Joint Pathology Center
Veterinary Pathology Services
Wednesday Slide Conference
2013-2014
Conference 1
11 September 2013

CASE I: 12-236 (JPC 4032712).

Signalment: 3-year-old male Labrador Retriever cross dog, (*Canis familiaris*).

History: The dog presented to the referring veterinarian with a one-day history of inappetance, unusual behavior, ataxia, falling over, vocalizing, incontinence, and apparent pain in the hips and back. The dog was hospitalized with rapid progression of clinical signs including rigidity of all four limbs, profuse salivation, opisthotonos, and nystagmus. Pentobarbital was administered. The dog later became unresponsive to sound and touch, and was found dead in the cage the next morning. A necropsy was performed by the referring veterinarian. Gross lesions were not observed.

Gross Pathology: Seven sections of brain were submitted in formalin for examination. There was moderate dilation of the lateral ventricles and marked dilation of the mesencephalic aqueduct and the most rostral aspects of the fourth ventricle. Occluding the lumen of the fourth ventricle there was a 0.8 cm, irregularly shaped, expansile, firm, opalescent mass. Multifocal areas of hemorrhage were noted in the adjacent cerebellum and brain stem.

Histopathologic Description: Brain, fourth ventricle: Filling approximately 80% of the fourth ventricle and in close association with the choroid plexus, there is a multilocular (not in all sections), expansile, unencapsulated and moderately well-defined mass. The wall of the mass is composed of well-differentiated stratified squamous epithelium which exhibits gradual keratinization through a granular cell layer resulting in the formation of multiple cysts filled with lamellar keratin. The cyst wall is discontinuous and free keratin spills into the lumen of the fourth ventricle. The squamous epithelium is supported by a moderate amount of fibrovascular stroma which frequently entraps the choroid plexus. In some areas the stroma is dense and brightly eosinophilic. Within the stroma there are modest numbers of inflammatory cells which mainly include lymphocytes and plasma cells. Infrequently there are stellate shaped cells with abundant dark brown, finely granular pigment which are interpreted to be melanocytes. Occasional hemosiderin laden macrophages are noted. There is marked rarefaction neuropil and hemorrhage. This is accompanied by numerous eosinophilic, spherical structures which are interpreted to be spheroids (swollen axons). Lining the lumen of the ventricle there are multiple round cells with abundant pale, foamy to slightly granular, eosinophilic cytoplasm, and one to multiple eccentric nuclei. These are interpreted to be gitter cells.

Intercellular bridges between keratinized cells are prominent. There are approximately five mitoses per 400x field with frequent bizarre mitotic figures. There is severe anisocytosis and anisokaryosis. Multifocally within the neoplasm there are large areas of necrosis, hemorrhage and a mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils and some macrophages. Many submucosal and subserosal vessels contain clusters of neoplastic cells (tumor emboli) as well as fibrin thrombi. Overlying the ulcerated mucosa, there is abundant fibrillar eosinophilic material (fibrin exudation) and hemorrhage, admixed with cellular and karyorrhectic debris (necrosis) and bacterial colonies. Throughout the mass, but especially along the serosa, there are multiple nodules or bands of abundant fibrous connective tissue (scirrhous response).

Contributor's Morphologic Diagnosis: Brain, fourth ventricle: Epidermoid cyst with hemorrhage, malacia, and acquired obstructive hydrocephalus.

Contributor's Comment: The central nervous system (CNS) develops from specialized ectoderm (neuroectoderm) which lies dorsal to the notochord throughout the axis of the embryo. Invagination of the neuroectoderm forms the neural groove and lateral processes referred to as the neural folds. Fusion of the neural folds results in the formation of the neural tube with the latter forming the ventricular system and central canal of the CNS. At the time of neural tube closure, the neuroectoderm separates from the surface ectoderm to form two distinct layers. The layer of nonneural ectoderm gives rise to structures such as the epidermis. Epidermoid cysts of the CNS are congenital lesions that are thought to be the result of inappropriate inclusion of this nonneural ectoderm at the time of closure of the neural tube.[4]

In humans, intracranial epidermoid cysts are a well-recognized entity and are thought to comprise up to 1.8% of all intracranial masses.[7] Epidermoid cysts of the CNS are uncommon in domestic animals with a handful of cases being reported in dogs, horses, mice, and rats.[2,4,9,10] In dogs, epidermoid cysts have been reported within the cranial cavity[4,6,11,12] and within the vertebral canal.[1,4] Intracranial masses are most common.

There are too few reports of intracranial epidermoid cysts in dogs to reliably identify a breed or sex predilection. Dogs with intracranial epidermoid cysts have ranged in age from 3 months to 8 years with the majority of dogs being less than 2 years old.[4] These findings may suggest a predilection for young dogs, and could be consistent with the presence of a congenital lesion. Intracranial epidermoid cysts are slow growing masses and in people typically do not cause clinical signs until fifth decade of life.[4] The slow, linear growing pattern of these masses may explain the wide age range reported in dogs for a purportedly congenital lesion.

Intracranial epidermoid cysts exhibit an expansile growth pattern through desquamation and accumulation of keratin.[7] While these lesions may be found as incidental findings at necropsy,[4] intracranial epidermoid cysts may cause clinical signs referable to compression of adjacent structures resulting in focal neurologic dysfunction or obstruction of CSF resulting in hydrocephalus.[4,6,11,12] In both humans and dogs, epidermoid cysts have a predilection for the caudal fossa which may be a reflection of the initial closure of the neural tube in the rhombencephalon. In dogs, intracranial epidermoid cysts have been reported in the fourth ventricle and cerebellopontine angle.[4] Based on their location in the caudal fossa, canine epidermoid cysts are frequently associated with vestibular and occasionally cerebellar signs.[4,6] In addition to the space occupying nature of these lesions, other sequelae reported in people include the development of chemical meningitis, and rarely malignant transformation to squamous cell carcinoma.[7,8] Malignant transformation has not been reported in dogs, and in the current case there was no evidence of squamous cell carcinoma in the sections examined.

Pathologic features of intracranial epidermoid cysts are similar to those that are routinely encountered in the skin. Microscopic examination reveals the presence of a cyst lined by stratified squamous epithelium

supported by connective tissue stroma and surrounding keratin. Portions of choroid plexus are often adhered to or are incorporated into the cyst.[4] Intracranial dermoid cysts have also been reported and also arise from the inappropriate inclusion of nonneural ectoderm in the CNS. Dermoid cysts can be differentiated from epidermoid cysts in that the former is lined by adnexal structures such as hair follicles, sebaceous glands, and sweat glands.[4] These features were not observed in the current mass favoring a diagnosis of epidermoid cyst over dermoid cyst.

JPC Diagnosis: Brain, 4th ventricle: Epidermoid cyst with granulomatous rhombencephalitis, encephalomalacia, edema and hemorrhage.

Conference Comment: Conference participants discussed the embryologic histogenesis and clinical signs of intracranial epidermoid cysts, as reviewed by the contributor in the above comments. Clinical signs of neurologic dysfunction are generally attributed to compression of adjacent neural structures[5]- indeed one of the more striking histologic features in this case is the degree of axonal degeneration and numerous, prominent spheroids noted within the adjacent neuropil. Cyst rupture with subsequent inflammation, known in human medicine as chemical meningoencephalitis, may also contribute to the clinical signs.[6] Although intracranial epidermoid cysts are more common (and of course, extracranial epidermoid cysts are the most common), intravertebral and intramedullary spinal cord cysts with progressive ataxia and paraparesis have also been described in dogs.[1,5]

Participants also examined dermoid cysts as a related, but more frequent finding in the CNS of dogs. Like their non-congenital, dermal counterparts, both epidermoid and dermoid intracranial cysts are lined by stratified squamous epithelium. Although they have a similar embryologic origin, the dermoid cyst is derived from a more pluripotent precursor cell and often produces adnexal structures, such as sebaceous glands, apocrine glands or hair follicles.[6] Additionally, Rhodesian ridgeback dogs that carry the autosomal dominant dorsal ridge trait have been shown to be predisposed to the congenital cutaneous defect known as dermoid sinus, which is a draining sinus at the dorsal midline that occasionally communicates with the subarachnoid space.[3] Typically these dogs do not have any clinical problems.

Intracranial epidermoid cysts occur in other veterinary species; in mice they often occur within the leptomeninges of the lumbar/sacral spinal cord or (less commonly) associated with the fourth ventricle.[2] There are generally no clinical signs in mice or rats with this lesion, which may be an indication that the cysts do not grow large enough to compress adjacent structures.[2] Intracranial epidermoid cysts in mice are thought to be strain dependent and are typically interpreted as incidental findings. Conversely, dermoid cysts in mice have rarely been associated with clinical neurologic dysfunction.[2] Epidermoid cysts have not been reported in the CNS of hamsters or cats.

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CASE II: 10-461 (JPC 4031864).

Signalment: 10-year-old male neutered Staffordshire terrier dog, (Canis familiaris).

History: Urinary bladder mass, hematochezia, hematemesis.

Gross Pathology: The urinary bladder is 1 cm thick, has emphysema of the wall, and has multiple 0.5-1.0 cm red nodules in the mucosa.

Histopathologic Description: Urinary bladder: The urinary bladder has severe transmural emphysema, severe submucosal edema, mild multifocal submucosal hemorrhage, and a mild, diffuse submucosal inflammation of lymphocytes, plasma cells, eosinophils and macrophages containing hemosiderin. A few macrophages and multinucleate giant cells are present around the air spaces.

Contributor's Morphologic Diagnosis: Urinary bladder: Emphysematous cystitis.

Contributor's Comment: Emphysema of the urinary bladder has been reported in humans, dogs, cattle and cats.[1,2] It occurs with infection of the bladder by gas-producing bacteria that ferment glucose in the urine. E. coli, Pseudomonas, Klebsiella, Proteus, Enterobacter sp., and Clostridium sp. are bacteria that have been isolated from cases of emphysematous cystitis in dogs and humans.[1,2] Glucosuria caused by diabetes mellitus is the most common cause of urinary bladder emphysema. The condition also occurs in non-diabetic humans and dogs associated with chronic recurrent cystitis, cortisone administration and primary glucosuria.[1,2]

JPC Diagnosis: Urinary bladder: Emphysema, transmural, multifocal to coalescing, marked, with mild granulomatous and lymphoplasmacytic cystitis, and submucosal hemorrhage and edema.

Conference Comment: Conference participants explored various mechanisms contributing to glucosuria and emphysematous cystitis, as summarized above by the contributor. Although diabetes mellitus is the most common underlying cause, proximal renal tubular disorders also result in glucosuria, and may induce emphysematous cystitis in several breeds of dog, including Basenjis, Labrador retrievers, Norwegian elkhounds, schnauzers and Shetland sheepdogs.[4] In Basenjis, this renal tubular abnormality,

known as canine Fanconi-like syndrome, is hereditary. It is characterized by impaired renal tubular reabsorption of glucose, phosphate, sodium, potassium, uric acid, amino acids and protein.[6] In humans, Fanconi-Bickel syndrome is due to a defect in the SLC2 gene, which results in defects of GLUT2, a member of the renal glucose transporter protein family.[5] It is suspected that there is a similar underlying defect triggering canine Fanconi-like syndrome, but this has not been proven. Acquired Fanconi-like syndrome in dogs has been associated with several drugs and toxins, such as gentamicin and ethylene glycol. Whether congenital or acquired, Fanconi-like syndrome results in glucosuria (with normal blood glucose), phosphaturia, aminoaciduria and proteinuria.[4] Primary (type II) renal tubular acidosis may also occur due to impaired bicarbonate reabsorption in renal proximal convoluted tubules, which leads to secretional, hyperchloremic metabolic acidosis with normal anion gap and alkaline urine.[3]

Primary renal glucosuria (without other reabsorption abnormalities) is an inherited disorder of Norwegian elkhounds.[4] It is caused by defects in SGLT2, from the sodium-glucose cotransporter family of proteins that is encoded by SLC5 genes in people.[5] As with Fanconi-like syndrome, the underlying genetic defect in dogs has not been established. In addition to primary glucosuria and diabetes, treatment with exogenous corticosteroids, and the subsequent antagonism of insulin, can lead to glucosuria (and thus, emphysematous cystitis) as well; once the resorptive capacity of the proximal renal tubule is exceeded, at a blood glucose concentration greater than 180mg/dL in dogs, glucose spills over into the urine.[3] Glucosuria may also occur in cattle treated with intravenous glucose.

Participants also reviewed other pathologic processes resulting in tissue emphysema. Clostridia are probably the most well-known gas producing organisms, as exemplified by Clostridium chauvoei induced necrohemorrhagic and emphysematous myositis (blackleg). Pulmonary emphysema is a non-specific change due to alveolar rupture in downer cattle or those with dyspnea or increased respiratory effort. Subcutaneous emphysema in any species could be a sequela to tracheal, bronchial or pulmonary trauma. In swine, intestinal emphysema (pneumatosis cystoides intestinalis) is an idiopathic, incidental finding at slaughter. Fetal death with maceration and emphysema is a fairly well described condition in ruminants. So called "gas bubble disease" in fish is caused by supersaturated levels of dissolved oxygen or nitrogen in the water of the tank. Lesions are secondary to the accumulation of gas bubbles in blood vasculature and tissues. Finally, postmortem gas production is also a familiar occurrence in veterinary pathology; it can be distinguished from antemortem emphysema by the absence of an inflammatory reaction.

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CASE III: 55860 (JPC 4032564).

Signalment: 1-year-old female blackbuck, (Antilope cervicapra cervicapra).

History: This blackbuck was housed in a large, mixed species enclosure. Two days prior to death she was isolating herself from the group, with some head shaking and stretching. In the afternoon prior to death she delivered a stillborn calf. The blackbuck was found dead on the following morning.

Gross Pathology: The diaphragm is loosely adhered to the ventral serosal surface of the spleen with tan fibrinous material. A small amount of fibrin is present on multiple loops of small intestine. An approximately 20 cm long region of small intestine (jejunum) is dark red to purple, and friable. A moderate amount of fibrin is present in the lumen orad and aborad to the affected intestine, and the mucosal surfaces are mildly petechiated. A mesenteric lymph node adjacent to the most severely affected loop of intestine is pink with a few white foci scattered throughout. The uterus contains a small amount of tan to grey material adhered to the endometrium. The vagina and cervix are markedly dilated and have red to dark red mucosal surfaces.

Laboratory Results: Bacterial culture of intestinal content was performed with the following results: Yersinia pseudotuberculosis 2+, Escherichia coli 3+, normal gram-positive flora 2+. Campylobacter, Salmonella, Shigella, Pleisiomonas, Edwardsiella, and Aeromonas sp. not isolated.

Histopathologic Description: Small intestine with adjacent fat and lymph node: The intestinal mucosa and submucosa are necrotic with extensive hemorrhage, infiltrates of neutrophils, and large clusters of gram-negative bacterial rods. Crypts are identified in most sections, but are absent in many areas. The superficial mucosa has large amounts of fibrinous exudate and necrotic debris with bacteria of various types. Autolysis hinders detailed evaluation of the superficial mucosa. Fibrin thrombi are present in many vessels (probable lymphatics). Adipose tissue and lymph node have similar areas of necrosis with large numbers of bacteria.

Contributor's Morphologic Diagnosis: Small intestine, lymph node, adipose tissue: Severe regionally extensive acute necrotizing enteritis, lymphadenitis, and steatitis with intralesional bacteria (etiology: Yersinia pseudotuberculosis).

Contributor's Comment: Yersinia pseudotuberculosis (YPT) is a gram-negative facultative anaerobic intracellular bacterium that is found worldwide. It causes sporadic disease in domestic animals and humans, and has been isolated from a wide range of species in both captive situations and the wild. In domestic animals infection usually results in sporadic cases, with occasional outbreaks. In wild rodents and birds epidemics are common. YPT may cause abortion in cattle, sheep, and goats.[1,2]

YPT, Yersinia enterocolitica (YE) and Yersinia pestis (YP) share 97% of their genomes as well as a tropism for lymph nodes. Despite this, manifestations of disease can be quite different for the three bacteria, presumably due to a combination of shared and unique chromosomal and plasmid-derived virulence factors.[3]

Less is known specifically about the pathogenesis of YPT infections than that of its close relative YE. As demonstrated with YE, following ingestion of contaminated food or water, bacteria adhere to distal small intestinal epithelial cells, cross the intestinal barrier using M cells which leads to bacterial replication in Peyer's patches. Spread to mesenteric and other distant lymph nodes is common. There is little host response to bacterial replication in the first 36-48 hours following infection. Subsequently, an influx of

activated phagocytes induces cytokine production and tissue necrosis. Septicemic spread from the distal ileum to the spleen and liver is relatively common.[3]

YPT is frequently isolated from feces of normal cattle. The organism may be shed in the feces by carrier animals in the group, or the environment may be contaminated by birds and rodents. The organism can survive and grow in cool environmental temperatures. Disease may be related to compromise of cell-mediated immunity and outbreaks may occur when animals are stressed (e.g., breeding, bad weather, poor nutritional condition). Clinical signs include mild diarrhea, severe hemorrhagic diarrhea, vague illness, or sudden death. Gross lesions may also be variable, with little noted in mild cases. More severe cases have hemorrhage in the intestine and enlarged hemorrhagic mesenteric lymph nodes. White foci of necrosis may be found in other organs, especially the liver and spleen.[1,2]

Diagnosis is based on the presence of characteristic lesions and isolation of the organism. Y. pseudotuberculosis prefers to grow at temperatures lower than the standard culture temperature of 37 degrees C, and since it may not be isolated using routine culture techniques, the laboratory should be notified when infection with this agent is suspected.[1,2]

Y. pseudotuberculosis also causes acute gastroenteritis and mesenteric lymphadenitis in humans. It is a food borne illness, and in the United States is much less common than Yersinia enterocolitica. In a review of FoodNet sites covering the period 1996-2007, 18 confirmed cases were identified, with an average annual incidence of 0.04 cases per 1,000,000 persons. Cases are probably underreported because less invasive forms are not recognized, and because of culture requirements for this organism. Infections in humans are less common in the United States than other countries.[4]

Our experience with this organism is similar to what is reported in the literature. We generally have seen sporadic cases, but this blackbuck was a case in an outbreak of 15 animals located in multiple enclosures, most of which are large and house multiple species. Attempts to isolate YPT from non-collection animals (e.g., wild rabbits and birds) were unsuccessful. In this blackbuck YPT was also isolated from the uterus, and mild fibrinous endometritis was present. Inflammation with bacteria was also seen in lung, liver, spleen, and urinary bladder.

JPC Diagnosis: Small intestine and mesenteric lymph node: Enteritis and lymphadenitis, necrosuppurative, subacute, diffuse, severe with mesenteric steatitis and numerous large colonies of bacilli.

Conference Comment: To open, participants conducted a cursory review of other types of pathogenic large colony-forming bacteria commonly encountered in veterinary medicine. Yersinia sp., Actinomyces sp., Actinobacillus sp., Corynebacterium sp., Staphylcoccus sp. and Streptococcus sp. are well known for the production of extensive bacterial colonies. Most are Gram-positive bacteria, with the exception of Yersinia sp. and Actinobacillus sp.

Next, the conference moderator expanded upon the contributor's adept description of the pathogenesis of Yersinia pseduotuberculosis (YPT) and participants further analyzed several of its virulence factors. Transmission of YPT is generally fecal-oral, with rare transmission via inhalation. Yersinia adhesion A protein, or YadA (encoded on the pYV virulence plasmid) and invasin (encoded by the chromosomal inv locus) facilitate bacterial contact for invasion of host small intestinal M-cells; YadA adheres to extracellular matrix proteins such as fibronectin, collagen and laminin, while invasin binds to host cell b1 integrins. Next, Yops (Yersinia outer membrane proteins) come into play. YopB & YopD form a pore in the host cell membrane which allows the type three secretion system (T3SS) injectisome, which is also encoded on the pYV virulence plasmid and has a structure similar to flagella, to inject effector Yops into the host cell.[3]

The RNA chaperone protein Hfq is important in the production of T3SS effectors as well as intracellular survival. The PhoP/Q system (chromosomally encoded) stimulates growth and survival in macrophages as well as delayed macrophage activation via reduction in the stimulatory capacity of LPS. Superantigenic toxin YPM contributes to systemic infection by inducing T-cell proliferation and cytokine production. Various Yop proteins inhibit the host inflammatory response via alteration of phagocyte function, inhibition of signal transduction and disruption of the cytoskeleton. YopM sequesters caspase-1, which blocks activation of pro-inflammatory cytokines and YadA inhibits the classic complement and lectin pathways. YopP/J induces apoptosis of host cells by inhibiting the inflammatory and pro-survival actions of LPS and directly activating caspases. So, overall, YPT infections are characterized by an initial "quiet" 36–48 hour period of bacterial replication with apoptosis and minimal host response, followed by a flood of phagocytic cells and an acute inflammatory response characterized by cytokine production and tissue necrosis.[3] This is illustrated by the abundant necrosis and large colonies of bacilli exhibited histologically.

To close, participants briefly discussed the role of exogenous superantigen in infection and immunity (such as the YPM toxin produced by *Y. pseudotuberculosis* in this case). Superantingens, which are often produced by Gram-negative bacteria, bind the V_b domain of the T-lymphocyte receptor (TCR) with the a chain of a class II major histocompatibility complex (MHC). This occurs outside of the normal antigen binding site and results in polyclonal T-lymphocyte activation regardless of antigen specificity, as well as massive cytokine release.[5]

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CASE IV: DR30 (JPC 4032701).

Signalment: 11-year-old male rhesus macaque, (Macaca mulatta).

History: Presented for chronic weight loss (9% loss in previous 10 weeks) and past history of vaccination with commercial Fluvax® and trauma to the hand that required surgical repair.

Gross Pathology: Thin male monkey presented with a bulging multinodular mass on the anterior pole of the right kidney measuring 1x2.3 cm. Suppurative exudate was apparent on cut section.

Laboratory Results: Previous viral testing indicated positive tests for CMV, LCV, RRV, and SV40. All previous TB skin tests were negative. CBC and clinical chemistry were unremarkable. Routine culture of the mass identified a mixture, in order of most abundant, of hemolytic, coagulase negative staphylococcus, Streptococcus fecalis, gamma and alpha hemolytic streptococcus, and Corynebacterium sp. Stains of smears for AFB were negative.

Histopathologic Description: Kidney: The kidney contains multiple variably sized sometimes confluent pyogranulomas that compress and ablate parenchyma from the medulla to the capsule. Pyogranulomas have thin peripheral concentric fibrous rings infiltrated with lymphocytes and plasma cells that surround sheets of epithelioid macrophages, numerous multinucleated giant cells, and a central core filled with large numbers of neutrophils and eosinophils. Within many of the granulomas and often associated with giant cells are spherical yeast-like cells measuring 10-20 microns in diameter with double-contoured walls and a smaller dense granular central nucleoid. Rare budding forms are observed. GMS stain highlights the outer wall. Gram stain demonstrates gram positive cocci within phagocytic cells. Proximal tubules have swollen epithelium with granular sometimes vacuolated cytoplasm, while scattered groups of ectatic distal tubules contain hyaline protein and cellular casts.

Contributor's Morphologic Diagnosis: Rhesus macaque, kidney: Pyogranulomatous inflammation with intralesional Blastomyces dermatitidis and Staphylococcus sp. and with tubular degeneration and cast formation.

Contributor's Comment: Blastomycosis is a disseminated or localized mycotic infection of primarily man and dogs in the Ohio and Mississippi River basins of North America and in Africa, and is caused by Blastomyces dermatitidis.[1,2] It is a dimorphic fungus found in soil in mycelial form but transforms into a pathogenic yeast at body temperature and is transmitted by inhalation or inoculation.[3] Common sites of infection are lung, skin, bone, male urogenital system, particularly prostate and testis[4] with less frequent distribution to liver, spleen, and lymph nodes.[5] It is rarely reported in monkeys; the first report in monkeys described an animal that originated from our colony.[6] We subsequently have seen several additional cases, which like the first report, developed lesions several years after leaving our breeding facility and contact with soil (Didier, unpublished). Serology[9] and PCR[11] may aid in the clinical diagnosis and recent work has associated clinical phenotypes of human infection with genetic variants of B. dermatitidis.[12]

JPC Diagnosis: Kidney: Pyogranulomas, multiple and coalescing, with renal capsular fibrosis, perirenal granulomatous steatitis and numerous yeasts, etiology consistent with Blastomyces dermatitidis.

Conference Comment: Participants summarized the key morphologic features used to differentiate Blastomyces dermatitidis from other pathogenic dimorphic fungi of veterinary significance, including Cryptococcus neoformans, Histoplasma capsulatum, Sporothrix schenckii, *Coccidioides immitis* and *posadasii* and *Penicillium marnefii*. B. dermatitidis appears in animal tissues, both extracellularly and intracellularly within macrophages and neutrophils, as an 8-20 µm round, yeast, with a double contoured, often refractile cell wall and broad-based budding. It stains with GMS, Gridleys and PAS. C. neoformans, which is also found in tissue in the yeast form, ranges from 2-20 µm and can be differentiated by its thick carminophilic capsule and narrow-based budding. Rare forms of *C. neoformans* lack a capsule, making definitive identification of these yeasts a challenge. H. capsulatum is smaller (2-6 µm) and typically intrahistiocytic, usually with many organisms in each macrophage and has narrow-based budding and a shrinkage-artifact "halo" on H&E sections; this yeast has no true capsule. S. schenckii appears in tissues as a 2-6 µm, pleomorphic, often cigar-shaped intracellular and extracellular yeast. It stains well with both PAS and GMS; however, yeasts are often difficult to see on H&E-stained sections in most species other than cats, in which they are usually readily visible. *C. immitis* and *posadasii* reproduce by endosporulation and have a characteristic tissue section appearance as 20-200 µm

spherules, with a double-contoured wall and numerous 2-5 µm endospores.[2] As emphasized by one participant, *Coccidioides* sp. is another fungal organism occasionally encountered in research monkeys.[7] *Penicillium marnefii*, which is emerging as an important pathogen of immunosuppressed humans in Southeast Asia, has also been identified in animals. *P. marnefii* is found both intracellularly and extracellularly; yeasts are 2-3 µm and multiply by binary fission, so elongated cells up to 13 µm are occasionally present.[10]

The most important virulence factor associated with *B. dermatitidis* is the adhesin BAD1. BAD1 has multiple functions and is secreted exclusively by the yeast form of *Blastomyces*. It mediates adhesion to host macrophages via calcium-dependent attachment to chitin in the yeast cell wall and binding to CR3 and CD14 on phagocytic cells. It also acts as an immunomodulator by suppressing the generation of TNF-α, a pro-inflammatory cytokine important in defense against fungal infections.[8]

One interesting aspect of this case is that this monkey developed clinical signs of blastomycosis several years after suspected exposure. Since the infective (mycelial) form of B. dermatitidis is generally found in soil, and this animal was housed entirely indoors, it is presumed that he was initially infected while housed outdoors at the breeding facility, and then harbored the organism for several years before developing clinical disease.[6] As pointed out by the contributor, there have been several similar reports, which suggest the potential for long periods of subclinical infection with *B. dermatitidis* before the manifestation of clinical disease.

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