The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

CONFERENCE 1
7 September 2005

Conference Moderator: COL Dale Dunn, DVM, Diplomate ACVP
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Washington, DC 20306

CASE I – 04W10854 (AFIP 2979950).

Signalment: Adult male mule deer (*Odocoileus hemionus*).

History: A blind alert deer was found in southeastern Wyoming in October by several hunters. The animal was reluctant to stand and when it did so it stumbled into trees. They shot it in the chest. A fresh carcass was submitted for examination.

Gross Pathology: Gross examination revealed a well-nourished adult (~3 - 5 year old) buck deer with bilateral corneal opacity. Internal organs were unremarkable.

Laboratory Results: *Yersinia pestis* was cultured from conjunctival sac and cornea. Retropharyngeal lymph nodes were positive for chronic wasting disease using a proprietary ELISA test. Samples of liver and lung were cultured aerobically, with negative results.

Contributor's Morphologic Diagnosis: Anterior segment, eye: 1. Severe acute diffuse endophthalmitis with myriad intracellular and extracellular coccobacilli.
2. Severe acute diffuse stromal keratitis with intralesional coccobacilli, limbal neovascularization, and non-occlusive fibrinous thrombosis.
3. Retinal detachment, with acute bacterial retinitis.

Contributor's Comment: The mule deer had bilateral ocular infection due to *Yersinia pestis*. In addition to these changes there were acute necrotizing inflammatory lesions in lung, adrenals, lymph node, and liver with intralesional bacteria, and disseminated intravascular coagulation.

Plague is unusual in big game animals and ungulates are generally considered resistant to the disease. There is a published report of plague in a free-ranging mule deer in Wyoming,[1] an unpublished, laboratory-confirmed case in a mule deer in Montana,[2] and bilateral plague-associated necrotizing panophthalmitis in a black-tailed deer in California.[3] Ocular plague has been seen in Colorado (Dr. M. Miller, Colorado Division of Wildlife, unpublished observations).

Numerous organisms are present in the anterior and posterior chambers, in the drainage angle, and between the pigmented epithelial cells at the posterior aspect of the iris. Unilateral plague endophthalmitis without evidence of disseminated disease was reported in a man who had recently handled poisoned rats.[4] He was treated successfully and with no loss of vision by intravenous and subconjunctival antibiotics.

Plague is an endemic disease of rodents in the western United States, with occasional spread into human and non-rodent populations via enzootic or amplification hosts. In addition to virulence factors common to the yersiniae that promote tissue invasion and resistance to successful phagocytosis, *Y. pestis* has additional virulence factors. These include pesticin, which serves as a plasminogen activator, murine toxin, and F_1- toxin, which forms a gel-like envelope around cells that protects them from phagocytosis. The florid inflammatory and necrotizing lesions present in this animal's eyes are presumably a consequence of these factors.

The animal's retropharyngeal lymph nodes were positive for chronic wasting disease. No lesions were present in the brain and the animal was in good nutritional condition. As CWD is common in mule deer in southeastern Wyoming, this may be an incidental finding.

AFIP Diagnosis: Eye: Endophthalmitis, suppurative, severe, with myriad coccobacilli, and moderate stromal keratitis, mule deer (*Odocoileus hemionus*), cervid.

Conference Comment: Conference attendees briefly reviewed the anatomy of the eye including the layers of the retina. Discussion was directed at developing a differential diagnosis for large, plaque-like colonies of bacteria. Residents at AFIP utilize the mnemonic "YACS" to develop a differential diagnosis when large colonies of bacteria are present in hematoxylin and eosin stained sections. YACS stands for:

Y *Yersinia sp*.
A *Actinomyces sp., Actinobacillus sp. Arcanobacter sp.*

C *Corynebacterium sp., Clostridium sp.*
S *Staphylococcus sp., Streptococcus sp.*

Attendees eliminated *Actinomyces sp.* and *Actinobacillus sp.* from the differential diagnosis based on the bacteria's morphology. *Actinobacillus sp.* is a Gram negative rod and *Actinomyces sp.* is a Gram positive rod which forms branching filaments. Gram stains were then used to eliminate *Staphylococcus sp*., *Streptococcus sp*., *Arcanobacter sp*., *Corynebacteria sp.* and *Clostridia sp*., all Gram positive bacteria, from the differential diagnosis. The only remaining bacterium from the differential diagnosis list was *Yersinia sp*., a Gram negative coccobacillus.

The contributor provides a concise summary of the virulence factors associated with *Y. pestis* infection. *Yersinia pestis*, the bacterium which causes "plague", is transmitted primarily by fleas and less commonly by the consumption of animals or inhalation of aerosolized droplets from animals with the pneumonic form of the disease. The three clinical manifestations of "plague" are the bubonic form, commonly associated with swollen, ruptured and draining inguinal and axillary lymph nodes or "bubos"; the rapidly fatal pneumonic form and the septicemic form.

Participants briefly discussed *Y. pestis* as a potential biological weapon. The Centers for Disease Control (CDC) lists *Y. pestis* as a Category A agent. Category A agents have the greatest potential for inflicting high numbers of human casualties, can be manufactured and disseminated on a large scale, require significant efforts in public health preparedness, and are most familiar to the public.

Contributor: Department of Veterinary Sciences, University of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070, USA. http://wyovet.uwyo.edu/

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CASE II - 1 (AFIP 2987474)

Signalment: 24-year-old male castrated Selle Français horse (*Equus caballus*)

History: The owner noticed blood droplets in the preputial opening of this horse and sent him to the veterinary hospital in Berne, Switzerland. The horse was in good body condition. He presented with a multilobulated, cauliflower-shaped, about 10 cm in diameter, partially ulcerated nodule on the penis (preputium). The clinical suspected diagnosis was a squamous cell carcinoma.

Gross Pathology: Provided.

Laboratory Results: None

Histopathologic Description: The subepithelial mucosa is expanded by numerous confluent granulomatous inflammatory foci that contain multiple tangential and cross sections of well preserved larval and adult nematodes and cellular debris surrounded by epithelioid macrophages, multinucleated giant cells and, at the periphery, by moderate numbers of plasma cells and lymphocytes admixed with fewer eosinophils. The nematodes are 10 to 25 μm in diameter with a smooth cuticle, platymyarian-meromyarian musculature, a long rhabditiform esophagus with terminal bulb, numerous deeply basophilic 2-3 μm internal structures, tubular digestive tract lined by low cuboidal epithelium, tapered tail and pseudocoelom that are often visible. The nematodes are interpreted as *Halicephalobus gingivalis*.

Contributor's Morphologic Diagnosis: Posthitis, nodular to diffuse, severe, chronic, granulomatous, with numerous larval and adult nematodes, etiology consistent with *Halicephalobus gingivalis*, Selle Français, equine.

Contributor's Comment: Halicephalobus gingivalis (Micronema deletrix) has been sporadically associated with equine infections. The organism has been reported in human infections and has also been the cause of extensive encephalitis in a bighorn sheep. Halicephalobus infection has been reported in Japan, Egypt, Switzerland, the Netherlands, the United Kingdom, Colombia, and the United States. Inflammation of the central nervous system is a consistent feature of infection in human beings and almost always occurs in equine infections (1, 4, 8, 11, 12). Vulnerable organs include brain (1) and spinal cord (4), optic nerve (9), lungs, skin, kidneys, maxilla, nasal cavities, bone and joints, prepuce and testicles (10). The brain is the most commonly involved tissue, followed in descending order by the kidneys, oral and nasal tissues, lymph nodes, lungs, spinal cord, and adrenal gland. There are also reports of involvement in the heart, liver, stomach, ganglion, bone, and prepuce. (2: Dunn DG, et al, 1993).

Halicephalobus is a parasite of which, to date, only females have been identified. This order of nematode (Rhabditida) can be found free-living in soil and humus. To date, identification of Halicephalobus in soil or other environmental samples has required labour-intensive microscopic identification of small adult nematodes present in the material (6: Nadler et al. 2003). Infections have been postulated to be associated with skin wounds. Of interest here is that viable organisms were noted in the sperm and urine of 2 stallions (5: Kinde H et al, 2000), suggesting the possibility of a urogenital route of infection. There are eight members of the genus, all of which are free-living saprophytes found in soil and decaying organic matter, except for H. gingivalis (7).

The identification of the parasite within tissues is based on the following criteria: a smooth, thin cuticle; platymyarian-meromyarian musculature; a pseudocoelom; a rhabditiform esophagus composed of a corpus, isthmus, and bulb; an intestinal tract composed of single nucleated, low cuboidal cells; flexed ovary and uterus; and a tapered tail. The flexed ovary and pointed tail differentiate this organism from other rhabditiforms.
Other rhabditid parasites infecting the horse include Pelodera strongyloides, Strongyloides westeri and Cephalobus sp. These nematodes must be differentiated from H. gingivalis in verminous cutaneous or mucocutaneous lesions. Differentiation is based upon location and severity of lesions and parasite morphology. Pelodera causes a self-limiting dermatitis normally confined to the ventral abdomen and limbs. The life-cycle of Strongyloides westeri involves cutaneous penetration by larvae; adults and eggs are not found in the skin. Cephalobus sp. can be distinguished from H gingivalis by its blunt posterior end and differences in the shape of the stoma and esophagus (Cephalobus has a greater ratio of corpus to isthmus).
Other causes of equine verminous encephalitis may include Hypoderma bovis, Hypoderma lineatum, Strongylus vulgaris, and Draschia megastoma. Setaria spp. are reported to be a common cause of cerebrospinal nematodiasis in horses in Asia. Other causes of ataxia in horses include trauma, degenerative myelopathy, Wobbler's syndrome, neoplasia, and various infectious agents. Differential diagnoses include also other infectious causes of granulomatous inflammation in skeletal, urinary, or central nervous system.

The pathogenesis, life-cycle, and route of infection of H. gingivalis are poorly understood. The route of infection in humans seems to be from manure contaminated skin lacerations. Speculated routes of infection in the horse include: skin and mucous membrane penetration in recumbent animals by the free-living form of the parasite in the soil with subsequent invasion of the sinuses and bones of the head, and/or hematogenous spread to internal parenchymal tissues; prenatal or transmammary infection of suckling foals (13: Wilkins P.A. 2001). The

nematode gains access to the brain via migration along vessels; continued migration within the brain causes necrosis and inflammation.

The typical clinical findings in horses include: lethargy, loss of condition, hyperesthesia, opisthotonos, nystagmus, ataxia, progressive neurologic signs, recumbency, and multiorgan dysfunction. Hyperglobulinemia and hyperfibrinogenemia (chronic inflammation); elevated creatine kinase (muscle injury from parasites, trauma, or recumbency); elevated creatinine (postrenal obstruction due to lower motor neuron disease if cauda equina neuritis). Abnormal clinical pathology values and clinical tests depending on organs affected and extent of disseminated lesions.

The typical gross findings are multifocal to diffuse, proliferative, firm, gray-white, granulomatous lesions.

The typical light microscopic findings include: Adult females, 15-20 mm in diameter, 250-430 mm in length, with a thin, smooth cuticle and tapered, pointed tail; platymyarian-meromyarian musculature; a pseudocoelom; a rhabditiform esophagus composed of a corpus, isthmus, and bulb; an intestinal tract lined by uninucleate, low cuboidal cells; and a single genital tube, with a dorsoflexed ovary and a ventroflexed uterus at the vulva.
Larvae, approximately 10 mm diameter, with a rhabditiform esophagus and tapered, pointed tail. Embryonated eggs are oval and average 15x35 mm.
Perivascular granulomatous inflammation with numerous adult and larval parasites; necrosis, vasculitis, and hemorrhage.
Normal tissue architecture may be replaced by dense collagen and fibroblasts, with infiltration of tissue by lymphocytes, plasma cells, epithelioid macrophages, multinucleate giant cells, eosinophils, and intralesional adult and larval nematodes.

H. gingivalis disseminated disease has been reported in a Grevy's zebra (Equus grevyi) (3).

AFIP Diagnosis: Prepuce (per contributor): Posthitis, granulomatous, nodular, severe, with numerous rhabditid adults, larvae, and eggs, etiology consistent with *Halicephalobus sp.*, Selle Français, equine.

Conference Comment: Conference attendees briefly reviewed the histomorphological characteristics of metazoan parasites. Metazoan parasites can be broken down into six morphologically distinct categories: nematodes, acanthocephalans, trematodes, cestodes, arthropods and pentastomes. Below is a simple table to identify the parasite to its group:

GROUP	GENERAL SHAPE	BODY CAVITY	DIGESTIVE TRACT	STRIATED MUSCLE	SPECIAL DIAGNOSTIC FEATURES
Cestode	Flattened dorso-ventrally	--	--	--	1. calcareous corpuscles 2. scolex 3. tegument
Trematode	Flattened dorso-ventrally	--	+	--	1. suckers 2. tegument 3. blind ceca 4. yolk gland 5. hermaphroditic (except Schistosomes)
Acanthocephalan	Spherical in section, flat when viable	+	--	--	1. hypodermis 2. lemniscus 3. two muscle layers 4. proboscis
Nematode	Spherical	+	+	--	1. cuticle 2. musculature
Arthropod	Tend to be spherical	+	+	+	1. chitinized exoskeleton 2. jointed appendages 3. tracheal tubes
Pentastomes	Spherical	+	+	+	1. chitinized exoskeleton 2. digestive glands 3. sclerotized openings

Once the organism has been identified as a nematode, it must be further classified into one of the following groups: Aphasmids or Phasmids. Aphasmids lack a tiny pair of sensory papillae (the phasmids) on the caudal end; however, these are not readily identifiable in histologic sections. The histologic features that distinguish them from phasmid nematodes are hypodermal bands with associated nuclei, and prominent esophageal glands that form a stichosome. The Phasmids consist of the Rhabditoids, Oxyurids, Ascarids, Strongyles, Spirurids, and Filarids. Both the Rhabditoids and Oxyurids have a rhabditoid esophagus composed of a corpus, isthmus and bulb. The Strongyles have a cuticle, which occasionally is ridged, and all have an intestine composed of few multinucleated cells and a prominent brush border. Spirurids can be very diverse, but all adult females in this group produce embryonated eggs. Filarial nematodes are small and can produce either eggs or free, distinctive larvae; microfilariae.

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. Dr. Gardiner adds," The reaction is what we would expect in cutaneous halicephalobiasis. The organisms are plentiful and are described well by the contributor. I would only offer this note. I'm not so sure that the species in question is *gingivalis.* I have, for a long time, suspected more than one species is involved in these cases. And, there have been lots of cases. The key in differentiating this parasite from others in histological section, is the characteristic rhabditiform esophagus and the dorsal reflection of the ovary….both can be seen readily in the section. The other parasites mentioned in the differential do not have

these characteristics." Dr. Gardiner also comments on other cases where viable organisms were found in sperm, "… it leaves speculation if the infection can be passed by artificial insemination. I wonder if the parasites become "dormant" when frozen down with the sperm, and then become viable when the sperm is brought to room temperature."

The contributor provides a thorough description of the pathogenesis, clinical, gross and histologic lesions associated with *Halicephalobus* infection as well as the nematode's distinguishing morphologic features and an extensive differential diagnosis list. Interestingly, adult females are parthenogenetic and thus can produce viable larvae without the necessity of mating which can result in large numbers of larvae as are seen in this case. We are grateful to Dr. Gardiner for his comments on this interesting case.

Contributor: Institute of Animal Pathology, University of Berne, Vetsuisse Faculty, Länggassstrasse 122, P.C. 8466 CH - 3001 Berne

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CASE III –05-255 (AFIP 2984136).

Signalment: 10 year-old, intact female Barn owl (*Tyto alba)*

History: This ten year-old Barn owl had the right wing amputated six years prior. The owl presented with a large growth at the amputation site. The animal died acutely soon after presentation.

Gross Pathology: Necropsy revealed an amputation site at the humeral-ulnar joint of the right wing. Along the medial aspect of the humerus there was a 3.5cm x 3cm x 2cm white-grey raised mass which contained a 1cm x 1.5cm firm red central ulcerated region exuding tan white material.

Laboratory Results: Cultures of the bone consisted of 30 colonies of *E. coli*, >100 colonies *Proteus mirabilis* and approximately 50 colonies of Enterococcus.

Histopathologic Description: Humeral bone: Most of the normal architecture of the humerus is obliterated by the presence of a proliferation of pleomorphic epithelial cells arranged in papillary projections, tubules and, less commonly, solid sheets. The cells have moderate amounts of lightly eosinophilic cytoplasm and large vesicular nuclei, often with a single nucleolus. There is little anisocytosis and anisokaryosis. Mitotic activity is rare. Epithelioid macrophages, some of which contain hemosiderin, are accompanied by necrotic bone, congested vasculature and hemorrhage.

Contributor's Morphologic Diagnosis: Humerus: Air Sac Adenocarcinoma.

Contributor's Comment: Neoplasms in barn owls are infrequent, although proventricular adenocarcinoma, papillary carcinoma of the thyroid gland, feather folliculoma, mucinous adenocarcinoma of the tongue, lymphoid neoplasia and cutaneous mast cell tumors have been reported (1,2). The histologic appearance of the humerus is consistent with an air sac adenocarcinoma. There is no other report of this tumor in barn owls or other raptors. A humeral mucinous adenocarcinoma of the air sacs has been reported in a salmon-crested cockatoo (*Cacatua moluccensis*) (1).

The air sacs are extrapulmonary extensions of the parabronchi that assist in air movement but not in gaseous exchange. They are lined by a single layer of epithelium supported by mesothelial serosa. Involvement of the air sacs in primary neoplasia is uncommon.

AFIP Diagnosis: Bone, humerus (per contributor): Adenocarcinoma, papillary, favor air sac origin, barn owl (*Tyto alba*), avian.

Conference Comment: Conference attendees generated considerable discussion on the origin of this neoplasm and what role the previous surgery may have had on the presence of air sac epithelium, bone and keratinizing epithelium (skin) being present in the same section. There is speculation that the lamellations of keratin were produced by a portion of squamous epithelium which invaginated into the healing surgical site possibly creating a focus of continuing keratinization. Gram-positive bacterial colonies are multifocally scattered throughout the keratin suggesting a nidus of infection within the neoplasm.

We believe this to be the same case as reported in reference #1; more can be read about it there as well. Of note, a similar case involving the humerus of a Moluccan cockatoo was presented as a Wednesday Slide Conference case in 2002. Interested parties are encouraged to review slide 53, conference 14 from the 2002-2003 slide set or to review the case on-line by visiting the Wednesday Slide Conference Results page and clicking on the appropriate year, conference, and slide.

Contributor: Department of Pathology, University of Tennessee Knoxville, Room A201, VTH, 2407 River Drive, Knoxville TN, 37996-8234

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CASE IV - 04135 (AFIP 2983613).

Signalment: 16 year old, male *Macaca fascicularis*

History: This wild caught Indonesian cynomolgus macaque was found reluctant to move and non-weight bearing on the left leg 16 months after arrival. Despite supportive treatment, it was found dead on the following day.

Gross Pathology: Purulent osteoarthritis accounted for the lameness, involving the left tibiotarsal and stifle joint. Multiple variably sized abscesses were present in the liver and kidneys which contained pale greenish-yellow, opaque, viscous fluid. The left seminal vesicle was approximately twice normal size and similar fluid exuded the cut surface. The bladder contained 2 ml of similar fluid.

Laboratory Results: A pure growth of *Burkholderia pseudomallei* was cultured from the bladder, tibiotarsal joint, and liver.

Histopathologic Description: Examined is a section of seminal vesicle which has largely undergone abscessation. Abundant neutrophils, many degenerate, eosinophilic granular material, pyknotic nuclei, and finely granular basophilic debris fill the vesicular lumina. The epithelial lining is variably effaced and replaced by this suppurative inflammatory exudate which affects all lobules. Up to 90% of the epithelium is lost with few remaining recognizable tubular structures lined by cuboidal epithelial cells. Interlobular connective tissue is expanded by edema, finely fibrillar eosinophilic material (fibrin), and neutrophils.

Contributor's Morphologic Diagnosis: Seminal Vesiculitis, Necrosuppurative, Diffuse, Subacute, Severe

Contributor's Comment: Melioidosis is an infectious disease caused by the motile, gram-negative environmental bacterium *Burkholderia pseudomallei.* The disease is generally restricted to tropical areas between latitudes 20˚N and 20˚S,

predominantly in Southeast Asia and Northern Australia. The organism is a saprophyte that can be isolated from soil and muddy water in endemic areas (1). Inhalation, ingestion and inoculation are recognized modes of acquisition for *B. pseudomallei* (2). Melioidosis has been reported in captive non-human primates, and in all cases the animals were imported from endemic areas with the exception of a chimpanzee that was thought to have acquired the infection in the laboratory (3,4,5,6,7). The time to development of overt disease ranged from 3 months to 10 years from the time of capture. Clinical signs were non-specific and varied depending on the primary system involved. The characteristic pathologic feature of melioidosis is abscessation of visceral organs, the subcutis, and regional lymph nodes (1). There is a high incidence of genitourinary infections in men in Australia, with prostatic abscesses occurring in 18% of males (2). The bacterium has a broad host range and melioidosis is commonly reported in sheep, goats and swine, and most mammals appear to be susceptible, although cattle, water buffalo, crocodiles, and birds are considered to be relatively resistant (8).

Melioidosis is considered an emerging disease with high impact on animals and humans. Due to the heightened awareness and threat of terrorist activity *Burkholderia pseudomallei* and *B. mallei*, the causative agent of glanders, have recently been classified as category B biological agents by the Centers for Disease Control and Prevention. Both organisms can be transmitted by aerosols, are naturally occurring in the environment in endemic regions, can be cultivated easily, and may cause severe, incapacitating or even fatal infections. Glanders and melioidosis have both been studied as potential agents of biological warfare, and *B. mallei* was used during both the first and second world wars (9).

AFIP Diagnosis: Seminal vesicle: Vesiculitis, suppurative, subacute, diffuse, severe, Cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: The contributor provides an excellent overview of melioidosis. Melioidosis affects several animal species as well as human beings and is commonly known as pseudoglanders due to its clinical and pathologic similarity to glanders. The lesions associated with pseudoglanders are typically granulomatous nodules with a caseous center often containing colonies of bacteria.[8] Although Gram negative, the coccobacilli are often difficult to identify in hematoxylin and eosin stained tissue sections. Methylene blue or Wright's stain can be used to help identify the bipolar morphology of the bacterium (9).

There are four clinical forms of melioidosis described in humans. The pulmonary form develops over one to two weeks following inhalation, and is characterized by non-specific symptoms of fever accompanied by sweating, rigor, cough, and chest-pain as well as ulcerative nodules within the nasal cavity. The septicemic form is

characterized by several of the same symptoms and can rapidly culminate in multi-organ failure and death within 7-10 days. Local infections occur in cuts and abrasions and result in grey-white, firm and often ulcerated nodules surrounded by hemorrhage. Nodules can become caseous or calcified and local lymph nodes can become swollen. The chronic form is characterized by multi-systemic abscesses (9).

Glanders is primarily an infectious disease of horses which is also zoonotic and can be transmitted to carnivores through the ingestion of infected horse meat. Although the pathogenesis is poorly understood, it is believed that following ingestion, the bacteria penetrate the oropharyngeal or intestinal mucosa, enter and spread through the lymphatics to the blood and multiple organs. Ulcerative lesions within the nasal mucosa develop into a characteristic star-shaped scar. The lungs can similarly be affected and contain randomly dispersed, miliary, nodular granulomas. Farcy is the cutaneous form of the disease, characterized by subcutaneous nodules with thickened lymphatics most commonly occurring on the limbs and ventral abdomen (10).

Contributor: Wake Forest University School of Medicine, Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157

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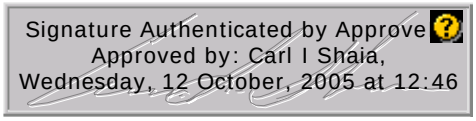

Carl I. Shaia, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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