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Department of Veterinary Pathology
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CASE I – Vet. Path. ZH S03-003.1 (AFIP 2936459).

Signalment: 32-year-old, female, captive black rhinoceros (*Diceros bicornis*), perissodactyl.

History: From the age of 15 years, this rhinoceros from the zoological garden, Zürich, showed intermittent reluctance to eat fibrous foods; it hypersalivated and ejected wads which the animal had chewed partially. The diet was Lucerne and grass hay (diet A). From the age of 30 years on, quidding was noticed constantly and the animal lost weight. Green meal pellets were added to the diet (diet B) to improve the fiber utilization. In February 2001, increasing foetor ex ore led to an examination of the oral cavity. Large amounts of dental plaque and calculus were removed. An abscess was discovered in the upper mandible between P1 and P2 and treated. After that, the animal showed an improvement in its food intake. The animal died at the age of 32 years in January 2003. Minor bleeding from the mouth was observed prior to death.

Gross Pathology: The animal was emaciated. There was a fistula in the tongue that extended from the dorsal surface to the base. The teeth were covered with thick dental plaque and calculus. The liver was firm, light brown and showed markedly sharp edges. The duodenal mucosa was a rusty brown.

Laboratory Results: Serology revealed a mean transferrin saturation of approximately 90% (Reference value 28%), and mean ferritin was 6046 ng/ml (Reference value 133 ng/ml).¹

Contributor's Morphologic Diagnoses: 1. Liver: Hepatitis, lymphoplasmacytic, chronic, moderate, with severe fibrosis, biliary duct proliferation, multifocal to

coalescing necrosis, hemorrhage and massive deposition of hemosiderin (Hemochromatosis).

2. Duodenum: Hemosiderin deposition in the submucosa, lamina propria and tips of villi, severe, diffuse (Hemosiderosis).

Contributor's Comment: Iron storage disease was suspected because of the high transferrin saturation and the high level of ferritin in the blood serum. The iron content of diet A and diet B were analyzed and it turned out that the addition of green meal pellets had increased the iron concentration from approximately 270 to approximately 590 mg/kg. Not only had the iron content been increased but also the minor concentration of tannins in the diet had provided more absorbable iron.⁴

Hemosiderosis is defined as a systemic overload of iron resulting in excessive deposition of hemosiderin in different organs or tissues. In contrast, hemochromatosis is also a deposition of hemosiderin but it is always combined with a morphological or functional disturbance of the organ, tissue or cell.⁵

Iron is essential for all living organisms, but it also may act as a potent toxin. Evolutionary mechanisms have therefore resulted in the adaptation of specific proteins for the uptake, transport (transferrin), utilization (hemoglobin), and storage of iron. Regulation of the uptake is essential because the mechanisms for excretion are limited. Iron is absorbed predominately in the duodenum and proximal jejunum. Here, the absorbed iron is bound to ferritin in the intestinal epithelial cells. The absorption rate and the release of iron-loaded ferritin into the blood are dependent on the plasma iron level. The remaining iron is lost when the epithelial cells are sloughed into the intestinal lumen. Most of the plasma iron is bound to transferrin.³

These mechanisms are referred to as "mucosal block" or, in human medicine, as feedback. This feedback is the most important limiting step in iron absorption and is designed to prevent iron uptake exceeding the range to which a species has adapted during its evolution. This species-specific range is the iron concentration in the animal's natural diet.

Chronically excessive dietary iron will lead to iron storage. The lack of dietary tannins in captivity could be responsible for excessive iron disease. Supportive for this hypothesis is the fact that not only black rhinoceroses suffer from iron storage disease, but also many different captive mammalian herbivores and birds that naturally consume large amounts of tannin. However, the analysis of postmortem examinations of exotic animals from different zoos suggested that it was not the content of tannins per se but rather the species' adaptation to a low iron natural diet that dispose them to excessive iron storage when they receive diets with high-iron content in captivity.^{1,2}

In contrast to these species, sera from captive foregut-fermenting browsers showed no elevation of transferrin saturation or ferritin levels. A possible explanation could be that the ingesta remain in the forestomachs longer whereby the tannins have more time to bind to the salivary and microbial proteins instead of the dietary iron. Furthermore, the anaerobic conditions of the forestomachs may reduce more normally unavailable Fe^{3+} ions to the more readily available Fe^{2+} ions. Thus, for such animals an effective iron absorption mechanism is unnecessary and the maintenance of controlled absorption for higher available iron ranges via feedback is important. There was no evidence of iron excess in the African white rhino (*Ceratotherium simum*) and the Indian rhinoceros (*Rhinoceros unicornis*). These grazers naturally consume large amounts of grass with a much higher concentration of iron and almost no tannins.^{1,2}

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, periportal to midzonal, chronic, diffuse, marked, with moderate biliary duct proliferation, fibrosis, and hemosiderosis, black rhinoceros (*Diceros bicornis*), perissodactyl.

Conference Comment: The contributor provides an excellent summary of the pathogenesis of hemochromatosis and defines the difference between hemosiderosis and hemochromatosis. Diffuse hemosiderosis is common in all species and when present is suggestive of excessive hemolytic activity relative to the reutilization rate of iron. Hemosiderosis is seen in hemolytic anemias; anemia of copper deficiency; cachexia; severe chronic passive congestion of the liver, lungs, or spleen and in areas of hemorrhage. Additionally, hemosiderin is normally present in the early neonatal period when fetal hemoglobin is being replaced by mature hemoglobin. The ferric iron component of hemosiderin can be demonstrated by staining with Prussian blue. Hemosiderin has a chemical structure identical to ferritin. Hemosiderin must be distinguished from lipofuscin, hematin, and bile pigments.^{6,7}

As pointed out by the contributor, tannins chelate iron forming a non-absorbable complex; therefore, decreased tannins in the diet increase the bioavailability of iron. Increased bioavailability of iron results in the increased accumulation of iron in tissues in animals' whose natural diet has high concentrations of tannins, phytates, fiber, polyphenolics, phosphates, and other compounds that chelate iron into insoluble complexes that normally pass through the gastrointestinal tract unabsorbed.^{1,2}

Hemochromatosis has also been reported in humans, hyraxes, lemurs, simians, prosimians, various avian species (mynahs, birds of paradise, toucans), gorillas,

tapirs, pikas, pinnipeds, cheetahs, snow leopards, siamangs, callithrichids, cattle (hereditary hemochromatosis of Salers cattle), and sheep.^{1,2,6,8}

Conference participants reviewed iron parameters useful in the diagnosis of hemochromatosis such as serum iron concentration, total iron binding capacity (TIBC), ferritin levels, transferrin levels, and percent saturation of transferrin.⁸

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CASE II – S2671-02 (AFIP 2890238).

Signalment: Seven-week-old, female, Arabian crossbred, equine.

History: This 7 week-old Anglo Arabian filly died while suffering from chronic respiratory disease and osteomyelitis. Radiography and ultrasound examination prior to death indicated what appeared to be multiple pulmonary abscessation and osteomyelitis of the distal left metacarpal bone. *Nocardia asteroides* had been isolated from a needle aspirate of the exudate from the suspected osteomyelitis lesion before death.

Gross Pathology: Severe, bilateral, subacute, diffuse fibrinonecrotic broncho-pneumonia involving the cranial and cardiac lobes. There appeared to be aplasia of the thymus as well as severe generalized hypoplasia of lymph nodes. The spleen was small (\pm 21x4x1,5cm) with inconspicuous lymphoid tissue. There was a moderate fibrinopurulent sialoadenitis of the mandibular salivary glands as well as a necropurulent osteomyelitis of the distal palmar surface of the left metacarpal bone.

Laboratory Results: Haematology (absolute counts):

WBC	6,500/ul (N 5,400–14,300)
Neutrophils	6,150/ul (N 2,260-8,580)
Band cells	7/ul (N 0-100)
Lymphocytes	30/ul (N 1,500–7,700)

There was a low absolute lymphocyte count of 30/ul compared to the normal range of 1,500–7,700/ul. However, the total white blood cell count was at the lower margin of the normal range.

Microbiology: A pathogenic smooth isolate of *Escherichia coli* and *Klebsiella pneumoniae* were isolated from several organs, including the lungs. *Nocardia asteroides* was isolated from the osteomyelitis lesion.

Genetic Analysis: DNA extracted from wax-embedded blocks of spleen tissue of this foal displayed the 158 base pair alleles characteristic of the severe combined immunodeficiency disease (SCID) genetic mutation using PCR amplification with specific DNA primers. DNA extracted from blood samples collected from the sire and dam of the foal were heterozygous for the 158 and 163 base pair alleles typical of carriers of severe combined immunodeficiency disease.

Contributor's Morphologic Diagnoses: 1. Pancreas: Moderate multifocal to coalescing, subacute, necropurulent pancreatitis associated with the presence of numerous large, amphophilic, intranuclear inclusion bodies, morphologically compatible with adenovirus infection.

2. Spleen: Severe diffuse lymphoid hypoplasia with an almost total absence of lymphoid tissue in the periarteriolar lymphoid follicles and sinusoids.

Contributor's Comment: Inclusion bodies appeared to be present within the nuclei of the pancreatic acinar and duct cells only. The presence of adenoviral inclusion bodies within the pancreas and their absence from the lung appears to be unusual when compared to previous reports of their presence within the lungs and pancreas. There was moderate necrosis and infiltration of neutrophils as well as scattered colonies of coccoid to bacilliform bacteria within the pancreas.¹

On transmission electron-microscopy of uranyl acetate- and lead citrate-stained sections of pancreas, characteristic adenovirus particles in crystalline array could be identified within the intranuclear inclusion bodies at 19000x magnification. The breed and the history of death from multiple infections at the early age of 7 weeks, in conjunction with severe lymphopaenia, lymphoid hypoplasia and positive PCR results are consistent with a diagnosis of SCID in Arabian and related horse breeds^{1,2,3,4,5,6,7}

The disease has been shown to be caused by an autosomal recessive trait expressed as severe B and T lymphocyte dysfunction at the level of the prolymphocytic bone marrow-derived and thymus dependent stem cells. The basic genetic lesion, induced by a recessive gene mutation, comprises a five base pair deletion in the gene responsible for B and T cell lymphopoiesis. This deletion has been shown to cause an inactivation of the gene for DNA-dependent kinase, catalytic subunit DNA-PK, which is required for the coding sequences of immunoglobulin and T cell antigen receptors during B and T cell lymphopoiesis.^{1,4,5,6,7}

The disease is believed to occur in approximately 0.2-2% of Arabian-bred foals of both sexes, but the incidence of phenotypically normal heterozygotes will be much higher. Carriers pass the gene on to 50% of their offspring, and affected foals are born from 25% of successful matings between carriers.^{1,4,5,7,8}

A specific test for the SCID mutation provides the ability to screen Arab horses used for breeding. DNA is extracted from appropriate samples (whole blood sample in EDTA or sodium citrate, hairs with roots or any other tissue) collected from the horse in question. Appropriately labeled DNA primers (forward primer 5'-AAG TTG GTC TTG TCA TTG AGC-3'; reverse primer 5'-TTT GTG ATG ATG TCA TCC CAG-3') that flank the gene region affected by the mutation are used in a PCR to amplify the affected region from the DNA sample. The fragment sizes can be determined using a genetic analyzer. The fragment size generated by the wild type allele is 163 base pairs and the fragment generated by the SCID allele is 158 base pairs.^{5,6,7}

Affected foals are normal at birth, but at 10 days of age frequently develop pneumonia and diarrhoea as a result of bacterial and adenovirus infections, as typified in this case. There is usually a severe lymphopaenia, as well as hypoplasia of all lymphoid tissue including thymus, spleen, and lymph nodes, with a marked

paucity of tissue- and circulating lymphocytes, as demonstrated in this case. The total white cell count is usually within the normal range as a result of compensation by increased neutrophils or immature leucocytes. Despite intensive veterinary care, foals rarely survive for longer than 3 months.¹

Although there were numerous adenovirus inclusions in the pancreas, no inclusions could be seen within the lungs. The identity of the adenovirus inclusions could be established by their characteristic morphology of crystalline array by transmission electron microscopy.²

AFIP Diagnoses: 1. Pancreas: Pancreatitis, necrotizing, multifocal to coalescing, marked, with intraepithelial basophilic intranuclear inclusion bodies, etiology consistent with adenovirus, Arabian crossbred (*Equus caballus*), equine.
2. Spleen, white pulp: Lymphoid hypoplasia, diffuse, severe.

Conference Comment: The contributor provides a thorough overview of severe combined immunodeficiency (SCID) in Arabian foals. As stated by the contributor, SCID in Arabian foals involves failure of maturation of both B- and T-lymphocytes. Neutrophils, macrophages, natural killer (NK) cells and the complement system function normally. In addition to adenovirus, SCID foals often succumb to secondary infections with *Pneumocystis carinii*, *Rhodococcus equi*, and *Cryptosporidium parvum*, or a variety of common equine bacterial pathogens.¹⁰

SCID has also been reported in dogs, mice and humans. Two molecular mechanisms account for SCID in dogs. X-linked SCID has been described in Bassett Hounds and a Cardigan Welsh Corgi resulting from a mutation in the gene encoding the γ -chain of the IL-2 receptor. The same γ -chain is also a component of the IL-4, IL-7, IL-9, IL-15, and IL-21 receptors. T-cell numbers may be nearly normal in affected dogs and their ability to produce IL-2 is not impaired. However, the defect in the γ -chain renders T cells unable to bind and respond to IL-2 resulting in T cells that are unresponsive to mitogenic stimuli, increased susceptibility to infection, thymic dysplasia, hypogammaglobulinemia, and a failure to grow normally. Jack Russell Terriers have a mutation within the DNA-PKcs gene. A spontaneous mutation in DNA-PKcs of BALB/C mice results in SCID, as well as experimentally induced mutations in recombinase-activating genes 1 and 2 (RAG1 and RAG2). The most common form of human SCID is X-linked with a mutation in the common γ -chain subunit of several cytokine receptors resulting in a profound defect in the earliest stages of lymphocyte development. Other forms show autosomal recessive inheritance and are usually due to an adenosine deaminase deficiency which may cause impaired DNA synthesis in lymphocytes.^{1,10,11}

There was some discussion as to whether the bacteria present are ante- or post-mortem. The location (intravascular), morphology (short rods), and lack of inflammation are supportive of post-mortem bacterial overgrowth. As stated previously, in SCID foals, neutrophils, macrophages, NK cells and the complement system function normally. However, the failure of maturation of B and T cells leads to ineffective cell mediated and humoral immunity and foals often succumb to a variety of common equine bacterial pathogens.¹²

Readers are encouraged to review reference 9 --- [Animal models molecular pathology of severe combined immunodeficiency in mice, horses, and dogs.](#)

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CASE III – 062172 (AFIP 3026810).

Signalment: Field vole (*Microtus arvalis*), male, one-year-old.

History: Animal captured near Lausanne (Switzerland) for breeding research, then submitted and euthanized for routine sanitary control of the colony to the platform of veterinary diagnosis of the University of Geneva (Switzerland). Graciously sent to the Department of Pathology of the Nantes Veterinary School for unreservedly pedagogic use by Dr. Laurence Fiette.

Gross Pathology: Infestation by fleas (*Nosophyllus sp*). Liver: Multiple multivesicular cysts invading the parenchyma, a few mm in diameter and containing whitish solid material.

Laboratory Results: No significant results from the routine sanitary control (annual FELASA list of agents).

Histopathologic Description: The liver is totally invaded by alveolar structures composed of numerous cysts of irregular shape, a few millimeters in diameter. The cysts contain numerous invaginated heads (protoscolices) and an eosinophilic material. The wall of these cysts has two layers. The external layer is acellular and fibrous. The inner layer is the germinal membrane producing the protoscolices. The presence of these cysts elicits a fibrous reaction of the liver tissue and a minimal mononuclear inflammatory reaction.

These structures are characteristic of the metacestode stage of *Echinococcus multilocularis*.

Contributor's Morphologic Diagnosis: Alveolar echinococcosis.

Other names of the disease: Alveolar hydatid disease, multilocular echinococcosis or multivesicular hydatidosis.

Contributor's Comment: Voles - *Microtus arvalis*, (field vole) and *Arvicola terrestris* (water vole) - and other Arvicolidae as well as small mammals (lemmings, shrews, mice) are natural intermediate hosts for *E. multilocularis* (Plathelminthes, Cestodes, Taeniidae). Intermediate hosts become contaminated by ingestion of eggs released from the definitive hosts to the environment. Foxes, and in some endemic areas, other species of wild carnivores (coyotes, wolves and raccoon dogs) are the definitive hosts in the sylvatic cycle. Dogs and cats are also definitive hosts in synanthropic or domestic cycles.

M. arvalis is a very sensitive intermediate host, in which cysts are fertile and contain numerous protoscolices. The metacestodes develop primarily in the liver, like in this case, but can be observed in other organs, especially the brain and lungs.

Besides rodents, humans and a number of mammals, may also acquire *E. multilocularis* and become aberrant hosts which do not play a role in the transmission cycle. Cysts have been reported in dogs, cats, domestic and wild pigs, horses, and primates.¹

The metacestode stage is characterized by an alveolar structure, composed of numerous small vesicles. This stage has a characteristic exogenous tumor-like proliferation, which leads to infiltration of the affected organs and, in progressive cases, to severe disease and even death.

In man, *E. multilocularis* infection is considered as the most severe helminthic infection, lethal without treatment.

This zoonosis is known in Northern America and Northern and Central Eurasia.

The spatial distribution of infected rodents is heterogeneous. The average prevalence of *E. multilocularis* metacestodes in rodents is generally low (<1%) in areas of endemic infection in central Europe. But they may be higher locally, in "hot spots" of intensive transmission. For example, up to 21 and 39% of *Arvicola terrestris* were found to be positive in high-endemicity areas.^{2,3}

Following the successful oral vaccination campaigns against rabies in Europe, the fox population densities have increased from 1985 onwards in many countries. Moreover, red foxes are now more and more frequent in urban areas not only in Great Britain as known since the 1930s, but in other countries including 20 of the 30 largest Swiss towns. Their number tends to increase as reported in Zurich, Switzerland (20-fold from 1985 to 1997).^{1,3}

Strong evidence for the existence of an urban cycle has been provided from many European cities. *E. multilocularis* prevalences in Zurich of 47% (61/129) in foxes from the city and of 67% (82/123) in foxes from the adjacent rural area are reported. Furthermore, metacestodes of *E. multilocularis* were found in 14% (19/135) of water voles (*A. terrestris*) in a Zurich city park, and two animals harbored protoscolices of the parasite. Therefore the establishment of urban cycles of *E. multilocularis* and potential risk for urban residents and pet animals has become an important issue.^{2,4}

In this animal, metacestodes of *T. taeniaeformis* (*Strobilocercus fasciolaris*) were also found. This co-infection has already been reported in rare cases.^{4,5}

AFIP Diagnosis: Liver: Hydatid cyst, field vole (*Microtus arvalis*), rodent.

Conference Comment: The contributor provides an excellent overview of *E. multilocularis*. Additional species of *Echinococcus* include *E. granulosus*, *E. oligarthus*, and *E. vogeli*. The latter two involve sylvatic cycles in Central and South America, with felids and canids as definitive hosts, respectively, and rodents as intermediate hosts. *E. vogeli* may infect humans.⁶

Readers are encouraged to compare this case to WSC 7, case 2, 2006-2007 *Echinococcus granulosus* in a mountain goat. In contrast to *E. multilocularis*, *E. granulosus* forms a unilocular hydatid cyst and is usually not invasive.

Skeletal muscle and esophagus were present in some sections.

This case was reviewed in consultation with Dr. Chris Gardiner.

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CASE IV – 051203 (AFIP 2983846).

Signalment: 6-year-old, male, Bernese Mountain Dog, canine.

History: Responsive anemia, splenomegaly.

Gross Pathology: Splenomegaly with infarction.

Histopathologic Description: Spleen: The red pulp is diffusely expanded by variably differentiated histiocytes with moderate to marked hemophagocytosis. The cells have abundant, pale eosinophilic, often coarsely vacuolated cytoplasm and oval and occasionally indented nuclei. The cytoplasm of histiocytes frequently contains hemosiderin and occasionally red blood cell precursors and granulocytes. Mitoses are 1-3 per HPF. There is mild anisocytosis and anisokaryosis. Marked extramedullary hematopoiesis is present. Extensive areas of red pulp infarction associated with thrombosis are present within some sections.

Immunohistochemistry: The neoplastic cells express CD11d, CD18 and CD45 antigens.

Contributor's Morphologic Diagnosis: Hemophagocytic histiocytic sarcoma, spleen, Bernese Mountain Dog, canine

Contributor's Comment: Histiocytic disorders of dogs include histiocytoma, localized histiocytic sarcoma (HS), disseminated HS (equivalent to malignant histiocytosis), and the reactive histiocytoses: cutaneous histiocytosis and systemic histiocytosis. A common element to these diseases is proliferation of dendritic cells (DC) of either Langerhans cell (epithelial DC) or interstitial DC lineage.¹

Hemophagocytic HS is a distinctive clinical and pathologic entity, marked by an aggressive clinical course dominated by splenomegaly, regenerative anemia,

thrombocytopenia, hypoalbuminemia, and hypocholesterolemia. Hemophagocytic histiocytic sarcomas of the spleen (and bone marrow) arise from splenic red pulp (and bone marrow) macrophages. Hemophagocytic HS is prevalent in the same breeds affected by localized and disseminated HS, which include Bernese Mountain Dog, Golden Retriever, Rottweiler and Labrador Retriever. Hemophagocytic HS initially involve spleen and bone marrow simultaneously and later spread to liver and lungs often via insidious intravascular invasion with minimal mass formation. The splenic lesions consist of diffuse splenomegaly often with additional, ill-defined masses and infarction. Neoplastic histiocytes dominantly expressed MHC class II and the leuko-integrin CD11d/CD18; expression of CD11c/CD18 and CD1c is far less prevalent. In contrast, localized and disseminated HS as previously reported dominantly expressed CD1c, CD11c, and MHC class II and lacked expression of CD11d, which supported their origin from interstitial DC.^{2,3}

The clinical presentation of hemophagocytic HS is often confused with immune-mediated hemolytic anemia (IMHA) or more specifically with Evan's syndrome, because thrombocytopenia usually occurs concurrently. The direct anti-globulin (Coombs) test is negative in dogs with hemophagocytic HS. In contrast, dogs with IMHA typically have higher serum bilirubin concentrations. Additionally, hypoalbuminemia and hypocholesterolemia were common in dogs with hemophagocytic HS, which also helped to differentiate it from IMHA.²

Hemophagocytic HS of dogs shares clinicopathologic and morphologic features with a rare subtype of malignant histiocytosis of humans, also referred to as histiocytic medullary reticulosis. In histiocytic medullary reticulosis, anemia, thrombocytopenia, and hyperbilirubinemia occur in association with diffuse hepatosplenomegaly and bone marrow infiltration. Cytologically atypical, hemophagocytic histiocytes expand the splenic red pulp and invade the hepatic sinusoids without significant mass formation.⁴

AFIP Diagnosis: Spleen: Hemophagocytic histiocytic sarcoma, Bernese Mountain Dog (*Canis familiaris*), canine.

Conference Comment: The contributor provides an excellent and thorough summary of hemophagocytic histiocytic sarcoma to include clinical presentation, associated clinical pathologic findings, breeds affected, biological behavior, and immunohistochemical findings.

As previously stated by the contributor, histiocytic disorders in the dog include histiocytoma, localized histiocytic sarcoma (HS), disseminated HS (equivalent to malignant histiocytosis), and the reactive histiocytoses: cutaneous histiocytosis and systemic histiocytosis. Histiocytoma is a localized tumor of epidermal

Langerhans cells. Localized and disseminated HS likely arise from interstitial dendritic cells prevalent in almost all organs and tissues with the exception of the brain. The reactive histiocytoses are complex disorders likely associated with disordered immune regulation characterized by infiltration or proliferation of lymphocytes and perivascular interstitial dendritic cells of the dermis and subcutis. As previously stated by the contributor, hemophagocytic HS is a proliferative disease of splenic red pulp and bone marrow macrophages; it is not a proliferative disorder of interstitial dendritic cells, as are most histiocytic sarcomas in the dog.^{2,3}

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<http://www.vetmed.ucdavis.edu/pmi/>

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