

The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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Conference Moderator:

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CASE I: 09/256 (AFIP 3148215).

Signalment: 16-year-old pony breed mare (*Equus caballus*).

History: The pony became ataxic one week before presenting at the clinic. The referring veterinarian found neurological signs affecting the tail and hindlimbs. At the Norwegian School of Veterinary Science clinic, neurological examination indicated that facial nerves (V and VII) were affected. Muscle tone in the tail and anus was reduced. Hindquarter skin had areas of hypersensitivity and areas of reduced sensitivity. Severe ataxia was found.

Gross Pathology: In the gluteal region there was edema and hemorrhages centered on nerves. The lumbosacral spinal cord (L5-S1) was firm and slightly irregular in thickness. There was hemorrhage in the epidural and subdural spaces and dark hemorrhagic foci in the spinal cord.

Histopathologic Description: Cauda equina: Nerves, extradural and intradural, are moderately to severely infiltrated by lymphocytes, plasma cells, epithelioid macrophages and fewer multinucleated giant cells. There is axonal degeneration and loss within the affected nerves. The nerve fibers are embedded in a collagen rich fibrous tissue (fibrosis) with a moderate lymphoplasmacytic infiltrate and focally extensive hemorrhages. Fibroplasia is also seen within nerves. Dorsal root ganglia (not present in all sections) have a moderate to severe lymphoplasmacytic infiltration and show axonal degeneration and loss as well as rare hypereosinophilic shrunken neurons (neuronal necrosis). Several cranial nerves and nerves associated with hemorrhages in the gluteal region had moderate lymphoplasmacytic infiltrates (tissue not submitted).

Contributor's Morphologic Diagnosis: Cauda equina: Neuritis and perineuritis, granulomatous, multifocal to diffuse with epineurial and perineurial fibrosis.

Contributor's Comment: Neuritis of the cauda equina, or polyneuritis equi, is a nonsuppurative inflammation mainly affecting the nerve trunks of the cauda equina in horses.(2) Clinically the disease is characterized by paralysis of the tail, reduced muscle tone of the anus and rectum, paralysis of the bladder (sometimes with urinary incontinence), paresthesia or anesthesia of the tail and perineum, and incoordination of the hindlimbs. There may also be involvement of cranial nerves.(2) Lesions are reported to be most severe in the sacral and coccygeal nerves, and are characterized by granulomatous inflammation, hemorrhage and fibrosis causing irregular thickening and discoloration of the nerve roots. The inflammation is usually more severe in the extradural parts of the nerves compared to the intradural nerve segments. Although more mildly, nerves outside the cauda equina, including cranial nerves, are commonly also affected.(2) In the present case, nerve associated hemorrhagic lesions were grossly visible in the gluteal region. A recent case report described biopsy of tail musculature as an aid in the antemortem diagnosis of polyneuritis equi.(1) The cause is unknown, but the morphology of the lesion suggests that it is an immune-mediated disorder.(2)

AFIP Diagnosis: Spinal cord, cauda equina: Polyradiculoneuritis, granulomatous, multifocally extensive, marked, with perineurial and epineurial fibrosis and nerve fiber loss.

Conference Comment: The contributor provides a succinct overview of this perplexing entity. Many favor the term *polyneuritis equi* (PNE) over *neuritis of the cauda equina* or *cauda equina syndrome* because it more accurately reflects the typical widespread distribution of inflammation affecting not only the cauda equina, but often spinal roots and cranial nerves as well.(3) As alluded to by the contributor, while the pathogenesis of the lesion remains obscure, Adenovirus I has been isolated from horses with PNE, and it is hypothesized that a viral infection may incite an autoimmune polyradiculoneuritis in horses with this condition.(3) *Halicephalobus gingivalis* has been reported as a cause in one case of PNE.(2)

Whatever the inciting etiology, an immune-mediated mechanism is suggested by similarities with Guillain-Barre syndrome (GBS) in humans and experimental allergic neuritis (EAN) in laboratory animals. Specifically, PNE, GBS and EAN are all characterized by demyelination in the proximal roots with invading mononuclear cells and macrophages stripping away segments of the myelin sheath. Furthermore, circulating antibodies to the P₂ myelin protein, the antigen that upon injection into laboratory animals produces EAN, have been demonstrated in horses with PNE. Nevertheless, the role of these antibodies in the pathogenesis of PNE is unknown; they may be causal or arise secondary to demyelination and inflammation.(3)

As mentioned by the contributor, a recent case report describes biopsy of the sacrocaudalis dorsalis lateralis muscle, the innervation of which arises in the cauda equina, for the antemortem diagnosis of PNE. In frozen biopsy specimens, intense lymphohistiocytic inflammation infiltrated and effaced terminal intramuscular nerve branches while sparing the myofibers, which exhibited angular atrophy of both muscle fiber types (neurogenic atrophy) and occasional hypertrophic or split fibers.(1)

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CASE II: 126998 (AFIP 3149866).

Signalment: 7-year-old, Thoroughbred cross mare (*Equus caballus*).

History: The horse presented with a sudden onset of anorexia, dysphagia and severe colic with no discernible gut sounds. Tachycardia (80 beats per minute), dehydration, patchy sweating and muscle tremors were also noted. On rectal examination the mucosa was dry and tacky and the content was dry with mucosal casts. Euthanasia was elected.

Gross Pathology: At necropsy, the stomach was distended with green fluid. Multiple linear red erosions were noted in the distal third of the esophagus. The small intestine was dilated with green fluid and gas, with pale red mucosal and serosal surfaces. The large and small colons were filled with large dry balls of fecal material coated with a black viscous substance (blood). There was multifocal reddening of the colonic mucosa.

Histopathologic Description: Many neurons in the coeliaco-mesenteric ganglion have a swollen, hyper eosinophilic, finely vacuolated cell body with significant reduction in or absence of Nissl granules and either a pyknotic nucleus or absence of nuclear detail. Others have a deeply eosinophilic, rounded cell body with absence of Nissl substance and a paracentral or peripheral pyknotic nucleus. Smaller numbers of neurons show margination of the Nissl granules with central deeply eosinophilic cytoplasm (central chromatolysis). There are numerous perineuronal axonal spheroids of various sizes. Many neurons contain small amounts of clumped, granular golden-brown pigment (lipofuscin).

Contributor's Morphologic Diagnosis: Coeliaco-mesenteric ganglion: Neuronal degeneration and necrosis, acute, multifocal, severe.

Contributor's Comment: These gross and histological lesions are considered diagnostic for equine dysautonomia, or "grass sickness", a disease that largely occurs in horses at pasture (of a wide variety of breeds) in the United Kingdom, western parts of continental Europe, and South America. The risk is highest for young horses (2 to 7 years of age). The greatest number of cases in the UK occurs in the spring. A similar syndrome has been described in cats in the same areas, in a few dogs, and in wild hares. Acute, subacute or chronic cases may be seen with typical clinical signs in horses including muscle tremors, abnormal sweating patterns, dysphagia, reflux of gastric contents, distended small intestinal loops, absence of gut sounds and absence of or abnormal (dry) feces. Megaesophagus occurs in more than 90% of feline and canine cases. Many horses die or are euthanized within the first two weeks but small numbers of animals have survived long-term. Chronic cases present with a more insidious onset of clinical signs.

In acute cases, which are more common, at post-mortem examination there is typically distension of the stomach, and there may be evidence of gastro-esophageal reflux, i.e. distal esophageal ulceration. The large colon is usually filled with firm fecal balls that have a black coating of blood products. Histological lesions are classically described as being found in postganglionic sympathetic and parasympathetic neurons; typically at post-mortem the coeliaco-mesenteric and cranial cervical ganglia are sampled in addition to intestinal tract specimens. Normal neuronal numbers in these sites have been published.(6) Lesions are also found in parasympathetic terminal cardiac ganglia and have been associated with a functional reduction in cardiac autonomic control.(5) Cytoplasmic vacuolation, chromatolysis and necrosis of neurons is noted, and numbers of neurons are reduced significantly.(1) More chromatolytic neurons are noted in acute than in chronic cases. However, lesions have also been reported in general somatic efferent and general visceral efferent lower motor neurons in the brainstem and spinal cord including chromatolysis of lower motor neurons of the general visceral efferent nucleus of cranial nerves III and X, and the general somatic efferent nuclei of cranial nerves III, V, VII and XII.(2) For antemortem diagnosis it is typical to submit a full-thickness biopsy specimen from the ileum as pathology in the intestinal tract is seen most consistently and severely in that segment, particularly in the submucosal plexuses.(1)

The cause is not known, but the most popular current theory being, with some circumstantial evidence, that this is a form of botulism (*Clostridium botulinum* type C).(4)

AFIP Diagnosis: Ganglion, celiaco-mesenteric (per contributor): Neuronal degeneration and necrosis, acute, diffuse, with satellite cell hypertrophy and proliferation, and minimal multifocal lymphocytic ganglioneuritis.

Conference Comment: During the conference, participants reviewed the key diagnostic features of this perplexing entity, as expertly synthesized in the contributor's comments. This condition highlights the importance of carefully considering the clinical history and presenting signs when collecting tissues at necropsy; an elevated index of suspicion for grass sickness should prompt the astute diagnostician to examine the celiaco-mesenteric ganglion. The conference moderator reminded participants to be cautious when evaluating autonomic ganglia microscopically, specifically to avoid misinterpreting the hypertrophic and hyperplastic satellite cells as lymphocytes. In this case, there is minimal lymphocytic ganglioneuritis, which is not always present in horses with grass sickness; more prominent are the numerous proliferating satellite cells, which can sometimes be mistaken for lymphocytes.

Conference participants based a discussion of chromatolysis on the example provided here. Chromatolysis is a histologically appreciable change in the soma resulting from dispersal of Nissl granules, which are composed of rough endoplasmic reticulum; chromatolysis may be classified as central or peripheral based on its location within the soma and is best demonstrated with special stains, such as cresyl violet. Cells undergoing central chromatolysis are usually swollen with a distinctly eccentric nucleus, while peripheral chromatolysis is more commonly associated with cell shrinkage. Chromatolysis is always considered to be a lesion; however, correctly identifying and interpreting it is predicated on knowing the normal distribution of Nissl granules at a given location.(3) In the autonomic ganglia, for instance, Nissl granules are normally concentrated at the periphery of the soma, so chromatolysis results in intense central eosinophilia, often with multiple fine clear vacuoles at the periphery of the soma, as is superbly demonstrated in this case.(7) Chromatolysis is a nonspecific reaction that essentially represents a metabolic adaptation to change. For instance, in cases of axonal injury, chromatolysis is evidence of the anabolic response required for regeneration, and has in this context been referred to as the *axon reaction*. When axonal regeneration is complete, the chromatolytic soma may pass through a densely basophilic phase before returning to normal. Alternatively, chromatolysis may precede cell death, the likelihood of which increases with increasing

proximity of the axonal lesion to the cell body. Chromatolysis is also a characteristic feature of numerous motor neurodegenerative conditions and perinatal copper deficiency in sheep and goats.(3)

The dysautonomias of horses, cats, dogs, and hares share not only compelling epidemiological and geographical commonalities, but also a similar distribution of central neuropathology, lending further credence to the suggestion of a common etiology.(2)

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CASE III: 07-5486029 (AFIP 3141621).

Signalment: 18-month-old, female Merino sheep (*Ovis aries*).

History: For each of the past five years, several male and female Merino sheep in the 12-18 months age range developed hindlimb incoordination. When the flock was driven, these sheep lagged behind and eventually collapsed; after a short time, they regained their feet. Over the next three months, they gradually lost hindlimb function and could only move using the forelimbs. They eventually became permanently recumbent and would have perished if not euthanized. Wasting was not a feature until late in the course of the disease when affected sheep were unable to obtain feed. Central nervous system (CNS) tissues from two of these sheep were examined, and a wide range of other tissues examined from the second sheep.

Gross Pathology: There were no obvious gross findings in either of the sheep.

Laboratory Results: A complete blood count and biochemistry was normal in the second sheep (not done on first sheep). Brain and liver tissue was subjected to PCR using primers to highly conserved regions of exon 1-9 from the gene for glial fibrillary acidic protein (GFAP). Numerous homozygous nucleotide variations in both the affected and control sheep were found, when compared to the published bovine sequence (no ovine sequences available), but these were considered to represent interspecies variations (see later discussion).

Histopathologic Description: In sheep #1 (forwarded here), there were large numbers of Rosenthal fibers (RF) in the brain and spinal cord, particularly in perivascular astrocytic end-feet, and in subpial and subependymal locations where astrocytes normally form a dense meshwork of processes. They often formed perpendicular arrays. Rosenthal fibers were found at all levels of the brain and spinal cord, although they were more numerous in white than gray matter and much more plentiful in the spinal cord and medulla than in the rostral brainstem and cerebellum. Rosenthal fibers were irregularly shaped, elongated or round, deeply eosinophilic intraastrocytic aggregates that varied in diameter from 4 to 20 μm . Hypertrophied astrocyte cell bodies accompanied and paralleled in density the number of RF and were thus more numerous in subpial and perivascular locations in the caudal brainstem and spinal cord. These astrocytes sometimes attained a diameter of 60 μm and contained large nuclei with

prominent nucleoli and pale-staining, hyaline cytoplasm. They were occasionally multi- (bi- or tri-) nucleated and rare nuclei contained large eosinophilic intranuclear inclusions consistent with cytoplasmic invaginations.

Within the cerebral cortex, RF were present in subpial locations. They were attended by low numbers of hypertrophied, and occasionally multinucleated astrocytes with stout fibrillar extensions into the pial membrane. Subcortical and central white matter did not contain RF, although there were a few hypertrophied astrocytes. However, RF density was high in the periventricular and subependymal regions around the third ventricle, and of lesser degree in the subependymal region of the temporal horns. The white matter of the hippocampus and corpus callosum contained occasional RF and mild astrocytic reaction. Perivascular RF were observed in the central gray matter.

The molecular layer of the cerebellum showed small numbers of subpial RF which, as in the cerebral gray matter, were attended by a few hypertrophied astrocytes. The granular layer, and more particularly the folial and deeper white matter, contained abundant RF and associated hypertrophied astrocytes.

In the pons and medulla, there were prominent subpial, subependymal and perivascular RF aggregates, accompanied by numerous hypertrophied astrocytes, with lesser numbers in the white matter. Ependymal cells were hypertrophied. α B-crystallin immunostaining showed positive cytoplasmic expression in many glia, including a subset of astrocytes with a ring-like perinuclear pattern of staining. Many RF were immunonegative, except for a thin rim of positive staining. Many of the α B-crystallin-positive astrocytes were GFAP-negative.

Rosenthal fibers reacted strongly with ubiquitin, but were vimentin-negative. GFAP gradually replaces vimentin in astrocytic IF during brain development, although both may be coexpressed in reactive astrocytes and some astrocytic subpopulations. Rosenthal fiber HSP70 expression resembled that for α B-crystallin but normal astrocytic perikarya, unlike the latter, did not stain for HSP70.

Within the spinal cord, RF formation was florid, generally being greatest in perivascular and subependymal locations. However, in some areas, gray matter contained nearly as many RF as white matter. There were also moderate to large numbers of hypertrophied astrocytes, small numbers of axonal spheroids, and minimal Wallerian degeneration.

All neuronal populations in the brain and spinal cord, including those in cortical, central, brainstem and spinal gray matter, as well as the pyramidal and granule cells of the hippocampus and cerebellar Purkinje and granule cells, were of normal morphology. Oligodendroglial nuclei also appeared normal.

In sheep #2, the neuroanatomical distribution of RF was similar to sheep #1, but they were greater in number and density. There was mild, Wallerian degeneration in the spinal cord, with ellipsoids (digestion chambers) containing axon fragments, and sometimes macrophages. Myelin ellipsoids were found in all funiculi, but were sometimes more prominent in, or largely restricted to, the ventral funiculi.

Electron Microscopy Results: At the ultrastructural level, within astrocytes, RF were electron-dense, rounded or elongated, coarsely granular structures of variable size with irregular contours. They were surrounded by densely aggregated sheaths of IF, some of which appeared to be continuous with the central osmiophilic mass.

Contributor's Morphologic Diagnosis: Brainstem (medulla or rostral colliculi): Moderate, multifocal (primarily perivascular/subpial/periependymal) Rosenthal fiber deposition, accompanied by moderate astrocytic hypertrophy.

Contributor's Comment: Alexander's disease (AD) is a rare (approximately 450 cases worldwide) and frequently fatal human neurological disorder. Diagnosis of AD can only be confirmed histologically and the signature lesion is the presence of large numbers of hypereosinophilic, intraastrocytic inclusions termed Rosenthal fibres (RF) that contain the heat shock proteins, α B crystallin and HSP27, as well as ubiquitin.(1) The swollen processes and perikarya of astrocytes containing these fibers are more numerous in perivascular, subpial and subependymal sites, often disposed in dense, perpendicular arrays. Alexander's disease is the only documented primary astrocytic disorder, and in approximately 90% of those cases examined at the molecular level various mutations in the glial fibrillary acidic protein (GFAP) gene, which encodes the major intermediate filament protein in these glia, have been found.(2) The phenotypic expression of AD depends on the age of onset and three forms (i.e. infantile, juvenile and adult) are recognized. Spontaneous encephalopathies resembling human AD have been reported in six juvenile dogs (onset at 9 weeks to 6 months of age) of both sexes and different breeds (two Labrador retrievers, two Bernese

mountain dogs, one Scottish terrier, and one miniature poodle) and one adult (4-year-old), female, White Alpine sheep.(1,3,4,7) In none of these cases was the GFAP region of the genome examined for mutations.

The histopathological, immunohistochemical and ultrastructural features of the RF encephalomyelopathy described in these two sheep resembled those found in human AD cases and the AD-like encephalopathies previously reported in several dog breeds and one sheep. The age of the present sheep, neuroanatomical distribution of RF, and paucity of myelin loss was similar to adult-onset AD in humans and the only other AD-like neuropathy reported in sheep, while canine cases resembled the human juvenile form of AD. However, this is the first case of an AD-like encephalopathy in domestic animals to be subjected to molecular pathological analysis. In almost all humans with AD, a heterozygous mutation has been identified in exons 1 to 8 of the GFAP gene, and since the sheep GFAP gene has not been sequenced, fragments corresponding to the highly conserved regions of exons 1 to 9 of the bovine GFAP gene were amplified. Polymerase chain reaction results revealed numerous homozygous nucleotide variations in both the affected and control sheep when compared to the published bovine sequence, but these were considered to represent interspecies variations.

It is hoped that further cases of ovine AD from this property will become available for molecular and pathological evaluation so that the strongly suspected genetic basis for the disease may be confirmed and characterized.

AFIP Diagnosis: Brain, cerebellum and medulla: Encephalopathy, bilaterally symmetrical, characterized by perivascular, subpial, and periependymal hypereosinophilic filament accumulation, astrocytosis, marked astrocytic hypertrophy, and multinucleated astrocytes (Rosenthal fiber encephalopathy).

Conference Comment: The contributor provides a very useful review of AD and a thorough description of the microscopic lesions and immunohistochemical findings in the sheep examined in this interesting case, which provides a dramatic example of striking astrocytosis. The conference moderator used this case to emphasize the importance of lesion distribution and pattern. Recognition of RF accumulation alone is insufficient for making a specific diagnosis, because a number of conditions (e.g. reactive astrocytosis, some astrocytomas) may feature RF formation; accurately characterizing RF distribution is therefore of paramount importance in making the correct diagnosis.(5) In this case, RF accumulation is noted in both the gray and white matter in a distinctly symmetrical pattern. The conference moderator emphasized that symmetry is often helpful in identifying lesions with a toxic, metabolic, nutritional, or degenerative etiology.

In humans, the infantile form of AD is characterized not only by RF accumulation, but by severe myelin changes in the frontal lobes, resulting in the characterization of AD as a leukodystrophy; this may be attributable to oligodendrocyte dysfunction secondary to the primary astrocytopathy. However, demyelination may or may not be present in the juvenile and adult forms, and pathology is primarily confined to the brainstem; thus, characterization as a leukodystrophy may not be appropriate in all cases.(3) As noted by the contributor, the lack of demyelination in the present case is reminiscent of the juvenile and adult forms of AD in humans.

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CASE IV: 08-2005 (AFIP 3133949).

Signalment: 5-month-old, female Maltese dog (*Canis familiaris*).

History: The dog failed to gain weight since obtained two months previously. Neurologic signs of circling, trembling, and loss of hearing and vision developed one month ago.

Gross Pathology: None reported.

Histopathologic Description: The cerebral cortex has severe, diffuse perivascular inflammation composed of lymphocytes and plasma cells, prominent astrocytosis (including many gemistocytic astrocytes), microgliosis, neuronal necrosis, and edema. Clusters of organisms, 1-3 μm in diameter, are present within blood vessels; these organisms are gram positive and consistent with *Encephalitozoon*.

The kidney has diffuse interstitial inflammation composed of lymphocytes and plasma cells within the cortex and similar, but milder inflammation, within the medulla. Extensive cortical tubular necrosis is present and occasional necrosis of medullary tubules is seen. Occasional clusters of organisms are present within cortical tubular epithelial cells and vascular lumina.

Contributor's Morphologic Diagnosis: 1. Encephalitis and meningitis, lymphoplasmacytic, diffuse, severe with intralésional protozoa consistent with *Encephalitozoon*.
2. Interstitial nephritis, lymphoplasmacytic, diffuse, severe with intralésional protozoa consistent with *Encephalitozoon*.

Contributor's Comment: *Encephalitozoon* is a microsporidian in the phylum Microspora and is considered to be a protozoan, although the organism has some features common to fungi. It is an obligate intracellular parasite with a wide host range, but disease is seen most commonly in rabbits and canids, particularly dogs and foxes. Spores are excreted in urine and feces and are resistant to environmental influences. Transmission is by ingestion, inhalation or transplacental. There are several species of *Encephalitozoon* but only *E. cuniculi* is reported in dogs.

Encephalitozoonosis is a systemic disease of neonatal dogs that tends to localize in the kidney and brain. Clinical signs are neurologic in origin. Gross lesions are often absent but radial streaks may be seen in the kidneys. Histopathology reveals a lymphoplasmacytic interstitial nephritis and meningoencephalitis. Spores may be visible in the lesions with hematoxylin and eosin (H&E) staining, but are few and difficult to see. The spores are rod-shaped, 1-3 μm in diameter, and form aggregates within a parasitophorous vacuole. They are located predominantly within endothelial cells but can be found in epithelial cells and macrophages. Spores are gram-positive and are easily visualized with a Gram's stain.

AFIP Diagnosis: 1. Brain, cerebrum and hippocampus: Meningoencephalitis, lymphoplasmacytic and histiocytic, multifocal, moderate, with neuronal necrosis, gliosis, and intraendothelial anisotropic microsporidia.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic and histiocytic, multifocal to coalescing, moderate to marked, with pyelitis, tubular degeneration and necrosis, and few anisotropic microsporidia.

Conference Comment: We thank Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP's Department of Veterinary Pathology, for reviewing this case. Conference participants discussed several potential causes of nonsuppurative encephalitis in dogs, including numerous viruses (e.g. canine distemper virus, canine and feline herpesviruses, rabies virus, avian influenza virus, West Nile virus, other arboviruses) and protozoa (e.g. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*). There is some slide variation in the number of microsporidian organisms present in this case, and in many slides they are difficult to observe with routine H&E staining. Tissue gram staining reveals numerous gram positive organisms in both the brain and kidney, underscoring the utility of special histochemical stains for the detection of pathogens in a diagnostic setting.

Discussion during the conference was primarily devoted to the comparative pathology of mammalian microsporidiosis in general, and encephalitozoonosis in particular. Microsporidia have an obligate intracellular life stage, and exist as environmentally resistant spores outside the host. The polar filament, the defining morphologic feature of the microsporidia, is a unique organelle that remains coiled within the spore until stimulated by some poorly-defined environmental signal to extrude. Upon extrusion, the polar filament penetrates a host cell and injects

infectious sporoplasm, which then divides to form meronts, which further differentiate into sporoblasts, sporonts, and spores; these may be contained within a parasitophorous vacuole, as in the case of *Encephalitozoon* spp., or may remain free in the cytoplasm, as in the case of *Enterocytozoon bieneusi*. Other typical microsporidian features include a proteinaceous exospore; a chitinous endospore; an anchoring disc at the anterior pole; an electron-lucent posterior vacuole; and a distinct lack of mitochondria, peroxisomes, and stacked Golgi membranes at all developmental stages. In addition to polar filament extrusion, phagocytosis of spores by host cells may also produce intracellular infection.(2)

Best characterized in rabbits, the species in which it was first identified, spontaneous encephalitozoonosis has also been described in numerous other species, including guinea pigs, mice, rats, hamsters, muskrats, ground shrews, goats, sheep, pigs, horses, dogs, foxes, cats, exotic carnivores, humans, and nonhuman primates. Generally, infection in immunocompetent rabbits, guinea pigs, mice, and squirrel monkeys is subclinical, whereas infection in domestic dogs, farm-raised blue foxes, and immunocompromised mice and humans results in clinical disease. As noted by the contributor and illustrated by this case, infection in carnivores often results in fulminating disease. In particular, domestic dogs in South Africa and the United States, and farm-raised blue fox kits in Scandinavian countries have been affected in outbreaks. Gross lesions include pale streaks extending from the renal cortex to the renal pelvis; edematous meninges; and thickened, tortuous, medium-sized to small arteries in the heart, intestines, and central nervous system, reminiscent of polyarteritis nodosa. Microscopic lesions include lymphoplasmacytic meningoencephalitis, lymphoplasmacytic nephritis, microgranulomatous hepatitis, and interstitial pneumonia.(2)

In rabbits, *E. cuniculi* is shed in the urine and the ingestion of infective urine is the primary route of infection. The brain and kidney are the organs primarily affected, and usually do not exhibit any gross abnormalities, although multifocal irregularly-shaped depressions in the renal cortex are sometimes present. Microscopic lesions in the brain include multifocal nonsuppurative meningoencephalitis, astrogliosis, and perivascular lymphocytic inflammation; lymphoplasmacytic interstitial nephritis is the typical renal lesion. The microsporidian organisms are primarily identified within epithelial cells, endothelial cells, and/or macrophages of affected tissues, but also are commonly found in the lens, lung, liver, and/or heart.(2)

In guinea pigs, as in rabbits, encephalitozoonosis is usually subclinical, but may result in multifocal necrotizing and granulomatous encephalitis and interstitial nephritis. In mice, *E. cuniculi* results in mononuclear inflammatory foci in the liver, lungs, and brain; the differential diagnosis includes *Clostridium piliforme*, *Corynebacterium kutscheri*, *Pseudomonas aeruginosa*, *Salmonella* species, mouse hepatitis virus (coronavirus), and ectromelia virus (mousepox). Infection in squirrel monkeys is also typically subclinical, but has been implicated in cases of granulomatous encephalitis, nonsuppurative meningitis, and vasculitis in immunosuppressed and neonatal animals; in utero infection is suspected in the latter. Interestingly, microsporidiosis in psittacines is reported with increasing frequency, and many cases are caused by *Encephalitozoon hellem*. Microsporidiosis is an emerging human disease, largely attributed to growing populations of immunocompromised hosts; *E. bieneusi* is the most frequently diagnosed microsporidian pathogen in humans, but disease is also attributed to *E. cuniculi*, *E. hellem*, and *Encephalitozoon intestinalis*.(2)

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