The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 2
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CASE I – DIV07040/0 (AFIP 2833324)

Signalment: 23 week-old, one male and one female, Tg.AC mouse

History: The control male and exposed female mice (the compound was non-carcinogenic) were killed in a moribund condition following 17 weeks on an alternative carcinogenicity study.

Gross Pathology: In both mice, a mandibular mass was associated with malocclusion.

Laboratory Results: N/A

Contributor's Morphologic Diagnosis: Ameloblastic odontoma

Contributor's Comment: The mass is a circumscribed, concentric neoplasm composed of two components. One component is composed of epithelial cells arranged in interdigitating cords surrounding stellate cells and a smaller area is composed of poorly differentiated tooth components.

Odontogenic tumors occur in a high percentage of control Tg.AC mice and have been reported at an incidence of 21% in males and 32% in females. The tumors may occur in mice as young as 10 weeks of age and they tend to occur most often in the mandible.

AFIP Diagnosis: Bone: Ameloblastic odontoma, Tg.AC strain, mouse, rodent.

Conference Comment: Ondotogenic tumors are uncommon in mice. There is a higher prevalence of these neoplasms in albumin-*ras*/albumin-*myc* transgenic mice and in Tg.AC mice. Tg.AC mice carry a v-Ha-*ras* oncogene that can result in the formation of ondotogenic tumors, therefore, they are used as an animal model for these tumors and tooth development.

There are three microscopic variations of odontogenic tumors in mice composed of: 1) mesenchymal cells, originating from the periodontal ligament;

2) abortive tooth structures, originating from ameloblasts and odontoblasts; and
3) well differentiated squamous cells bounded by a loose stroma. In this case, there are two cell lines, ameloblasts located at the periphery around a central area of stellate reticulum, and a smaller component of odontoblasts. The submitted sections vary, and along with the neoplasm and bone, may include tongue, skeletal muscle, oral mucosa, haired skin, and salivary gland.

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CASE II - 37570 (AFIP 2593741)

Signalment: Adult, hooded, female, rat, *Rattus norvegicus*, 100.8 gm

History: Animal belongs to a private owner who raises rats to feed to his snakes. Owner maintains up to several hundred rats. Owner purchased about 460 rats from a seller in Florida. In less than a month, owner reported losing 1-2 rats daily, and multiple animals with "nose bleeds" and/or lumps around the neck. Clinical exam showed animals in poor condition: roughened haircoat, tachypnea, dyspnea, nasal discharge, porphyrin staining. Animals that were euthanized, rapidly succumbed to metofane.

Gross Pathology: The lungs were tan and had a cobblestone appearance. There were several firm, white foci within the lung lobes. When the right lung was incised, creamy white material was contained within.

Laboratory Results:
Microbiology (lung culture): *Bordetella bronchiseptica, Escherichia coli, Staphylococcus aureus*
Impression smears (lung): many polymorphonuclear cells seen as well as Gram-positive coccobacilli
Serology (different animal):
ELISA
Cilia-Associated Respiratory Bacillus (+)

Mycoplasma pulmonis (+)
Rat Coronavirus, Sialodacryadenitis (+)

IFA

Cilia-Associated Respiratory Bacillus (+)
Parvovirus (+)
Rat Coronavirus, Sialodacryadenitis (+)

Contributor's Morphologic Diagnoses: Lung, pleuropneumonia, suppurative, diffuse, chronic, severe, with bronchiectasis and multiple abscessation and Gram-positive cocci

Etiology: Mixed bacterial infection with possible underlying viral component.

Contributor's Comment: This animal was one of seven submitted from a private owner reporting animal losses. All animals showed varying degrees of lung pathology. Culture of the lung tissue from this animal yielded several bacterial species (*Bordetella bronchiseptica, Escherichia coli, Staphylococcus aureus*), which can cause suppurative pneumonia and tracheitis. Due to financial constraints, serology was done on only one animal from the submitted group. That animal was positive for cilia-associated respiratory (CAR) bacillus, *Mycoplasma pulmonis*, and rat coronavirus (RCV)/sialodacryadenitis (SDA) virus antibodies. That animal was also IFA positive for parvovirus. These agents have been seen historically and are rarely seen in modern rodent facilities. Their presence suggests major husbandry issues.

AFIP Diagnosis: Lung: Bronchopneumonia, necrotizing, chronic-suppurative, diffuse, severe, with bronchiectasis, peribronchiolar lymphoplasmacytic infiltrates, pleuritis, and colonies of coccobacilli, hooded rat (*Rattus norvegicus*), rodent.

Conference Comment: *Mycoplasma pulmonis* commonly causes chronic bronchopneumonia characterized by bronchiectasis, lymphoid hyperplasia, atelectasis, abscessation, and consolidation. *M. pulmonis* is transmitted *in utero*, by direct contact, or via aerosols. Cilia-associated respiratory (CAR) bacillus is a Gram-negative, motile, filamentous bacterium that colonizes the ciliated epithelium of the respiratory tract, causing ciliary stasis, goblet cell hyperplasia, bronchiectasis, bronchiolitis, and mucopurulent bronchopneumonia. CAR bacillus can be transmitted between species and can amplify the diseases caused by other respiratory pathogens. *Bordetella bronchiseptica* is an inhabitant of the respiratory tract of many animals, and can cause suppurative bronchopneumonia. *B. bronchiseptica* is also an important factor in the pathogenesis of porcine atrophic rhinitis and infectious canine tracheobronchitis. *B. bronchiseptica* is a Gram-negative, motile, coccobaccilus; transmission is via direct contact or aerosol. Sialodacryadenitis virus is a coronavirus, which is positive-sense, single stranded, enveloped RNA virus. It commonly causes self-limiting, lymphoplasmacytic inflammation and necrosis of the salivary and nasolacrimal glands of rats. This disease has high morbidity, but low mortality; and transmission is through direct contact, fomites and aerosols. Parvoviruses are single stranded, nonenveloped DNA viruses. Rat parvovirus commonly causes subclinical infections in adult rodents; but in the fetus and neonate, it may result in cerebellar hypoplasia, hepatitis, periodontal

deformities, and fetal death. This parvovirus is transmitted by direct contact and fomites.

Conference participants included *Corynebacterium kutscheri, Streptococcus pneumoniae*, and Sendai virus (Paramyxoviridae) in their differential diagnosis.

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CASE III – A02-6644-21 (AFIP 2834605)

Signalment: 10 week-old, female, crossbreed, *Sus scrofa*, porcine.

History: Loss of body condition, reduced appetite, signs of thumping and coughing following transfer from barn to nursery.

Gross Pathology: Marked icterus, marked edema of the serosa of the colon and the mesocolon. Caudal lobes of lungs were diffusely non-collapsed, mottled tan, and edematous with cranio-ventral atelectasis.

Laboratory Results:
Porcine reproductive and respiratory syndrome (PRRS) virus isolated from the serum.
Porcine circovirus positive by immunohistochemistry in liver, small intestine, and mesenteric lymph node.
Porcine circovirus type-2 positive by PCR on tonsil and spleen.

Contributor's Morphologic Diagnosis: Liver; Necrotizing hepatitis, diffuse, severe, associated with Ito cell proliferation, apoptotic bodies, bile stasis, and bridging fibrosis.

Contributor's Comment: Postweaning multisystemic wasting syndrome (PMWS) is a recently emerged disease of nursery and grower pigs associated with type-2 porcine circovirus (PCV-2) infection. A proposed case definition of PMWS requires that a pig has all of the following: 1) clinical signs characterized by wasting/failure to thrive, with or without dyspnea and icterus; 2) histologic lesions characterized by depletion of lymphoid tissues and/or lymphohistiocytic to granulomatous inflammation in any organ, typically lungs, liver, and/or lymphoid tissues; and 3) PCV-2 within characteristic lesions. Immunohistochemistry and in situ hybridization applied to formalin-fixed tissues are the preferred methods for demonstrating PCV-2 antigen or genome, respectively, within PMWS lesions. Since available evidence indicates that PCV-2 infection is much more common than PMWS, demonstrating PCV-2 exposure via serology or infection via isolation or PCR without localizing PCV-2 within characteristic lesions cannot constitute a diagnosis of PMWS.

The pathogenesis of hepatitis caused by PCV-2 is not clear, but probably Kupffer cells and other cells of monocyte-macrophage lineage are first infected by PCV-2. Infection probably extends from those cells to contiguous hepatocytes. Infected hepatocytes are usually in the periportal region where mitotic activity is higher, likely due to the requirement of circoviruses to infect host cells in mitosis for replication of this virus. Pigs infected with PCV-2 alone develop subclinical disease. In contrast, pigs coinfected with PCV-2 and porcine parvovirus (PPV) exhibit a fulminant and fatal disease. The proposed pathogenesis in dually infected pigs is as follows: both PPV and PCV-2 initially localize in the regional histiocytes of the oropharynx. PPV spreads rapidly beyond the inoculation site, chiefly by cell-associated viremia, and replicates in lymphoid tissues, thereby stimulating proliferative response in both lymphocytes and macrophages. PCV-2 is also slowly replicating in macrophages, but effective immunity to PCV-2 is not established. During the immunoproliferative phase of PPV convalescence, activated macrophages and possibly other immune cells support heightened PCV-2 replication. PCV-2 virion replication is upregulated and cell-to-cell spread is facilitated by the activation mediated increased phagocytic and fusigenic capabilities of macrophages. Then the PCV-2 virus is further disseminated in macrophages to different targets. It has been suggested that activation of the cells of the immune system during the early phase of the PCV-2 infection is the "pivotal event" for expression of virulence by PCV-2.

AFIP Diagnosis: Liver: Hepatitis, lymphohistiocytic, diffuse, moderate, with extensive hepatocellular degeneration, necrosis (apoptosis) and loss, perilobular fibrosis, and canalicular bile stasis, crossbreed pig, porcine.

Conference Comment: The family Circoviridae includes one genus, *Circovirus*, a single stranded, circular ambisense or positive-sense DNA virus that is non-enveloped, and17-22 nm in diameter, and has icosahedral symmetry. Circoviruses cause anemia in chickens, psittacine beak and feather disease, and infections in pigeons and swine. Except for fetuses, in which infection may result in stillbirth, porcine circovirus 1 is considered nonpathogenic for pigs. The contributor has provided a concise summary of porcine circovirus 2. Chicken anemia virus causes acute, immunosuppressive disease in young chickens, resulting in anemia, lymphoid atrophy, and bone marrow aplasia. Psittacine beak and feather disease is a debilitating, progressive disease of young psittacines, primarily cockatoos, that results in malformed feathers and beak, feather loss, and immunosuppression, which can lead to secondary infections and death. Pigeon circovirus infection causes lymphocytolysis leading to immunosuppression in young pigeons. Porcine parvovirus is the causative agent of SMEDI (stillbirth, mummification, embryonic death, infertility) disease. *Parvovirus* is a single stranded DNA virus that is non-enveloped, measures 25 nm in diameter, and has icosahedral symmetry.

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CASE IV - N01-670 (AFIP 2839005)

Signalment: 13 year-old, female, Thoroughbred, equine.

History: The horse was vaccinated for West Nile Virus and 4 months pregnant. The mare developed sudden onset of neurologic disease with grain refusal and lip weakness. There was flaccid paralysis of the tongue, lips, and jaws and the horse was unable to swallow. The body temperature reached 103.9 degrees Fahrenheit. The horse began to struggle and to not arise in the stall due to hind leg weakness. Due to poor prognosis, the horse was euthanized and necropsied. The clinical diagnosis was West Nile Virus encephalitis.

Gross Pathology: The body was that of a grey Thoroughbred mare in good body and postmortem conditions. Most organs examined were within expected limits. There were a few focal 1 mm areas of red discoloration on a hemisection of the brainstem, ventral to the cerebellum. The entire spinal cord was removed.

Laboratory Results: Immunofluorescence testing for rabies virus was negative.

Contributor's Morphologic Diagnosis: Meningoencephalomyelitis, lymphocytic and neutrophilic, with neuronal necrosis, subacute, multifocal, severe, cerebrum, cerebellum and spinal cord.

Contributor's Comment: The inflammation as seen in the submitted brain stem is very severe. The pattern of inflammation is compatible with a neurotropic viral etiology, most notable West Nile Virus (WNV) or eastern equine encephalomyelitis virus (EEE). Typically, the lesions of WNV are within the grey matter of the brain and the grey and white matter substances of the entire equine spinal cord, with most severity in the lumbosacral segment. The lesions in EEE are more located in the white matter substance as far as the spinal cord is concerned. They are distributed within the medulla, brain stem and midbrain as far as the brain is concerned. Response to EEE is neutrophilic, and can be so in WNV, but is more lymphocytic in less advanced cases of WNV. Immunohistochemistry was performed and strongly positive in most glial cells and numerous fibers. A similar stain was negative for EEE virus antigen.

West Nile Virus encephalomyelitis surfaced in 2001 for the first time in horses in Florida. There were approximately 200 confirmed equine cases of WNV in Florida in 2001, most of them in the Panhandle and North Florida. About 14 horses that came to necropsy at the University of Florida were confirmed to have WNV infection. Most of these horses were unvaccinated and demised in August through October.

West Nile Virus (WNV) is a member of the genus *Flavivirus* and was originally isolated from the blood of a woman with fever in the West Nile province of Uganda in 1937. The virus is transmitted by many species of mosquitoes. The mosquitoes transmit the virus to susceptible mammals and birds. The horse is considered a dead-end host of this flavivirus. Outbreaks of WNV in horses have been described in Italy and Southern France as well as in Africa and Asia. The virus was isolated for the first time in the USA in 1999. The incidence of WNV infection was particularly high in crows. The first cases of WNV infection in Florida were reported in May of 2001 in the Panhandle in birds and horses. The clinical signs observed in the affected horses are similar to EEE and Equine Protozoal Myelitis (EPM). The histologic changes in the spinal cord, if subtle, can mimic those seen in EPM. Occasional red blood cell extravasation may suggest infection with Equine Herpes Virus (EHV-1). EHV-1 induces ischemic myeloencephalopathy. Generally, there is prevalent inflammatory involvement of the brain stem and the ventral horns of the lumbosacral spinal cord in equine WNV infection. Of course, equine rabies infection needs to be always excluded in the differential diagnosis.

Immunohistochemistry, virus isolation and RT-PCR techniques were employed to make the specific etiologic diagnosis in this case.

As to the public health concern, fewer than 1%of cases in humans develop to fatal meningoencephalitis. Immunosuppression is a risk factor. Appropriate protective gear including three pairs of gloves and face shields should be used when collecting samples and when performing necropsies. The main concern for transmission to humans is viral contact with open wounds and mucous membranes.

AFIP Diagnosis: Brainstem: Meningoencephalitis, lymphohistiocytic and neutrophilic, multifocal, moderate, with necrosis, hemorrhage, and gliosis, Thoroughbred, equine.

Conference Comment: West Nile Virus, a member of Flaviviridae, is an enveloped, single stranded, positive-sense RNA virus. Other diseases caused by *Flaviviruses* include Japanese encephalitis, Murray Valley (Australian) encephalitis, St. Louis encephalitis, Wesselsbron Disease, Dengue, Yellow Fever, and Louping Ill. The contributor has provided a concise comparison of West Nile Virus encephalitis and Eastern equine encephalitis.

Conference participants included rabies, Japanese encephalitis, Borna disease, and equine herpesvirus 1 infection in their differential diagnosis for this case. Rabies virus, a member of Rhabdoviridae, is an enveloped, negative-sense, single stranded RNA virus that measures 70 x 170 nm. It causes nonsuppurative inflammation of the central nervous system, microgliosis, neuronal degeneration, neuronal intracytoplasmic inclusions (Negri bodies), and ganglioneuritis. Japanese encephalitis virus causes nonsuppurative leptomeningoencephalitis, neuronal degeneration, hemorrhage and necrosis. Borna disease virus, a member of Bornaviridae, is an enveloped, negative-sense, single stranded RNA virus that is 90 nm in diameter. It causes nonsuppurative encephalomyelitis without neuronal necrosis, and has intranuclear eosinophilic inclusion bodies (Joest-Degen bodies). Equine herpesvirus 1 is an alphaherpesvirus, and 150 nm in diameter, with double stranded DNA; it can cause encephalitis that is

characterized by vasculitis, intranuclear inclusion bodies, and minimal neutrophilic and lymphocytic inflammation.

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