed by histopathology and immunohistochemistry following postmortem examination. Histopathology revealed an unencapsulated, multilobulated, moderately cellular neoplasm that effaced the first cervical vertebra and was composed of polygonal cells arranged in solid sheets supported by a moderate amount of fibrous to myxomatous to chondromatous stroma. These cells were often markedly vacuolated, typical of the physaliphorous cells described in chordomas. No mitoses were observed. Immunohistochemistry for cytokeratin and vimentin expression was performed on decalcified sections of the neoplasm, resulting in weak to moderate, patchy, cytoplasmic expression of both markers.

Chordomas are the most common neoplasm of the musculoskeletal system of the ferret, but have also been described in other species, such as rats, cats, humans, mink and dogs. The embryonic notochord degenerates early in fetal development and remains as the nucleus pulposus within intervertebral disks. In some cases, residual notochord cells remain outside the intervertebral disks and may become a chordoma.

In ferrets, chordomas arise primarily in or adjacent to the caudal vertebra, but they also have been described elsewhere along the spine. Chordomas are locally aggressive, destroy the vertebral body, and invade adjacent tissues. Cutaneous metastases of chordomas have been reported in ferrets. Although chordomas of the tail may be treated by amputation, at other locations along the axial skeleton, chordomas may not be amenable to surgical therapy and can cause spinal cord compression, as seen in this case.

The main differential diagnosis for a chordoma is chondrosarcoma. The large, severely vacuolated, physaliphorous cells of chordomas are not a feature of chondrosarcoma; however, differentiation of these tumors is further aided by immunohistochemistry. Chordomas express
vimentin, cytokeratin, and S-100 protein, while chondrosarcomas do not express cytokeratin. Combining the midline location of the tumor, cytological criteria such as physaliphorous cells, and immunohistochemical expression of cytokeratin confirms a diagnosis of chordoma.

**JPC Diagnosis:** Cervical vertebrae: Chordoma.

**Conference Comment:** Chordomas are fairly well described in humans; however, they are rare in domestic animals, with the exception of ferrets. In both humans and rats, chordomas are more common in aged males. In humans they can occur anywhere along the vertebral column, often extend into soft tissue, and are divided into three types: conventional, chondroid and dedifferentiated.

Conventional chordomas are slow-growing, but locally invasive with a high rate of recurrence, especially in those that arise from the sacrococcygeal region or the vertebrae. Chondroid chordomas tend to originate from the sphen-o-occipital region and generally behave more benignly. Conversely, dedifferentiated chordomas are rare (less than 5% of cases), with histologic features of a high-grade spindle cell sarcoma and aggressive biologic behavior. The distinction between conventional, chondroid and dedifferentiated types is important prognostically as
survival rates are up to three times higher with chondroid chordomas. 

Conference participants briefly discussed the occurrence of chordomas in various veterinary species. In ferrets, the most common location is on the tip of the tail; however, in most domestic species, chordomas tend to occur in the sacrococcygeal and cervical regions. In dogs, chordomas have also been reported in the brain, spinal cord, and skin. Of the two reported cases of feline chordoma, one was initially diagnosed as chronic granulomatous inflammation due to the interpretation of the characteristic physaliphorous cells as atypical, foamy macrophages, but was later shown to be a classic chordoma, which subsequently metastasized to multiple lymph nodes. The second feline case was classified histologically and immunohistochemically as a chondroid chordoma. Chondroid chordomas similar to the human subtype have been reported in ferrets, mink, cats and dogs. Similarly to human medicine, the distinction between conventional and chondroid chordomas may also have some degree of prognostic significance in animals. Although all chordomas are potentially locally invasive, chondroid chordomas in ferrets, dogs and cats have not been reported to metastasize, while conventional chordomas in rats and cats (but not dogs) seem to have a higher rate of metastasis. Additionally, chordomas arising from the tail appear to have a good prognosis in all cases.

An alternate spelling of “physaliferous” may be seen in the literature, and is considered a correct spelling as well.

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http://www.tufts.edu/vet/dbs/pathology.html

**References:**

CASE II: N12-247 (JPC 4032703).

Signalment: 1-year-old male rat snake (Pantherophis sp.)

History: Presented for inappetence and weight loss.

Gross Pathology: The mucosal surface of the stomach is diffusely tan and appears moderately thickened with a wall thickness of approximately 5 mm and increased prominence of rugal folds.

Histopathologic Description: The gastric mucosa is diffusely moderately to markedly thickened, with prominent rugal folds. Gastric pits lined by luminal epithelial cells are moderately to markedly elongated and frequently mildly dilated and branched or irregularly shaped. There is moderate to marked proliferation (hyperplasia) of mucus neck cells and aggregates of mucus neck cells multifocally replace granular cells within deeper portions of gastric glands disrupting the normal glandular architecture. There is multifocal piling (hyperplasia) of luminal epithelial and mucus neck cells and low numbers of mitotic figures are observed within these cell populations (averaging one per 400X field) and these mitotic figures extend to the luminal aspect of gastric pits. Low to moderate numbers of gastric glands are mildly dilated. Low numbers of gastric glands lined by remaining granular cells have multifocal attenuation (atrophy) of the glandular epithelium. Large numbers of approximately 3-6 µm diameter protozoa with amphophilic cytoplasm and distinct basophilic nuclei are adherent to the luminal epithelium and epithelium lining gastric pits and glands and are frequently admixed with small amounts of mucin and low numbers of necrotic epithelial cells. The lamina propria is diffusely expanded by mildly to moderately increased amounts of fibrous connective tissue that separates adjacent glands. Low to moderate numbers of lymphocytes and plasma cells and low numbers of heterophils are scattered within the lamina propria and are present in multifocal, nodular aggregates that surround gastric glands. Low numbers of lymphocytes and plasma cells are present within the luminal and glandular epithelium. The submucosa is multifocally expanded by small to moderate amounts of pale amphophilic granular to wispy material (edema), mildly increased amounts of fibrous connective tissue and small numbers of similar inflammatory cells.

2-1. Stomach, rat snake: The stomach wall is diffusely thickened and thrown into prominent rugal folds. (HE 0.63X)
Contributor’s Morphologic Diagnosis: Stomach: Hypertrophic gastritis, diffuse, moderate, chronic, with myriad intraluminal protozoa consistent with *Cryptosporidium* sp.

Contributor’s Comment: A number of *Cryptosporidium* sp. infect reptiles, including *C. serpentis*, *C. varanii* (*saurophilum*), and other unidentified species of *Cryptosporidium*. Despite this, each species of *Cryptosporidium* is relatively species-specific; species from mammals do not infect snakes with experimental transmission. *Cryptosporidium* infection commonly causes proliferative gastritis, progressive weight loss, and eventual death. In lizards, enteritis is more common, although gastritis can occur as well.

Histopathology is considered the gold standard for diagnosis, as it enables differentiation of pathogenic *Cryptosporidium* from those ingested with prey, which makes fecal testing difficult. However, recent advances in molecular biology make fecal testing more useful.

JPC Diagnosis: Stomach: Gastritis, proliferative, diffuse, moderate, with marked mucus cell hyperplasia and metaplasia, rare mucosal epithelial necrosis and numerous intraepithelial and luminal apicomplexan schizonts.

Conference Comment: Cryptosporidia are obligate intracellular coccidians of the phylum *Apicomplexa*. Apicomplexans are so named because the sporozoites possess an “apical complex,” containing rhoptries, micronemes, a conoid and a polar ring associated with microtubules, all of which are evident ultrastructurally. These organisms reside within parasitophorous vacuoles along the epithelial microvillus border, and thus occupy an intracellular yet extracytoplasmic domain; at the junction with the host cell there are finger-like folds of parasitic cytoplasm formed within an electron dense attachment zone, known as the “feeder organelle.” Infected hosts shed sporulated, thick-walled fecal oocysts that contain four sporozoites and can remain viable for several months. Upon ingestion, sporozoites excyst and invade the microvillus border of gastrointestinal, biliary, or respiratory epithelial cells, where they undergo asexual multiplication (schizogony/merogony) and gametogony resulting in macro- and microgamonts. Finally, fertilization produces two types of oocysts, which sporulate within the host. Thick-walled oocysts exit the host, while thin-walled oocysts are involved in autoinfection; this ability to self-infect accounts for the chronicity and severity observed in some cases of cryptosporidiosis.

*Cryptosporidium* infects both immune-competent and immune-suppressed animals. Some cryptosporidia of veterinary importance are listed in table 1. *Cryptosporidium* spp. have a comparatively low minimum infective dose, are relatively resistant to normal concentrations of chlorination and can cause life-threatening diarrhea in immune-deficient humans. Several species, most notoriously *C. parvum*, are zoonotic; however the
most frequent mode of human transmission is actually human-to-human.\textsuperscript{7} Whereas dairy calves are an important potential source of \emph{C. parvum}, adult cattle typically shed non-zoonotic species. \emph{C. meleagridis}, especially from turkeys, has also been identified as zoonotic. \emph{C. hominis} and \emph{C. parvum} are the most commonly identified species in humans.\textsuperscript{5} Interestingly, \emph{Cryptosporidium} sp. instigated the largest ever recorded waterborne disease outbreak, affecting 400,000 people (approximately one quarter of the population) in Milwaukee, Wisconsin in 1993.\textsuperscript{7} Initially, contamination of the municipal drinking water was blamed on contaminated run-off from nearby cattle farms and abattoirs; however \emph{C. hominis} was the primary isolate from water samples, with lower concentrations of \emph{C. parvum}, suggesting that the majority of the contamination was likely secondary to human sewage.\textsuperscript{5}

\textbf{Table 1.} Selected \emph{Cryptosporidium} sp.\textsuperscript{3,8,9}

<table>
<thead>
<tr>
<th>Species</th>
<th>Major Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>\emph{C. parvum}</td>
<td>Mammals (human and bovine genotypes)</td>
</tr>
<tr>
<td>\emph{C. hominis}</td>
<td>Humans</td>
</tr>
<tr>
<td>\emph{C. andersoni}</td>
<td>Cattle, camels</td>
</tr>
<tr>
<td>\emph{C. suis}</td>
<td>Pigs</td>
</tr>
<tr>
<td>\emph{C. felis}</td>
<td>Cats</td>
</tr>
<tr>
<td>\emph{C. canis}</td>
<td>Dogs</td>
</tr>
<tr>
<td>\emph{C. muris}</td>
<td>Rodents, camels</td>
</tr>
<tr>
<td>\emph{C. wrairi}</td>
<td>Guinea pigs</td>
</tr>
<tr>
<td>\emph{C. cuniculus}</td>
<td>Rabbins</td>
</tr>
<tr>
<td>\emph{C. baileyi, C. meleagridis}</td>
<td>Birds</td>
</tr>
<tr>
<td>\emph{C. serpentis, C. crotali}</td>
<td>Snakes</td>
</tr>
<tr>
<td>\emph{C. varanii}</td>
<td>Lizards, snakes</td>
</tr>
<tr>
<td>\emph{C. ducismarci}</td>
<td>Tortoises</td>
</tr>
<tr>
<td>\emph{C. molnari}</td>
<td>Fish</td>
</tr>
<tr>
<td>\emph{C. fayeri, C. macropodum}</td>
<td>Kangaroos</td>
</tr>
</tbody>
</table>

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CASE III: WSC 2012 #1 (JPC 4019387).

Signalment: 8-week-old male Flemish Giant rabbit (*Oryctolagus cuniculus*).

History: A 400-animal rabbitry experienced a mortality of 20 rabbits in one month. Clinical signs included sudden onset of marked lethargy and head tilt. Rabbits died 24-48 hours after showing signs of illness. Clinical signs were observed in all age groups, although mostly young rabbits were affected. The breeder was treating the herd with sodium sulfamethazine and amprolium solution in water for coccidiosis.

Gross Pathology: Five, 8-week-old rabbits (3 male, 2 female) were submitted for necropsy. The rabbits were in poor body condition, e.g. were very thin and lacked body fat. There was tan/red mottling of the renal cortices, and the cortical surfaces of the kidneys were irregular. The cut surfaces of kidneys had many pale areas extending from the cortex to the medulla. The lungs were moderately congested and edematous. The spleen was moderately enlarged and the liver was moderately congested. The stomach contained a small amount of green mucoid material. In many areas, the mucosa of the small and large intestine was moderately congested.

Laboratory Results: Fecal flotation was positive for *Eimeria* sp. oocysts. Aerobic bacterial culture of the kidney yielded *E. coli*.

A sample of frozen kidney was positive by PCR testing for *Encephalitozoon cuniculi*.

Histopathologic Description: Kidney: In many areas of the cortex and medulla there are interstitial infiltrates of moderate to large numbers of macrophages and lymphocytes and plasma cells. These interstitial infiltrates frequently surround ectatic tubules, which are lined by attenuated, degenerate, or necrotic epithelium; occasionally, tubules are lined by swollen renal tubular epithelial cells that contain numerous, intracytoplasmic, 1.5 x 3 µm refractile spores. Often the lumina of affected tubules contain sloughed tubular epithelial cells, cellular debris, and numerous intracellular and extracellular 1.5 x 3 µm refractile spores. Moderate numbers of tubules have cellular casts in their lumina.

Affecting the gray and white matter of the cerebrum and the cerebellum (slide not provided), there are scattered, nodular infiltrates of moderate numbers of macrophages (which contain 1.5 x 3 µm refractile spores in their cytoplasm) and activated microglia, few lymphocytes and plasma cells, and rare neutrophils together with small amounts of necrotic debris. Rarely there are 10-100 µm in diameter cysts which contain numerous, 1.5 x 3 µm refractile spores; these cysts are present both in areas of inflammation and in areas free of inflammation. The leptomeninges in many areas are moderately expanded by a similar inflammatory infiltrate. Vessels are lined by reactive endothelium and there

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3-1. Kidney, rabbit: The cortical interstitium is expanded by pyramidal rays of basophilic inflammatory cells. Vasculature is congested. Renal tubules are ectatic multifocally. (HE 0.63)

3-2. Kidney, rabbit: Tubules are multifocally necrotic, and the intervening interstitium is expanded by aggregates of macrophages surrounded by large numbers of lymphocytes and plasma cells. (HE 256X)
is a small amount of hemorrhage in the leptomeninges.

The spores in the kidney and the brain stained gram positive with the Brown and Brenn's gram stain and faintly acid-fast-positive with Ziehl-Neelsen acid-fast stain.

**Contributor’s Morphologic Diagnosis:** Kidney: Marked, multifocal, granulomatous, interstitial nephritis, with tubular ectasia, degeneration and necrosis, and intraleisional microsporidial spores, etiology consistent with *Encephalitozoon cuniculi*.

**Contributor’s Comment:** In this case, 4 out of 5 rabbits had kidney and brain lesions that were similar and severe; one had mild lesions but had severe enteric coccidiosis. A Gram stain was used to assist in identifying spores and their features, which were compatible with those of microsporea. PCR was performed on a sample of frozen kidney from one of the rabbits to confirm the presence of DNA of *Encephalitozoon cuniculi*.

*Encephalitozoon cuniculi* is a microscopic parasite that belongs to the phylum Microspora, which encompasses obligate intracellular, spore-forming, single-celled parasites with a direct life cycle. Microsporidia are characterized by a severe reduction, or even absence, of cellular components typical of eukaryotes such as mitochondria, Golgi apparatus and flagella. This simplistic cellular organization has made it difficult to infer the evolutionary relationship of Microsporidia to other eukaryotes. It is now widely acknowledged that features of Microsporidia previously recognized as primitive are instead highly derived adaptations to their obligate parasitic lifestyle. Microsporidia were initially thought to be protozoa but subsequent molecular biological evidence suggests that they are more closely related to fungi than protozoa. These findings include the presence of a particular mitochondrial heat shock protein more closely related to that of fungi, alpha- and beta-tubulins that are closely related in composition to those of fungi, and the presence of chitin and trehalose, which are typical components of fungi.

*Encephalitozoon cuniculi* infections have been reported in many mammalian species, e.g. rabbits, SCID mice, guinea pigs, alpacas, neonatal foxes, neonatal dogs, and immune compromised people. Severe disease is rare except in immune compromised mammals. Three strains have been identified: strain I was found in rabbits and humans, strain II in rodents and blue foxes, and strain III in dogs and humans. Identification of strains in humans and animals suggest possible zoonotic transmission. Although strain III of *E. cuniculi* is found in humans and dogs, no direct evidence suggests that dogs can transmit the disease to humans.

*Encephalitozoon cuniculi* transmission occurs through ingestion, by inhalation of contaminated urine or feces shed by infected hosts, transplacentally, and rarely by penetration across
injured epithelium. Microsporidia extrude a polar tube and inject the infective sporoplasm into the host cell in response to the appropriate environmental stimuli from the host. Subsequently, development proceeds by two processes, i.e. merogony and sporogony. During merogony the sporoplasm divides and generates numerous proliferative forms called meronts. Sporogony produces intermediate stages known as sporonts, which produce sporoblasts that will mature into spores and eventually be released into extracellular space.

In naturally infected rabbits, *E. cuniculi* infections are often subclinical. Occasionally infected rabbits will display neurologic signs of ataxia, opisthotonos, torticollis, hyperaesthesia, or paralysis. This is especially true in young rabbits. Dwarf rabbits are also especially susceptible. Focal, irregular, depressed areas in the renal cortex can be seen in chronically infected rabbits; however, gross lesions are usually absent in infected rabbits. The parasite is able to infect a large variety of cell types, such as neurons, epithelial cells of ependyma and choroid plexus, renal tubular epithelium, endothelium and macrophages. Characteristic foci of granulomatous inflammation and organisms are observed in brain, kidney, lung, adrenal gland, and liver. Histologically, multifocal nonsuppurative meningoencephalitis with astrogliosis and perivascular lymphocytic infiltration and focal to segmental lymphocytic-plasmacytic interstitial nephritis with variable amounts of fibrosis are reported. Early lesions in the kidney display focal to segmental granulomatous interstitial nephritis with degenerated epithelial cells and mononuclear cell infiltration. Lesions minimally involve glomeruli. In the lung, focal to diffuse interstitial pneumonia with mononuclear cell infiltration may occur. Hepatic lesions are characterized by a focal granulomatous inflammatory response with periportal lymphocytic infiltration. Multifocal lymphocytic infiltrates may also occur in the myocardium. With the Gram stain parasites are seen as gram-positive, 1.3-1.5 μm, rod-shaped organisms in the intracellular parasitophorous vacuoles of cells. Encephalitozoonosis in rabbits should be
differentiated from infection by *Pasteurella multocida*, *Listeria monocytogenes*, and *Toxoplasma gondii*. Electron microscopy can reveal the organisms in parasitophorous vacuoles as well as the distinctive polar filaments.3

The incidence of encephalitozoonosis is on the decline in many areas, particularly in well-managed rabbitries. Regular serological testing will readily identify infected animals. Since seroconversion precedes renal shedding, infected animals can be identified before they are shedding organisms in the urine.8

**JPC Diagnosis:** Kidney: Nephritis, tubulointerstitial, histiocytic & lymphoplasmacytic, chronic, multifocal, moderate with intratubular microsporidian spores.

**Conference Comment:** As noted in the contributor’s thorough examination of the life cycle and pathogenesis of *E. cuniculi*, microsporidia are obligate intracellular, unicellular eukaryotes that are most closely related to fungi, specifically zygomycetes. They have one of the smallest known genomes and exist extracellularly only as small, thick-walled spores with a coiled polar filament.6 Developing spores can be packaged within a parasitophorous vacuole (*Encephalitozoon* spp.) or can remain within the cytoplasm (*Enterocytozoon bieneusi*, *Nosema* spp.).10 The first recorded microsporidian parasite, *Nosema bombycis*, devastated the European silk-worm industry in the 1850s,2 while currently, *Nosema apis* is a significant problem in honey bees, *Enterocytozoon salmonis* causes disease in chinook salmon6 and *Encephalitozoon hellum* affects pigeons and exotic birds such as budgerigars and parrots.7 Several other microsporidian species, including *Encephalitozoon intestinalis*, *E. hellum*, *Enterocytozoon bieneusi*, and *Vittaforma cornea* have been identified as opportunistic pathogens of humans, especially immunocompromised patients.10 Additionally, a recent study suggests that Crohn’s disease (CD) patients are at risk for microsporidiosis and that microsporidiosis may be involved in the etiology of CD.1

**References:**

**Contributing Institution:** University of Connecticut
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CASE IV: 12-0399 (JPC 4025191).

Signalment: 2-year-old female goat, breed unspecified (Capra hircus).

History: Two female goats presented with multifocal to coalescing progressive ulcerative and crusting lesions on the commissures of the lips. Both goats were recently sent to another farm to be bred. The owner reports no change in appetite or behavior.

Gross Pathology: Portions of the lip were submitted for histopathological evaluation. Gross lesions consisted of multifocal to coalescing proliferative and ulcerative cheilitis.

Histopathologic Description: Haired skin, lip: There was extensive multifocal epidermal hyperplasia with acanthosis up to 10 times normal thickness with elongated, anastomosing rete ridges. Multifocally within the stratum spinosum, keratinocytes were markedly swollen and often contained clear intracytoplasmic vacuoles (ballooning degeneration) and pyknotic nuclei. Multifocally, rare keratinocytes contained one or more 2-6 µm, round to oval, eosinophilic intracytoplasmic inclusion bodies. Within the superficial stratum spinosum and stratum corneum, there was neutrophilic transmigration and multifocally there were microabscesses composed of aggregates of degenerate neutrophils admixed with eosinophilic intracytoplasmic vacuoles (ballooning degeneration) and pyknotic nuclei. Overlying the affected epidermis was a thick serocellular crust composed of keratin, proteinaceous fluid, degenerate neutrophils, and rare mixed bacteria. The dermis there were numerous dilated small caliber blood vessels separated by increased clear space and fibrin (edema), and moderate numbers of perivascular neutrophils, histiocytes, and lymphocytes.

Contributor’s Morphologic Diagnosis: Haired skin, lip: Cheilitis, proliferative and necrotizing, multifocally extensive, severe, with epidermal hyperplasia, hyperkeratosis, rare epidermal eosinophilic intracytoplasmic inclusion bodies, and intraepidermal and intracorneal microabscesses, breed unspecified, caprine.

Contributor’s Comment: Contagious ecthyma, also called contagious pustular dermatitis, is a zoonotic disease with worldwide distribution that affects sheep, goats, and man.1-9,13,14,16,17 The causative agent is a dsDNA parapoxvirus (PPV).1,3,5-9,16 PPV, also known as orf virus, gains entry through abraded skin and replicates in epidermal cells.1,3,6-9,16,17 Skin lesions progress in an orderly fashion through multiple stages: erythema, macule, papule, vesicle, pustule, scab, and scar.1-6,8,9,13,16,17 Infection is confined to the squamous epithelium and may involve the oral cavity, eyelids, teats, and coronary band, and predispose affected animals to secondary infections.1,2,4,13,14,16,18 Very rarely, lesions extend to the squamous epithelium of the esophagus, rumen and omasum, causing ulcerative gastroenteritis.1,2,4,18 Residual skin lesions are not infective once the scab falls off, but substantial amounts of infective virus are shed within scabs which can remain infective for years.1,3-8,16 The disease has high morbidity and low mortality but can cause significant debilitation due to the inability of affected animals to suckle or graze.4,6-9,13 Nursing animals often transfer the virus to adults, typically affecting the teats and udder.1,3,4,6,7,9,13,14,17 There are several commercial vaccines for orf virus which contain virulent virus.8,13 These vaccines are valuable because they limit the severity of disease, but they do not prevent infection, induce lesions, and contribute to maintenance of infective virus in the environment, so are only recommended for use in endemically infected herds.8,13 Gross lesions are characterized by multifocal to coalescing proliferative and ulcerative dermatitis which is localized to mucocutaneous junctions, particularly around the mouth and nares.1,3,5,9,14 Histologic lesions are characterized by ballooning degeneration, exuberant epidermal hyperplasia with intracorneal pustules, and eosinophilic intracytoplasmic inclusion bodies within the stratum spinosum that are only briefly detectable at the vesicular stage.1,3,4,8,9,17 There are frequently superimposed bacterial infections in the affected skin.13

Sheep previously exposed to orf virus can be repeatedly infected although the severity of the lesions and time to resolution diminishes with each subsequent infection.1,6,8,13 This indicates that the host immune response can control the severity of disease but cannot prevent reinfection.6 The reason is due, at least in part, to the presence of at least five immunomodulatory proteins expressed by orf virus that subvert or suppress elements of the host immune and inflammatory response, including:2,5-8,11
Orf virus interferon resistance protein (OVIFNR): Host cells generally attempt to stop viral replication through interferon-induced activation of the double stranded RNA-dependent kinase (PKR) pathway. In the presence of viral double-stranded RNA, the PKR pathway is induced by interferon and binds to viral dsRNA which inhibits virus (and host cell) protein translation, effectively blocking virus replication. The orf virus encodes a protein, OVIFNR, which is a homologue of vaccinia virus E3L gene product, competitively binds to dsRNA and disallows activation of PKR, which ultimately promotes a permissive state for viral replication, leading to sustained protein synthesis and completion of the virus life cycle. In other words, the OVIFNR protein prevents activation of PKR and protects the virus from the antiviral effects of interferons, allowing viral replication.

Granulocyte/macrophage colony-stimulating factor (GM-CSF) inhibitory factor (GIF): GIF binds to and inhibits the biological activity of the cytokines GMCSF and interleukin2 (IL-2). In non-hematopoietic tissues, GMCSF is involved in the recruitment and activation of macrophages and neutrophils. GMCSF also supports the recruitment and antigen-presenting function of dendritic cells (DC). IL2 is required for T cell proliferation and activation, for example, the augmentation of T cell IF gamma production and to expand CTL and NK cell populations. Inhibition of these two important cytokines significantly impedes the development of both innate and acquired immune responses.

Orf viral interleukin 10 (ORFV-IL-10): The initiation of an acquired immune response to a virus requires that antigen be taken up by DC at the periphery, and then processed and transported to lymphoid tissue to be presented to T cells. For this to occur, DC must first be activated by signals transmitted through the interaction of viral products with pattern recognition receptors expressed on the surface of the DC. DC therefore form the vital bridge between the innate and acquired arms of the immune response. IL-10 is an immunoregulatory cytokine that inhibits both innate and adaptive
immune responses and is produced by macrophages and dendritic cells in response to microbial products.\textsuperscript{17} It is produced by multiple T cell subsets, including all three populations of helper T cells (Th1, Th2, and Th17).\textsuperscript{17} IL10 is able to down regulate MHC class II and co-stimulatory molecule expression on DC and macrophages, thus decreasing antigen presentation.\textsuperscript{7,17} Orf virus-encoded interleukin-10 (ORFVIL10), a homologue of IL-10, has specifically been shown to suppress the migration of Langerhans cells (LC) and inhibit DC function in addition to suppressing inflammation and the innate immune response.\textsuperscript{11} ORFV-IL-10 has the capacity to impair the initiation of an acquired immune response and hence inhibit the generation of immunological memory necessary for immunity of subsequent exposure.\textsuperscript{11} ORFV-IL-10 has the capacity to impair the initiation of an acquired immune response and hence inhibit the generation of immunological memory necessary for immunity of subsequent exposure.\textsuperscript{11} ORFV-IL-10 has the capacity to impair the initiation of an acquired immune response and hence inhibit the generation of immunological memory necessary for immunity of subsequent exposure.\textsuperscript{11}

(5) Orf virus vascular endothelial growth factor (VEGF): Orf virus VEGF functions in the same way as cellular VEGFs, causing vascular proliferation and angiogenesis.\textsuperscript{5,6,8,16} Orf virus lesions in sheep are characterized by vascular proliferation and dilation as well as epidermal proliferation.\textsuperscript{5,8,16} The induction of epidermal proliferation by orf virus is probably important in supplying an abundance of target epidermal cells for virus replication.\textsuperscript{5,8,16} Additionally, host cell VEGF has the ability to inhibit the development and maturation of dendritic cells.\textsuperscript{5,8} If viral VEGF also has this ability, it would benefit the virus because of the important role that dendritic cells play in the generation and maintenance of immune responses.\textsuperscript{5}

JPC Diagnosis: Mucocutaneous junction: Epithelial hyperplasia, diffuse, severe, with marked acanthosis, focally extensive necrosis, ballooning degeneration and rare intracytoplasmic viral inclusions.

Conference Comment: The contributor provides an excellent, comprehensive review of parapoxviruses. This genus causes epitheliotropic disease in a number of species of veterinary importance, in addition to sheep and goats. Papular stomatitis virus causes proliferative to ulcerative lesions on the lips and oral mucosa of cattle, while pseudocowpox virus causes similar lesions on the teats of dairy cattle and on the muzzles and mouths of nursing calves.\textsuperscript{13} A parapoxvirus has been blamed for the sporadic occurrence of pustular facial dermatitis in camels\textsuperscript{13} as well as New Zealand farmed red deer.\textsuperscript{10} Additionally, outbreaks of contagious ecthyma have resulted in serious economical loss in Norwegian reindeer.\textsuperscript{10} Sealpox virus, which produces self-limiting ulcerative dermal nodules in some pinniped species, has also been tentatively classified as a parapoxvirus.\textsuperscript{15} The red squirrel population in the United Kingdom has been devastated by what was initially classified as a parapoxvirus, but is more likely a closely related poxvirus from the subfamily Chordopoxvirinae. American grey squirrels, which are relatively resistant to this virulent squirrelpox virus, likely
serve as a reservoir for disease in the more susceptible red squirrel population.12

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References: