Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 11

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Conference Moderator:
Charles W. Bradley, VMD, DACVP
Assistant Professor, Pathobiology
University of Pennsylvania School of Veterinary Medicine
4005 MJR-VHUP
3900 Delancey Street
Philadelphia, PA, 19104

CASE I: P15/141 JPC 4066260)

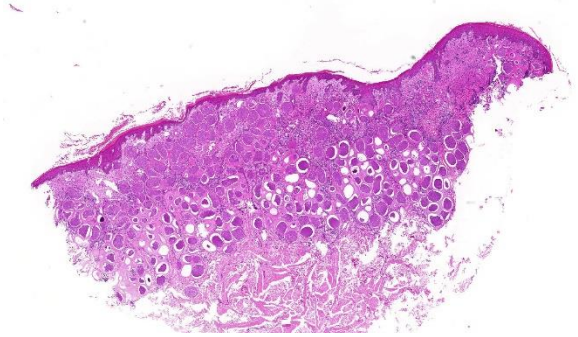
Signalment: 2.5-year-old, Aubrac bull, *Bos taurus*

History: The bull presented to Veterinary Teaching Hospital with history of chronic skin lesions of unknown reason.

Gross Pathology: Multifocal areas of alopecia

Laboratory results: PCR positive for *B. besnoiti*, ELISA positive for *B. besnoiti*

Microscopic Description:
Haired skin. The epidermis displays low to moderate, diffuse, orthokeratotic hyperkeratosis, moderate, diffuse, epidermal hyperplasia characterized by acanthosis and irregular rete ridge formation, rare, multifocal, individual, intraepidermal macrophages, eosinophils and neutrophils (exocytosis), and rare, multifocal, discrete, shrunken, hypereosinophilic keratinocytes with pyknotic nuclei (apoptosis). The superficial dermis and a variable part of the adjacent deep dermis are markedly and diffusely expanded by a moderate, coalescing, perivascular, and nodular to diffuse infiltration of macrophages, plasma cells, lymphocytes, eosinophilic granulocytes and to a locally variable extent also neutrophilic granulocytes, as well as a low to moderate, coalescing amount of bundles of fibroblasts and fibrocytes within a collagenous stroma (fibrosis). There is a marked coalescing to diffuse loss of



Haired skin, ox. The dermis is diffusely expanded by numerous apicomplexan tissue cysts ranging from 200-400um. (HE, 5X)

follicular and adnexal structures (alopecia). Within the superficial and upper deep dermis are numerous round protozoal cysts characterized by a diameter of ~ 250 µm, a ~ 12 µm thick, distinctly bordered, pale eosinophilic, hyaline outer capsule, a subcapsular ~ 10 µm thick ring of eosinophilic cytoplasm containing multiple fusiform nuclei, and a central round vacuole containing myriads of densely packed, ~ 4 µm in diameter, distinctly bordered, crescent-shaped bradyzoites with pale eosinophilic cytoplasm and a central, hyperchromatic nucleus. The dermal collagenous stroma, adjacent to the cysts show a mild, laminar zone of shrinkage and hypereosinophilia (compression atrophy).

Contributor's Morphologic Diagnoses: Haired skin. Dermatitis nodular to diffuse, granulomatous and eosinophilic, chronic, coalescing, moderate with numerous intradermal, intracellular, apicomplexan cysts (etiology consistent with *Besnoitia besnoiti)*, adjacent laminar compression atrophy, dermal fibrosis, alopecia, epidermal hyperplasia and orthokeratotic hyperkeratosis.

Contributor's Comment: Etiology: The apicomplexan protozoan parasite *Besnoitia besnoiti* (*B. besnoiti*; Family: *Sarcocystidae*) is the etiologic agent of bovine besnoitiosis (Synonyms: bovine cutaneous globidiosis, bovine cutaneous sarcosporidiosis, elephant skin disease of cattle) and has been described first by Cadéac in 1884.[1] The closely related Besnoitia species *B. caprae*, *B. bennetti* and *B. tarandi* induce a comparable disease mainly in goats, equids and wild ruminants, respectively (Table 1).[11]

Besnoitiosis is an endemic disease in the southern part of Europe, the subtropical areas of Asia, and sub-Saharan Africa, but has also been reported as an emerging disease in the central and northern part of Europe.[1,5,7,8] In endemic areas seroprevalence rates are ~ 50% whereas the incidence of clinical cases of 1-10% per year, as well as the mortality rate of less than 1% are obviously low. Prevalence and incidence may be higher in areas where the disease is emerging.[1,11]

B. besnoiti is commonly believed to have a heteroxenous life cycle, however until today only homoxenous transmission from intermediate to intermediate host has been shown experimentally. The most important intermediate host are cattle, but wildebeest, kudu and impala are also affected.[11] The epidemiological importance of wild ruminants in northern Europe is currently unknown. Although roe deer and red deer can be seropositive in areas of endemic bovine besnoitiosis, the strong cross reactivity of *B. besnoiti*, *B. tarandi* and *B. bennetti* prevents differentiation of these species employing serological methods.[6] Although many authors have suggested that the final host of *B. besnoiti* could be the domestic cat or wild carnivores, attempts to proof this hypothesis have been unsuccessful so far.[10] Therefore the final host of *B. besnoiti* remains currently unknown, as is true for most other species of the genus *Besnoitia* (Table 1).

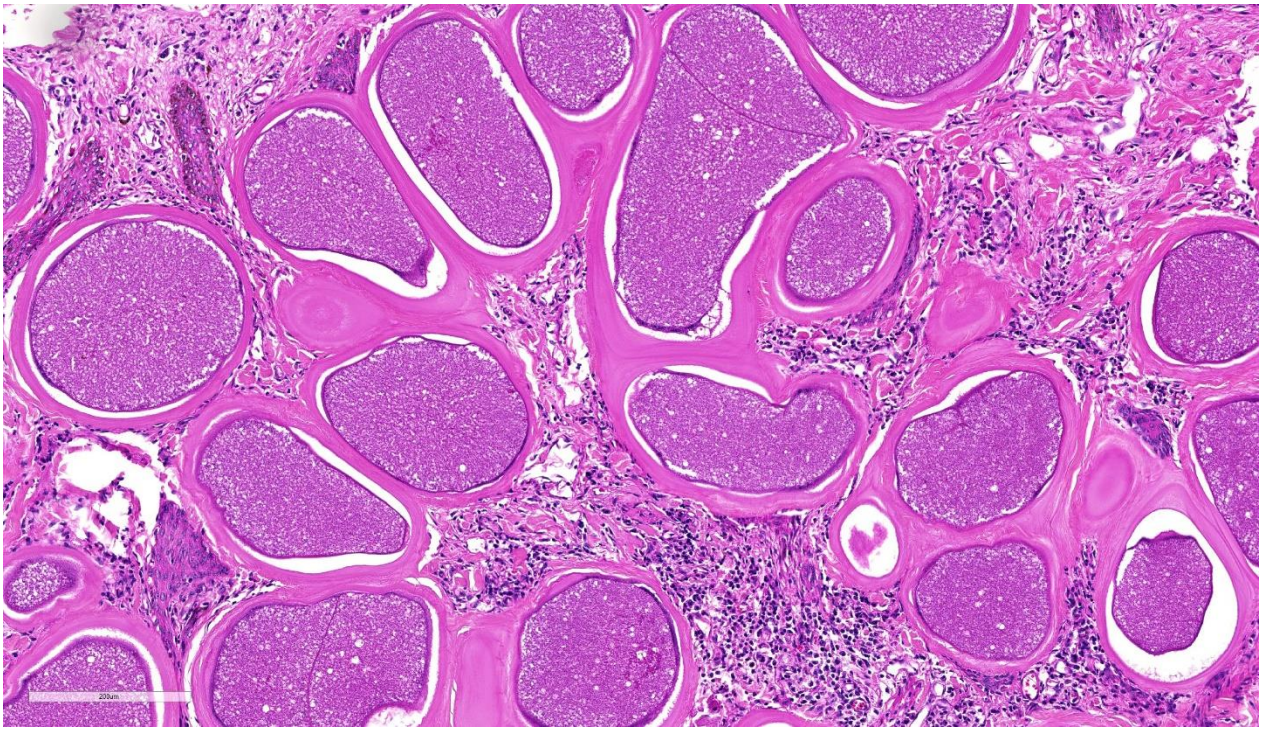
Whether transmission from the currently unknown definitive host to the intermediate host occurs in via oocytes shed in the feces remains unknown. In contrast, horizontal mechanical transfer between intermediate hosts by blood-sucking insects including tsetse flies (*Glossina brevipalpis*), tabanids (*Tabanocella denticornis, Atylotus nigromaculatus, Haematopota albihirta*), mosquitoes (*Culex simpsoni, Culex spp.*) and stable flies (*Stomoxys calcitrans*) has been experimentally proven and is suggested to be an important mode of transmission.[5]

Furthermore, transmission by other arthropods, iatrogenic transfer and direct contact including sexual transfer are possible other routes of transmission.[1]

Rapid asexual intracellular proliferation of *B. besnoiti* tachyzoites occurs mainly within the intermediate host´s endothelial cells during the acute phase after the first infection. This leads to vasculitis and thrombosis of capillaries and smaller veins, especially within the dermis, and subsequent generalized subcutaneous edema.[2,8] One to two weeks after the onset of the acute phase, the infection reaches the subacute to chronic stage which is characterized by evolving intracellular tissue cysts containing myriads of bradyzoites within vimentin-positive, MAC387-negative, mesenchymal cells (suggested to be fibroblasts or myofibroblasts), especially within the dermis and submucosa, as well as various other tissues.[8] A detailed description of the sequential steps of tissue cyst formation can be found in Langenmayer et al. (2015).[8]

The clinical disease can be subdivided into two phases. The acute phase ("anasarca phase") of bovine besnoitiosis occurs 11-13 days after infection and lasts for 6-10 days, and is characterized by fever, subcutaneous edema, lymphadenitis, conjunctivitis, nasal discharge, salivation, laminitis and depression.[2,8]

In contrast, the chronic phase (scleroderma phase) is characterized by macroscopically detectable tissue cysts in connective tissues, especially the pathognomonic pin-head sized cysts in the dermis and submucosa of conjunctiva, nasal cavity and vagina, as well



Haired skin, ox. Tissue cysts are surrounding by the thick hyaline wall ranging from 10 to 20um. Within cyst the wall, a thin rim of cell cytoplasm with peripheralized nuclei surrounds a parasitophorous vacuole contain innumerable zoites. Tissue cysts are separated by moderate numbers of lymphocytes, histiocytes, and eosinophils, as well as down projecting rete ridges of the hyperplastic epithelium (HE, 159X)

as lichenification, hyperkeratosis and alopecia.[5,8]

Overt clinical disease most often affects 2- to 4-year old adults and chronically infected animals may partially recover but are thought to remain infected for the rest of their lifes.[1] Although only a small percentage of less than 1% of the affected cattle die, bovine besnoitiosis may lead to major economic losses due to weight loss and decreased milk production, abortion, male infertility, and reduced value of the hides. Currently there are no therapeutic treatment options available. An attenuated live vaccine is available in some countries for prophylaxis.

Macroscopic changes in the acute phase of besnoitiosis are rather unspecific and include coalescing to generalized subcutaneous edema (anasarca), multifocal to coalescing, lymphohistioplasmacytic and eosinophilic perivascular dermatitis, dermal hemorrhages, generalized swelling of lymph nodes (lymphadenopathy), and swollen testicles (orchitis).[2,8]

The characteristic tissue cysts start to develop in the subacute stage concurrent with the decline of dermal edema. The tissue cysts become clearly macroscopically visible as pin-head sized, pearl white tissue cysts with a diameter of up to 1 mm within the conjunctiva and mucous membranes of the vagina, nose, pharynx and upper respiratory tract in the chronic phase of besnoitiosis.[8] The dermis exhibits multifocal to coalescing palpable indurations with a diameter of initially 3-5 mm and later up to 1 cm, especially at the teats, eyelids, neck and limbs, as well as hypotrichosis, alopecia, seborrhea, lichenification and partially erosions, exudations and crusts. Other affected tissues include the connective tissue of the subcutis, intermuscular fascia, mesentery and scrotum.[2,4,5]
Histologically, crescent-shaped 6-7,5 x 2,5-3,9 µm sized *B. besnoiti* tachyzoites with eosinophilic cytoplasm and a round basophilic nucleus may be visible within endothelial cells, blood and lymph vessels, and extracellular spaces during the acute stage. Notably, these tachyzoites are indistinguishable from *Neospora caninum* or *Toxoplasma gondii* by light microscopy.[2,4,8]

The *B. besnoiti* tissue cysts of the chronic phase exhibit a diameter of up to 600 µm, and a characteristic double-walled morphology which allows differentiation from the similar cysts of *Sarcocystis spp.* and *Eimeria spp.*[11] The outer 10-12 µm, pale eosinophilic, hyaline cyst wall is comprised of host-derived collagenous material and its outer surface blends irregularly into the surrounding connective tissue. The inner cyst wall is a thin pale gray-bluish band with distinct histochemical staining characteristics in between the outer cyst wall and the outer cell membrane of the host cell. The outer cyst wall stains blue with Masson´s trichrome and pale white with Giemsa stain, whereas the inner cyst wall stains pale white with Masson´s trichrome stain and deeply violet with Giemsa stain.[8] The large host cell is multinucleated and forms a peripheral rim of cytoplasm, which in turn encompasses the central parasitophorous vacuole. The parasitophorous vacuole is filled with thousands of 6,0-7,5 x 1,9-2,3 µm sized bradyzoites.[2] The cysts are often surrounded by a granulomatous and eosinophilic, nodular to diffuse dermatitis with fibrosis, compression atrophy of adnexa, and hyperkeratosis.[4,7] Occasional multinucleated giant cells of the foreign body type can be present in the inflammatory infiltrate.[8] Other lesions include focal or multifocal myositis, keratitis, periostitis, endosteitis,

lymphadenitis, pneumonia, periorchitis, orchitis, epididymitis, arteritis, perineuritis, and laminitis.[4,8]

A comprehensive ultrastructural description of the tachyzoites, bradyzoites and tissue cysts in bovine besnoitiosis has been published recently.[9]

The gold standard for the diagnosis of bovine besnoitiosis seems to be the histologic demonstration of the pathognomonic *Besnoitia spp.* tissue cysts. Obvious microscopic differentials for *B. besnoiti* include the indistinguishable cysts other species of the genus *Besnoitia* as well as the confusable cysts of *Sarcocystis spp., Eimeria spp.,* and the sporangia of the fungal agents *Rhinosporidium seeberi*, *Emmonsia crescens*, *Sporothrix schenkii*, *Coccidioides immitis* and *Loboa loboi.*[3]

Immunohistochemistry can be used to differentiate *B. besnoiti* bradyzoites and cysts from those of *Toxoplasma gondii*, *Neospora caninum* and, although with minor cross reactivity, also *Sarcocystis spp.*[8] Due to the serological cross reactivity of the various species of the genus *Besnoitia*, it seems reasonable that it is also not possible to differentiate between these species using immunohistochemistry.[6] Therefore molecular genetic methods are the method of choice if a diagnosis at the species level is needed.[11]

Table 1. Species of the genus Besnoitia and their main hosts.

Species name	Main intermediate host	Main final host
Besnoitia besnoiti	Cattle, kudu, blue wildebeest, impala	?
Besnoitia caprae	Goat	?
Besnoitia tarandi	Reindeer, caribou, mule deer, roe deer, muskox	?
Besnoitia bennetti	Horse, donkey, mule, (zebra)	?
Besnoitia jellisoni	Mouse, rat, other rodents	?
Besnoitia akodoni	Montane grass mouse	?
Besnoitia oryctofelisi	Rabbit	Cat
Besnoitia darlingi	Virginia opossum	Cat
Babesia neotomofelis	Southern planes woodrat	Cat
Besnoitia wallacei	?	Cat

Data based on the review of Olias et al. (2011).[11]

Contributing Institution:
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, 17493 Greifswald-Insel Riems, Germany (www.fli.bund.de)

JPC Diagnosis: Haired skin: Dermatitis, lymphoplasmacytic, histiocytic, and eosinophilic, diffuse, moderate, with numerous apicomplexan cysts and mild diffuse epidermal hyperplasia and hyperkeratosis.

JPC Comment: The contributor has provided a thorough discussion of this disease which is currently considered of great economic importance in cattle in Europe. Historically, the disease was first described by Cadeac in 1884 and named "Telephanitiasis el l"anasarque du bouef.' Although initially misdiagnosed by Besnoit

(a professor of veterinary medicine in Toulouse) and Robin as a species of *Sarcocystis* (*S. besnoiti*) in 1912, and in 1916, Franco and Borges proposed the name *Besnoitia besnoiti*. The disease is gone by many names, including bovine subcutaneous globidiosis, bovine cutaneous cervical spongiosis, and elephant skin disease of cattle.

While the definitive host of *B besnoiti* remains unproven, domestic and wild felines have been shown to be the definitive host for *Besnoitia* species of rodents. A recent publication by Verma et al. has identified the bobcat (*Lynx rufus)* as the definitive host of *B. darlingi*, a species which parasitizes the opossum.

While orchitis and a marked decrease in fertility in bulls has been well documented this disease, chronically infected cows may still become pregnant, and give birth, with no reports to date of vertical transmission of *B. besnoiti.* Subsequent rearing is problematic, as the disease causes a negative impact on milk production, as well as the calf's nursing opening painful wounds on infected teats.[2] Another clinical finding in chronic cases of disease in cattle include laminitis, likely as a result of interference with dermal vasculature and pressure put on epidermal laminae by the presence of tissue cysts. Inappropriate weight bearing ultimately results in rotation of P3 and the development of sole ulcers.[5]

On a microscopic level, the composition of the two cysts walls, the inner cyst wall (which lies outside the host cell membrane), and the hyalin outer cyst wall (composed of type I collagen as evidenced by a deep blue staining on a Masson's trichrome and orange birefringence on picrosirius red), was recently described by Langenmeyer et al. The pale white staining of the ICW on Masson's and a blue-green color in picrosirius red/Alcian blue strongly suggests that the ICW is composed of elements of the extracellular matrix. This interesting histochemical discovery further supports myofibroblasts as the cell of origin for tissue cysts as myofibroblasts are capable of producing both extracellular matrix components as well as type I collagen.

To date, no member of the genus *Besnoitia* have been demonstrated to elicit disease in humans.

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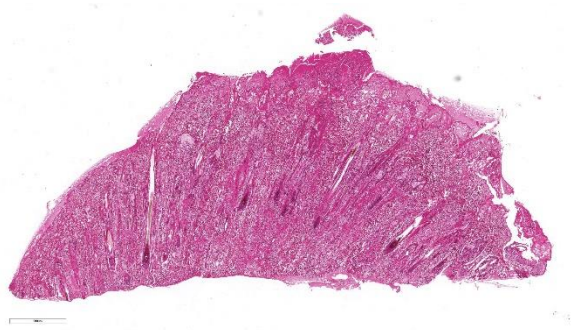
CASE II: Case #1 (JPC 4085967)

Signalment: 16-month-old, female, European shorthair cat



Haired skin: At subgross examination, the submitted section of skin is ulcerated and the dermis is hypercellular. (HE, %x)

History: The cat showed a non-healing wound at the right forelimb over a few weeks. A treatment with NSAID´s and antibiotics didn't show any improvement. Therefore a biopsy was taken for microscopic investigation.

Gross Pathology: The specimens showed a moderate alopecia and some crusts.

Laboratory results: Molecular biological examination:

- PCR for Cowpox virus: positive

Microscopic Description:

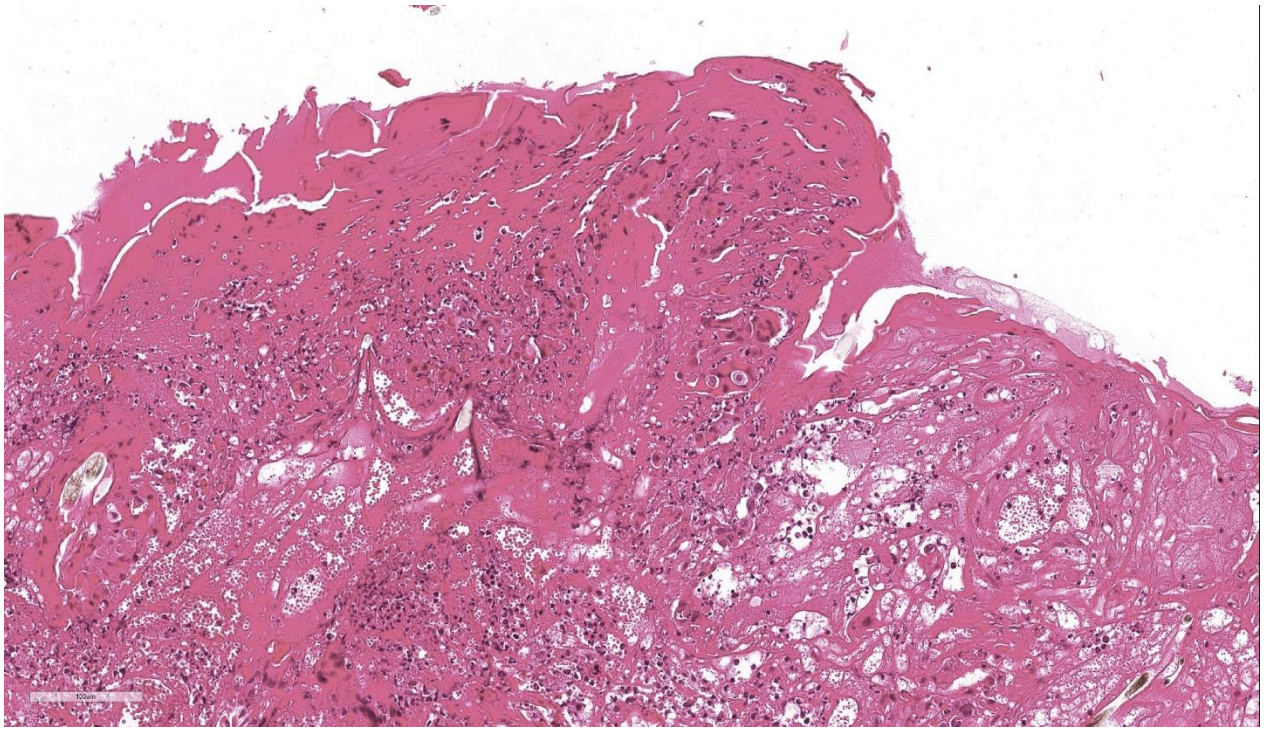
- Epidermis: diffusely covered by plasmatic components; nearly diffuse severe erosions and ulcerations multifocal with serocellular crusts containing a high mount of neutrophils; multifocal moderate intracellular edema up to ballooning degeneration and necrosis of the keratinocytes; multifocal eosinophilic intracytoplasmic inclusion bodies within the keratinocytes
- Dermis: severe infiltration with marked numbers of neutrophils, some lymphocytes, plasma cells and macrophages; multifocal severe necrosis with degenerate neutrophils

and cellular and karyorrhectic debris; multifocal moderate hemorrhages

- Adnexa: disseminated severe perifollicular infiltration with marked numbers of neutrophils, some lymphocytes, plasma cells and macrophages, multifocal with complete destruction of the adnexa; multifocal eosinophilic intracytoplasmic inclusion bodies within the keratinocytes of the hair follicle

Contributor's Morphologic Diagnoses: Haired skin: Dermatitis, purulent and necrotizing, erosive and ulcerative, severe, with eosinophilic intracytoplasmic inclusion bodies within keratinocytes

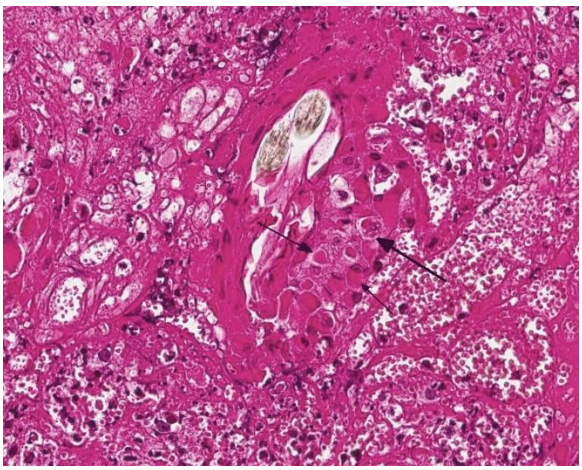
Contributor's Comment: Etiology: The cowpox virus is a member of the genus Orthopoxvirus in the family Poxviridae. The reservoir hosts are rodents from which the virus spreads to other animals, for example cows, cats (including large felids), zoo animals (rhinoceroses, elephants, okapis) and humans.[9] In cats, it usually causes a regional viral dermatitis and is often a more severe disease than in cattle or humans. There is an increased incidence in the autumn, which may reflect the increased size and activity of wild rodents in this time of the year.[1]



Haired skin, cat. There is diffuse full thickness necrosis of the epithelium of the superficial dermis whitch extends into the follicular epithelium. Large pools of serocellular exudate expand the intercellular space. The dermis is infiltrated by large numbers of degenerate neutrophils admixed with cellular debris, hemorrhage, edema, and fibrin. (HE, 182X)

The primary lesion after cutaneous infection is usually an ulcerating, erythematous, crusted macule or plaque on the head, neck, or forelegs that occurs after 3-6 days.[9] After a viremia, pyrexia, anorexia and depression widespread secondary lesions develop within 10 days to a few weeks.[1,5] Like the primary lesion, these first appear as small erythematous macules, ultimately forming ulcerated papules. Sometimes, vesicles are found on the tongue, the mouth or inner aspect of the pinna. Approximately 20 per cent of affected cats show a mild infection of the upper respiratory tract. After three weeks the cutaneous scabs dry and fall off.[2] Typical microscopic findings are ulceration, serocellular crusts, ballooning degeneration of keratinocytes in epidermis and hair follicles with large intracytoplasmic eosinophilic inclusions and a secondary necrosis and infiltration with inflammatory cells.[6]

There are reports about some cases with more severe clinical signs, especially with a severe necrotizing or interstitial pneumonia, necrotizing rhinitis and/or generalized skin lesions. These are either described as atypical infections or are associated with secondary bacterial infection or an underlying immunosuppression like FIV, parvovirus and FeLV infections, debilitative disease or treatment with corticosteroids.[8,12,13,14] Macroscopic differential diagnoses for this condition are facial and nasal dermatitis and stomatitis associated to feline herpesvirus 1, cutaneous lymphoma, autoimmune dermatoses, such as pemphigus foliaceus, cutaneous bacterial, fungal or parasitic infections and physicochemical injuries.[7,8] Because of the intracytoplasmic inclusion bodies, there are no histological differential diagnoses.



Furthermore, cowpox virus infection is zoonotic and there exist several reports of the virus being transmitted from cats to human beings.[8,14,15] Lesions are similar to those in cats and are usually associated with a cat scratch.[8,14]

Contributing Institution:
Institut für Veterinär-Pathologie, Veterinärmedizinische Fakultät Leipzig, Universität Leipzig,

Germany (http://www.vetmed.uni-leipzig.de/ik/wpathologie)

JPC Diagnosis: Haired skin: Dermatitis, necrotizing, diffuse, severe with epithelial ballooning degeneration,and numerous intracytoplasmic viral inclusions.

Haired skin, cat. There is diffuse full thickness necrosis of the epithelium of the superficial dermis which extends into the follicular epithelium. Large pools of serocellular exudate expand the intercellular space. The dermis is infiltrated by large numbers of degenerate neutrophils admixed with cellular debris, hemorrhage, edema, and fibrin. (HE, 182X)

JPC Comment: Few medical or veterinary students have not heard the story of Edward Jenner, the milkmaids, and his discovery of the correlation between inoculation with cowpox and resistance to smallpox. In a time in which myths fall quickly and hard, the long-held attribution of one of the early

vaccine discoveries has been recently called into question. A 2018 article in the *New England Journal of Medicine*[3] ascribes the important observation to the physician towhom Jenner was actually apprenticed, Dr. John Fuster, who two years earlier in 1768, began inoculating people against smallpox." It was a conversation with a farmer that Fuster inoculated ineffectually that led to the connection between smallpox and cowpox, when the farmer exclaimed, "I have had the Cow Pox lately to a violent degree, if that's any odds." Jenner confided about the finding to a friend later that year, but would not travel to London to gain fame as the inventor of the vaccine until 1770.

Even the identity of the virus used by Jenner et al. has been called into question. While the story of the "unrivaled" (i.e., unpocked) complexions of the milkmaids in the area at that time is legend, even the use of the cowpox virus in early vaccinations has been called in to question. The widespread exchange and mixing of vaccines and "lymph" in the US in the early 1900s resulted in vaccines today that likely would contain viral determinants of many vaccines in use around the world at that time. Interestingly, no trace of cowpox has been identified in vaccines tested from that time or today's derivatives. The active virus appears to be the vaccinia virus, closely related to cowpox, but instead isolated from horses.[5]

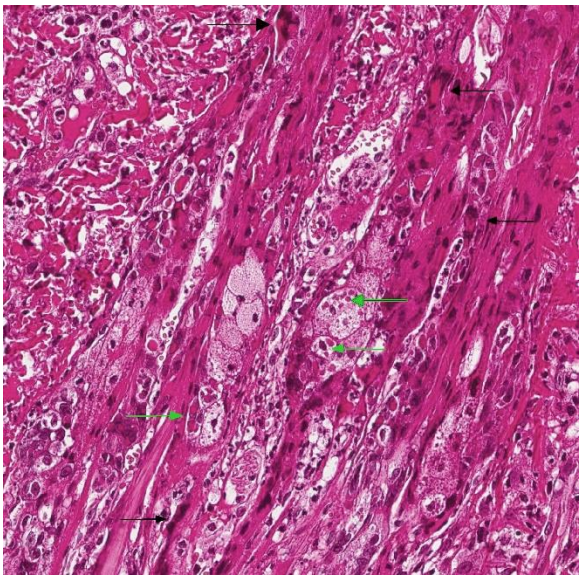
Today, cowpox viruses are distributed throughout Asia and Europe, except for Ireland. Wild rodents, predominantly voles, are the reservoir of these viruses; hunting cats are accidentally infected by their prey.[9] Historically, cattle (likely infected by rodent contact) have been the primary source for zoonotic infections; however, within the last decade, cats have been primarily described as the source of human infections. Wild rodents are the primary source for the cat; pet rats have been rarely been incriminated in human infections.[9]

While generally considered to be a cutaneous infection, fatal generalized systemic infections with cowpox virus have been seen in immunosuppressed cats, as well as a single human case report following immunosuppressive corticosteroid therapy. The effects of concurrent immunosuppressive viruses such as file T virus, retrovirus, parvovirus may also exerts adverse effects by promulgated concurrent bacterial infections.[9]

Haired skin, cat. Within surviving follicular epithelium, there are numerous multinucleated viral syncytia (black arrows). Degenerating sebaceous epithelium also contains intracytoplasmic viral inclusions. (HE, 351X)

A number of atypical cases of cowpox in cats have been identified in the literature. Necrotizing and proliferative pneumonia has been seen in the absence of cutaneous lesions in domestic cats and wild felids. A series of cases in cats in which the cutaneous lesions were present on the hindlimbs or tail was reported by Jungwirth et al.[10] In a Denmark zoo which has reported seasonal recurrence of cowpox virus outbreaks in captive cheetahs, two of nine (22%) died of generalized disease or pulmonary infection.[17]

Finally, a fatal outbreak of a novel orthopoxvirus was reported in 2017, in



which phylogenetic analysis revealed the virus to be distantly related to cowpox, and more closely related to ectromelia virus.[4] Autopsied macaques demonstrated interstitial pneumonia with necrosis of bronchial epithelium; lymphoid necrosis of the spleen and lymph node, and numerous ulcers in the skin and upper GI tract. Rodents in the facility demonstrated serologic (IGG) to the putative poxvirus, but the virus was not isolated from trapped rodents. A subsequent report of cutaneous infection by a virus within the same cluster as this putative novel orthomyxovirus was subsequently reported.[11]

The attendees noted the presence of numerous multinucleated epithelial cells, which are not commonly seen in poxviral infections; their significance or etiology in this case is not apparent. The large areas of profound necrosis of the epidermis led some attendees to question whether vascular lesions, such as may be seen in other orthopoxviral infections, such as ectromelia, might have been present.

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CASE III: E2337 (JPC 4090973)

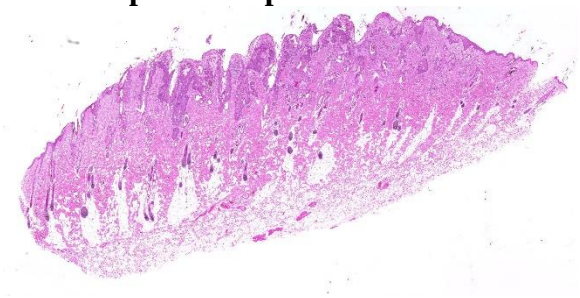
Signalment: 13-year old female domestic European shorthair cat (*Felis catus*)

History: The affected skin displayed a plaque-like lesion with irregular edges.

Gross Pathology: The tissue sample submitted for histopathological examination had an extension of 1,2 cm consisting of skin and subcutis.

Laboratory results: None given.

Microscopic Description:



Haired skin, cat. Higher magnification demonstrates the profound epidermal hyperplasia with nodular downward growth of rete ridges. Erosion of the epidermis has resulted in marked intercellular edema, and there is marked dermal inflammation. (HE, 88X)

Haired skin: There is an area of irregular epidermal hyperplasia with formation of rete ridges, also affecting the hair follicle infundibulum; part of the lesion is covered by a serocellular crust. There is a mild hyperkeratosis and mild to severe hyperpigmentation. In most areas, the basement membrane is intact with neoplastic cells confined to the epidermis with dysplasia of all layers with loss of the polarity of the cell nuclei and loss of the normal stratification of the keratinocytes. Some keratinocytes are small with hyperchromatic nuclei, others are rather large with vesicular nuclei.

Groups of cells are dorsoventrally protracted and bent in one direction, exhibiting a "wind-blown" appearance. Nuclei are large, round to oval, centrally placed, and vesicular with finely stippled chromatin and one to

two prominent round magenta nucleoli. There is a mild to moderate anisocytosis and anisokaryosis. The number of mitotic figures range from 0 to 4 per high power field. Multifocally, dark basophilic round structures are present within the neoplastic cells, interpreted as apoptotic bodies. In the dermis a mild, perivascular infiltration of neutrophils, lymphocytes and macrophages is present and few mast cells are observed. Multifocally there is moderate dermal fibrosis.

Contributor's Morphologic Diagnoses: Epidermal hyperplasia and dysplasia, focally extensive, severe (Bowenoid *in situ* carcinoma – BISC)

Contributor's Comment: The morphologic findings are compatible with a Bowenoid *in situ* carcinoma (BISC) or Bowen-like disease, an uncommon premalignant lesion in middle-aged to old cats, that has been rarely reported in dogs.[5,6]

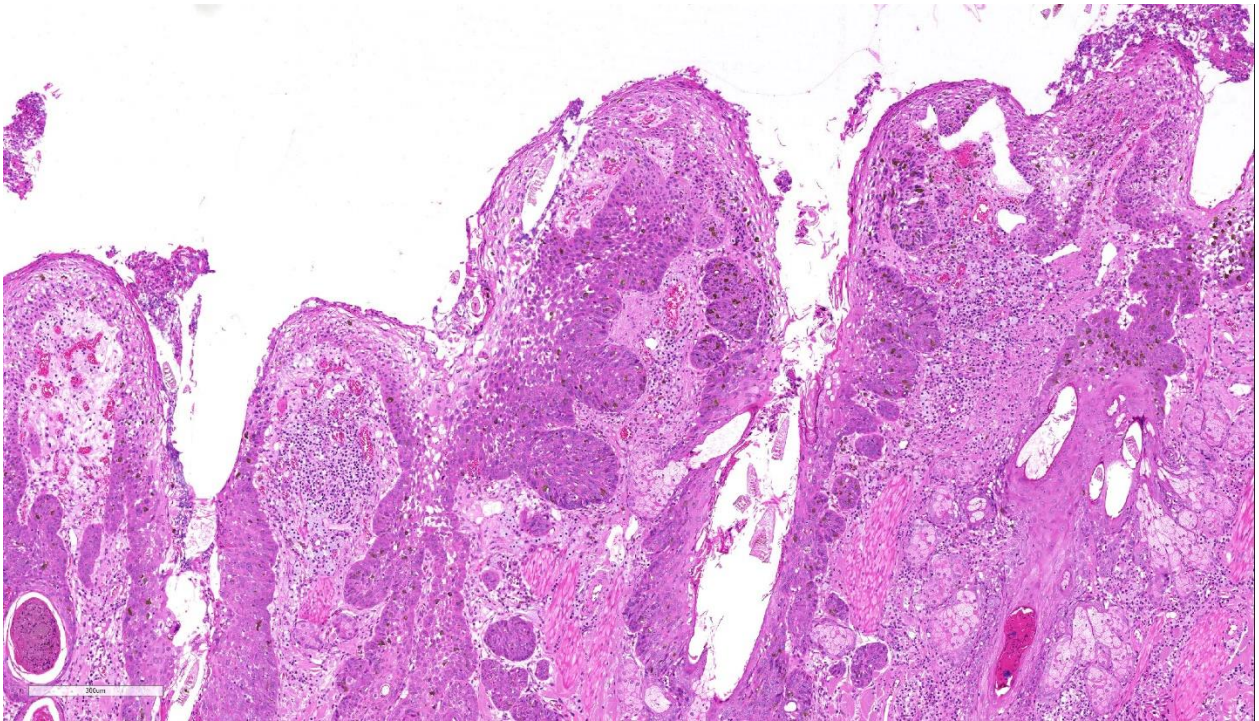
Grossly, irregular, slightly elevated to heavily-crusted plaques and verrucous or papillary lesions up to 5.0 cm in diameter are found on haired, pigmented or non-pigmented skin at any site of the body.[3,5,6] Usually, multiple lesions occur, but also solitary lesions are seen infrequently.[4] Lesions are reported to be chronic, not painful, and only mild pruritus is noted.[3] There is neither a causal correlation to sunlight exposure nor a breed or sex predilection.[5,6]

Several studies suspect that papillomavirus infection may be associated with feline BISC *in situ*.[3,7,8] In two studies, papillomavirus-antigen was detected in 11% and 48% of BISC *in situ*, respectively, using immunohistochemistry.[7,8]

Contributing Institution:
Institute of Veterinary Pathology at the Centre for Clinical Veterinary Medicine LMU, Munich
Veterinaerstr. 13; 80539 Munich Germany

JPC Diagnosis: Haired skin: Bowenoid squamous cell carcinoma *in situ*.

JPC Comment: John Templeton Bowen, MD was a quiet professor of dermatology who spent his days at Harvard Medical School dodging patients, preferring to read slides. In 1912, he published a paper on a particular form of precancerous chronic dermatosis. As his proposed name of



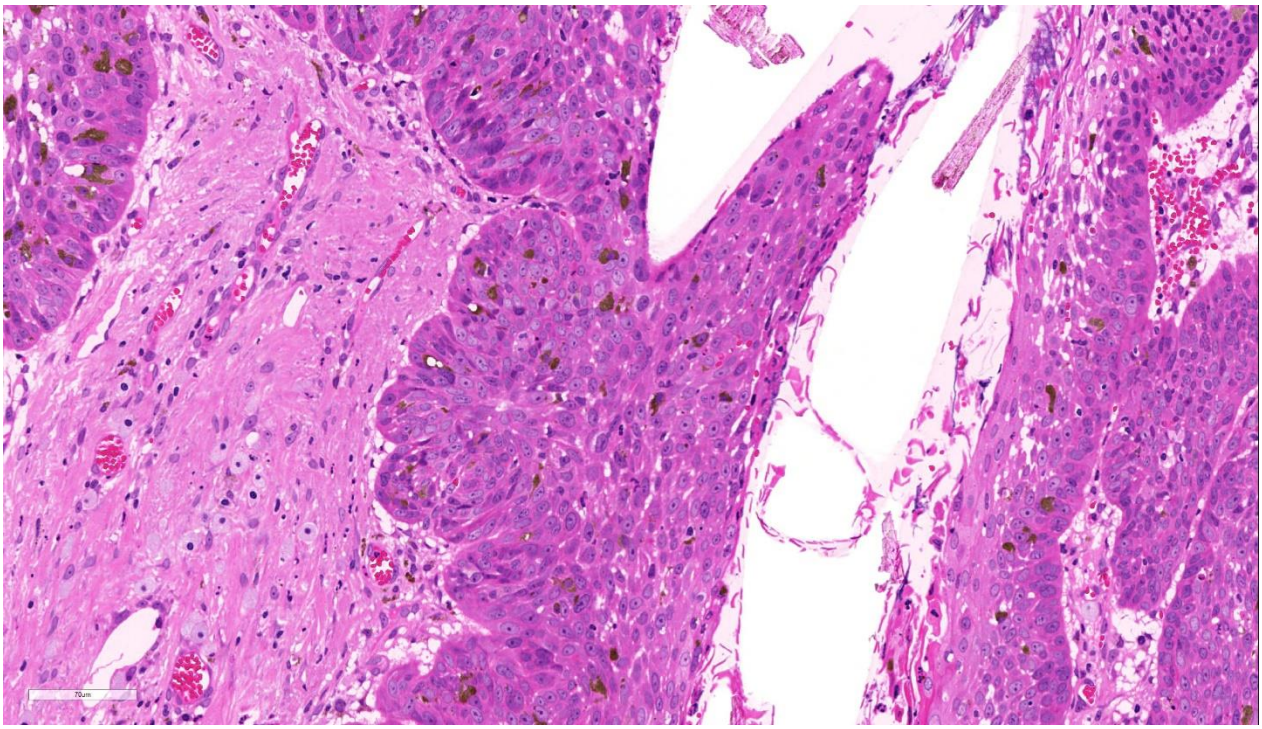
Haired skin, cat. At subgross magnification, there is marked thickening of the epidermis and follicular epithelium within the center of the section. (HE, 88X)

"dyskeratosis lenticularis et discoides" didn't appear to catch on widely, Dr. Jean Darier (who already had Darier's disease named after him, so he wasn't really in the game anymore) suggested in his rather aptly named 1914 textbook on dermatology, *A Textbook on Dermatology,* that this condition simply be called Bowen's disease.[1] Bowenoid papulosis, a proliferative lesion of the genitals resulting from infection by human papillomavirus 16 or 18, was named in Bowen's honor in 1977, 37 years after his death, when he couldn't refuse.

In humans, Bowen's disease is the eponym commonly applied to cutaneous squamous cell carcinoma *in situ.* It appears as a sharply marginated erythematous scaly or verrucous, often pigmented plaque. It may be appear in sun-exposed areas of skin, or other areas where it may be associated with HPV infection or exposure to inorganic arsenic.[9]

In the cat, Bowenoid *in situ* carcinoma (BISC) presents as one or multiple thickened areas of the epidermis, which may present as a thick plate of keratin due to its propensity for overlying orthokeratotic or parakeratotic hyperkeratosis.[4] This keratinization may extend into follicles which may become dilated and plugged with keratin debris. Over time, BISCs may progress to a nodular mass which bulges into the underlying dermis.[4]

The histologic features of the risks are characteristic. Poorly lesions display basal cell crowding and a loss of nuclear polarity and stratification. Cells within the stratum spinosum and even more superficial levels are enlarged with prominent nuclei and nucleoli are more consistent with cells of the basal layer. Mitoses may be present at any level in the dermis. Alternatively, as seen in this case discs may demonstrate a more basaloid pattern, with proliferation of epithelial cells with minimal cytoplasm and



Haired skin, cat. Within the affected epidermal and follicular epithelium, there is inappropriate maturation (dysplasia) with a similarity of nuclei at all levels, lack of keratohyaline granules, and mitotic figures at all levels of the epidermis. (HE, 137X)

large often hyperchromatic nuclei. Melanization of these of the epithelial cells as well as macrophages in the underlying dermis is often seen in these lesions.[4]

Feline papilloma virus has been repeatedly isolated from lesions in this type, and cytopathic effects, such as koilocyte formation, associated with papillomavirus infection may be seen in early lesions. Similar changes are rarely seen in advanced lesions.[4]

Cats that develop one BISC are likely to develop additional lesions over time. Histologic differentials include invasive squamous cell carcinoma and actinic keratosis. The differentiation between a BISC and a SCC is made by careful examination to insure that all cells are confined within the basement membrane. Differentiation between actinic keratosis and a BISC may be more difficult unless papilloma virus-induced cell changes are present. As opposed to the BISC, actinic keratosis demonstrates random loss of nuclear polarity of the basal cells only, a poorly defined interface between normal and affected epidermis, and most importantly a lack of thickening and dysplasia of the follicular infundibulum.[4]

The association between feline papilloma virus infection in the cat in the development of BISC is well-known and consistent with an emerging body of knowledge associating papillomavirus infection and malignant transformation of epithelial neoplasms in cats and many other species. In the cat, *Felis catus* papilloma virus 2 and 3 (FcPV-2 and -3) have been isolated from BISC lesions. Other lesions associated with feline papillomavirus infection including feline viral plaques (FcPV-1 and -2) and FcPV-2 been detected not only in BISCs but in up to 50% of invasive SCC lesions in this species. Feline sarcoid is a rare neoplasms of the skin of the nose limbs, or digits of young to middle-aged cats; however, the papillomavirus that has been isolated from these lesions has extensive similarity in the DNA to bovine papillomavirus 1 and 2 and may represent a novel bovine papilloma virus.

The moderator discussed the requirements and methods for differentiation between papillomavirus-induced lesion and similar lesions that might be induced by actinic damage. The moderator suggested that the cellular proliferation extending deep into hair follicles as well as the bulbous outgrowth of neoplastic cells perpendicularly outward from the affected follicles is more suggestive of viral induction than actinic induction.

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CASE IV: 16-859 ((JPC 4084013)

Signalment: 8-year-old castrated male domestic shorthair cat, *Felis catus*

History: Presented with a 1cm mass around the right lower canine. The referring veterinarian removed the mass, tooth, and part of the mandible.

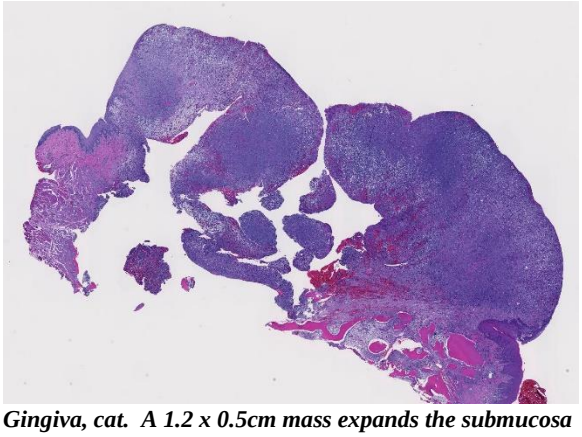
Gross Pathology: None given.

Laboratory results: None given.

Microscopic Description:
There is almost diffuse gingival ulceration with necrosis of the exposed lamina propria, fibrin deposition, and neutrophil infiltration. Underlying this there is early granulation tissue proliferation that blends into a deeper, poorly-demarcated population of plump spindle cells arranged in streams and admixed with large numbers of multinucleated giant cells. Cells are occasionally separated by amorphous eosinophilic material (presumed osteoid). The nuclei have finely stippled chromatin and a central prominent nucleolus. This area is surrounded by fibrosis with scattered lymphocytes and plasma cells. Occasionally there are central areas of necrosis with hemorrhage, neutrophils, and rarely a raft of filamentous bacteria is present (in some slides). At the periphery of the mass there are fragments of pre-existing lamellar bone with varying amounts of woven bone. Bone fragments are lined by primarily by osteoblasts with rare osteoclasts in Howship's lacunae. There are also tooth fragments surrounded by fibrosis. The remaining gingival epithelium is hyperplastic.

Contributor's Morphologic Diagnoses: Gingiva: Peripheral giant cell granuloma

Contributor's Comment: Peripheral giant cell granulomas were previously referred to as giant cell epulides.[7] These are ulcerated masses composed of a poorly demarcated proliferation of fibroblasts and blood vessels (resembling granulation tissue) with prominent multinucleated giant cells. Osteoid-like material may be present. Cellular pleomorphism, mitoses and anaplastic cells are absent. Unlike the fibromatous epulis and acanthomatous



Gingiva, cat. A 1.2 x 0.5cm mass expands the submucosa and elevates the overlying ulcerate mucosa. (HE, 5X)

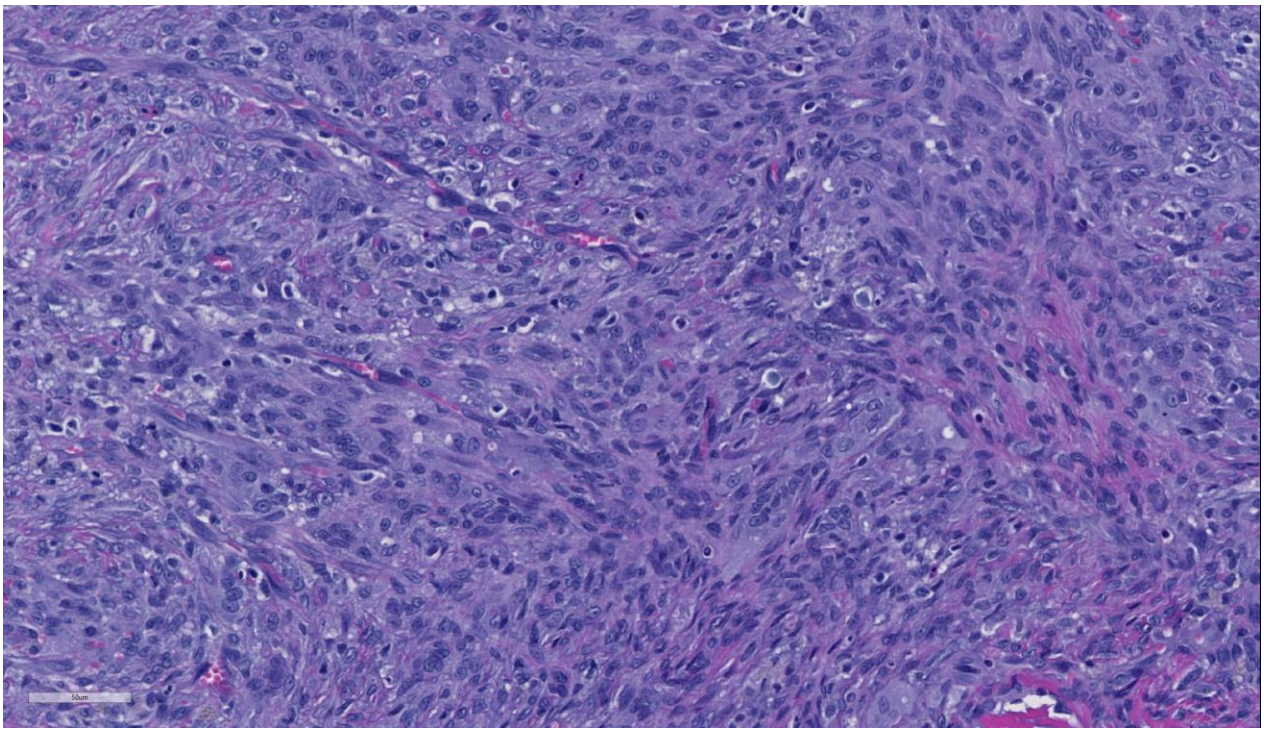
ameloblastoma, these tumors lack an epithelial component, although the gingival epithelium adjacent to the areas of ulceration is, as expected, hyperplastic.[1]

In cats, peripheral giant cell granulomas (giant cell epulis) are the second most common gingival tumor (28.8%) after fibromatous epulis (57.7%).The reported range for age of onset is 4-19 years. Compared to the fibromatous epulis, giant cell epulides are more likely to be ulcerated, grow more rapidly, rapidly recur following excision, and are more likely to result in the death or euthanasia of the cat.[1]

Immunohistochemical stains have shown the multinucleated giant cells to be positive for vimentin, tartrate-resistant acid phosphatase (TRAP; osteoclast marker) and the polyclonal antibody receptor activator of nuclear factor κβ (RANK; involved in osteoclast maturation)[3] while being negative for smooth muscle actin, MIB-1 (proliferation marker) and factor VIII. These findings suggest an osteoclastic, as opposed to macrophage, origin for the multinucleated giant cells.[1]

Peripheral giant cell granulomas are regarded as hyperplastic (reactive) and have occurred at sites of tooth extraction.[7]

Contributing Institution:
University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences http://www.vet.utk.edu/departments/path/index.php



Gingiva, cat. The lesion is composed primarily of plump fibroblasts forming tightly packed short streams and bundles oriented in various planes. (HE, 400X)

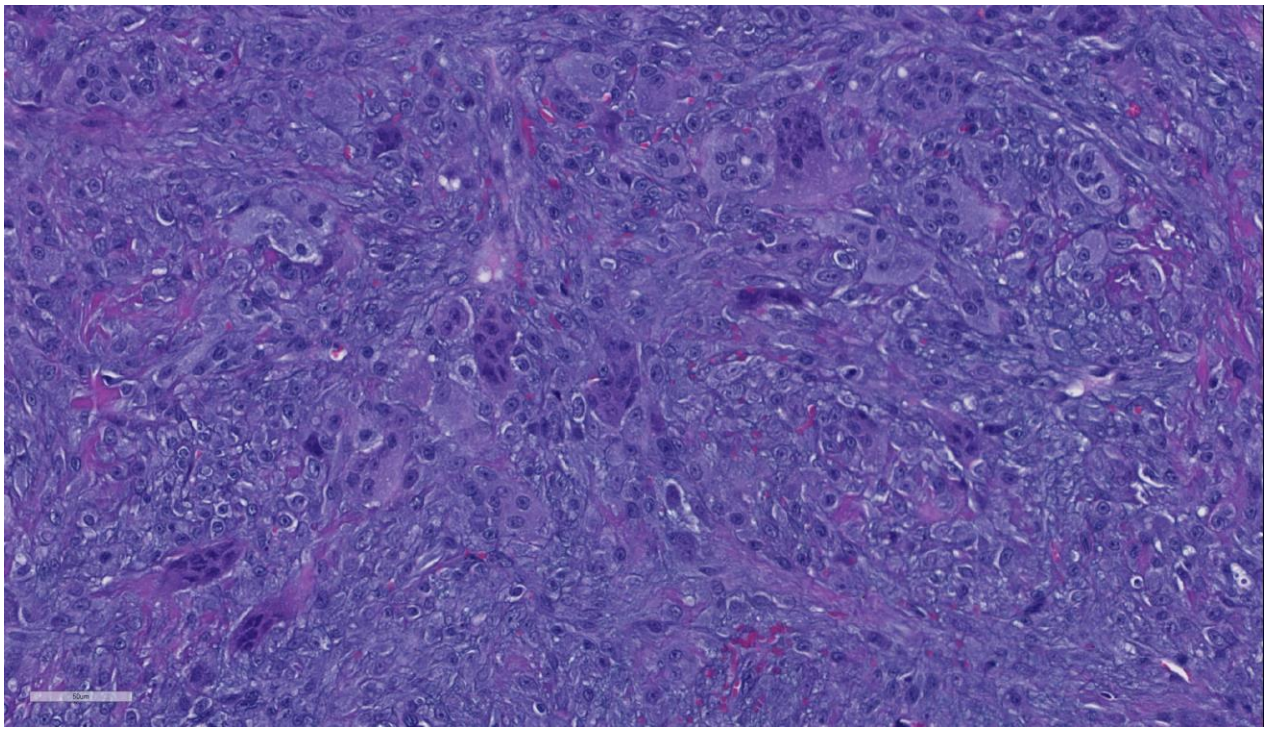
JPC Diagnosis: Gingiva: Peripheral giant cell granuloma.

JPC Comment:

The contributor does an excellent job of describing this poorly-named reactive lesion. The term "peripheral giant cell granuloma" has been lifted from the human literature, although this lesion bears no resemblance to a true granuloma. The term "peripheral" is used to differentiate this lesion from its identical intraosseous or "central" counterpart. It has been classified previously as an epulis[3], another relatively non-specific term for a "tumor-like mass on the gingiva" which also appears to be falling from common usage today.

in the human and tends to be seen more commonly in females than in males. . The appearance of multinucleated giant cells is unusual and the reason for the presence of this cell type remains unknown; they appear to be nonfunctional in the usual sets of either phagocytosis or bone resorption. Islands of metaplastic bone may occasionally be seen in this lesion.

A number of peripheral giant cell granulomas have been published in the dog as well[2,4] An article by DeSoutter et al.[4]



Gingiva, cat. Numerous multinucleated polygonal cells are scattered throughout the lesion. (HE, 400X)

In the human, where this lesion is best defined, it represents an exuberant reparative response to local trauma or irritation. These lesions are seen exclusively in the gingiva and presumably arises from the periodontal ligament or periosteum. They typically present as red-blue, broad-based masses in the gingiva around the incisors and premolars (a location that is shared in the cat and dog as well).[5] They are seen at any age

demonstrates an almost identical lesion in the area of the incisors or premolars (the characteristic lesion in humans as well) described as an "classic" peripheral giant cell granuloma, as well as a number of open "collision" peripheral giant cell granulomas, with features of both a peripheral giant cell granuloma and a fibromatous epulis of periodontal ligament origin. In contrast to the lesion in cats, peripheral giant cell

granulomas in the dog behave more like fibromatous epulides of periodontal ligament origin and seldom recur after excision. Multinucleated cells in the canine lesion also exhibits positive staining with tartrate-resistant acid phosphatase (TRAP).

The ultimate diagnosis of this case engendered spirited discussion. A number of attendees identified osteoid amidst the spindle cell population, favoring a diagnosis of osteosarcoma in this case. Consultation from the oral pathology human subspecialists confirmed the lesion to a peripheral giant cell granuloma; the presence of osteoid is not uncommon in their experience. The presence of large islands of dental and woven bone in one area of the section, was attributed to products of the extracted tooth and section of manible surrounding it..

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