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CASE I – 3070 (AFIP 2694900)

Signalment: Adult, female, Bunte Deutsche Edelziege, goat.

History: This animal came from a herd of 85 animals in Northern Germany. In November 1998, an outbreak of severe pneumonia was observed in fourteen animals. This goat first showed clinical signs on November 18th. She was treated with amoxicillin. After initial improvement her condition deteriorated and she was euthanized on November 22nd.

Gross Pathology: The animal was in good general condition. Upon opening the thoracic cavity a severe acute fibrinous pleuritis affecting about 50% of the left caudal lung lobe and the pleural lining of the pericardium was noted. There was also a fibrinous to necrotizing bronchopneumonia in the affected lung lobe. The mediastinal lymph nodes were severely swollen. The spleen was also severely swollen. Multiple erosions were present in the pyloric region of the abomasum.

Laboratory Results: Microbiological examination revealed high numbers of mycoplasma that were identified as *Mycoplasma mycoides* subsp. *capri*, few *Escherichia coli*, few alpha-hemolytic streptococci and few staphylococci.

Contributor's Morphologic Diagnosis: Lung: 1. Bronchopneumonia, severe, acute, fibrinopurulent to necrotizing, with multiple thrombi and necrotizing vasculitis. 2. Pleuritis, moderate to severe, fibrinopurulent.

Lymph node: Lymphadenitis, severe, acute, fibrinopurulent, with multifocal necrosis.

Contributor's Comment: Both sections of lung are from the altered left lung lobe. In one of the sections very early lesions of fibrinous pneumonia are present. They are characterized by severe congestion, large amounts of fibrin and moderate numbers of neutrophils in the alveoli and numerous fibrin thrombi. On the surface a severe pleuritis with large amounts of fibrin and numerous neutrophils is present. In the other sections of lung, multifocal areas of necrosis surrounded by neutrophils occur in addition. Thrombi of fibrin and neutrophils occlude numerous blood vessels. The walls of several of these blood vessels are necrotic. In the regional lymph node, the lymphatics in the capsule are dilated and contain fibrin and neutrophils. The connective tissue is infiltrated by neutrophils. The subcapsular sinus is packed with fibrin and neutrophils. In the cortex multifocal areas of necrosis demarcated by neutrophils occur.

Mycoplasma mycoides subspecies *capri* was isolated from the lung. This agent has been associated with severe outbreaks of caprine pleuropneumonia beside *Mycoplasma mycoides* subsp. *mycoides* (large colony type) and *Mycoplasma* species F38. Caprine pleuropneumonia is the most important form of respiratory mycoplasmosis in goats. The disease occurs mainly in Africa, the Middle East and Western Asia. In these countries differentiation the *Mycoplasma mycoides* subspecies is of great importance.

Only five animals were introduced into this herd in Northern Germany between 1997 and 1999. It can be assumed that *Mycoplasma mycoides* subsp. *capri* was introduced by two animals imported from France. This was the second time that *Mycoplasma mycoides* could be isolated from goats in Germany.

AFIP Diagnoses: 1. Lung: Pleuropneumonia, fibrinosuppurative and necrotizing, subacute, diffuse, severe, with vasculitis and thrombosis, Bunte Deutsche Edelziege, goat, caprine.
2. Lymph node: Lymphadenitis, fibrinosuppurative and necrotizing, subacute, diffuse, severe.

Conference Comment: Classic contagious caprine pleuropneumonia (CCPP) is caused by *Mycoplasma capricolum* subsp. *capripneumoniae*. The condition was originally described in 1881 but the causative agent wasn't characterized until 1976. Originally identified as *Mycoplasma* sp. type F38, the current nomenclature was proposed in 1993. The disease is restricted to goats, affects only the lungs, and is transmitted by direct aerogenous routes. The gross lesions are similar to those in contagious bovine pleuropneumonia (*M. mycoides* subsp. *mycoides* small colony type); differences with CCPP include the absence of sequestra and less pronounced thickening of the interlobular septae. *M. capricolum* subsp. *capripneumoniae* is difficult to isolate, and hence before 1976, CCPP had been attributed to other more easily isolated *Mycoplasma* sp. within the mycoides "cluster" of organisms.

Organisms within the mycoides "cluster" that are associated with caprine pneumonia include: *M*. *mycoides* subsp. *mycoides* large colony type, *M*. *mycoides* subsp. *capri*, and *M*. *capricoloum* subsp. *capricoloum*. In contrast to the causative agent of CCPP, these organisms are easily grown; infect both goats and sheep; and can be transmitted through infected milk, by mechanical transmission, or through aerogenous routes. The constellation of lesions produced by these organisms is referred to by the pneumonic "MAKePS". The MAKePS syndrome includes mastitis, arthritis, keratitis, pneumonia, and septicemia presenting as single conditions or in combination.

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CASE II – 7600 (AFIP 2791132)

Signalment: One-day-old, crossbred pig.

History: A ten-month-old gilt farrowed nine piglets. Of these, one was mummified, three had tremors, and two were infirm and unable to suck. The three piglets with tremors were euthanized and necropsied the next day. The gilt had no clinical signs and had been vaccinated for swine fever and erysipelas, but not for Japanese encephalitis (JE) and Aujeszky's disease (AD). Previous abortions and stillbirths had not occurred at this farm.

Gross Pathology: The brain was hyperemic. The liver was pale and fragile.

Laboratory Results: No virus was isolated from the brains of three piglets. Serum titer for JE virus (JEV) from the submitted case was 1:320. Serum antibody to AD virus was not detected by the latex agglutination test. JEV antigen was detected in the several neurons and glial cells in the cerebral lesions by the streptavidin-biotin immunoperoxidase (SAB) method.

Contributor's Morphologic Diagnosis: Cerebrum: Nonpurulent meningoencephalitis, with severe necrosis of nerve cells and multiple neuronophagia, swine.

Contributor's Comment: JEV is a mosquito-borne flavivirus and is distributed through much of eastern Asia from Russia to Indonesia (north and south) and from Japan to India (east and west). JEV causes encephalitis and reproductive failures in animals and humans, and the pig is the most important natural amplifying animal for JEV.

Abortion and stillbirth with mummified fetuses of different size are the features of JE in a pig. A mummified fetus was observed in this case. The serum antibody titer showed that the piglet was infected with JE, because the newborn piglets had not ingested colostrum.

The histopathological findings in this case are typical of JE in piglets. These include necrosis of nerve cells, neuronophagia, glial nodules, small malacic lesions, perivascular cuffing and mild meningitis in the cerebrum. JEV antigen was detected in the nerve cells and glial cells in the cerebrum by SAB method. Most of the cells in the glial nodules and neuronophagia were not positive for GFAP.

AFIP Diagnosis: Cerebrum: Meningoencephalitis, nonsuppurative, multifocal, moderate, with neuronal necrosis and gliosis, crossbreed pig, porcine.

Conference Comment: Adult swine infected with JEV typically do not develop any major pathological changes or clinical signs other than reproductive loss despite high viremias. Varying degrees of abnormality occur in developing fetuses, and while the exact pathogenesis is unclear, timing appears to be significant. The degree of lesion severity seems to be related to the development of immunocompetence in the fetus. Infection of the dam during the mid-third of gestation (i.e. day 40-60) results in obvious pathogenic effects, while infection late in gestation (i.e. after day 85) results in few. Gross lesions noted in aborted and stillborn piglets include hydrocephalus, cerebellar hypoplasia, and spinal hypomyelinogenesis.

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CASE III – 01-0360 (AFIP 2789200)

Signalment: Nine-month-old feedlot steer

History: A nine-month-old feedlot steer showed nervous symptoms of ataxia, collapse, muscular twitching, opisthotonus, and nystagmus whereafter euthanasia was effected by intravenous injection of barbiturate within c. 7 days after the onset of symptoms.

Gross Pathology: There was severe dehydration, emaciation, and rumen stasis, as well as hyperaemia of the abomasum and intestinal tract. The entire brain appeared red with extensive haemorrhage in the subdural space and under the pia mater in the region of the cerebellar peduncles. On transverse sections, there were numerous petechiae and few ecchymoses within the brain stem in the same region. No other significant changes were described.

Laboratory Results: Bacterial isolation was negative.

Contributor's Morphologic Diagnosis: Brain: Haemorrhage, multifocal; meningo-encephalitis with perivascular lymphocyte infiltration, lymphocytic vasculitis and thrombosis with small (c. 2-5 um in diameter) basophilic, irregular, granular bodies morphologically compatible with *Theileria* sp. schizonts (Koch's bodies) within the cytoplasm of a small percentage of lymphoid cells.

Etiology: *Theileria* sp. infection: suspected *Theileria taurotragi*.

Contributor's Comment: The history, clinical signs, macroscopic, and histopathological lesions correspond to the description of bovine cerebral theileriosis or Turning sickness. This condition was first described in Uganda in 1936, and is considered to be an atypical form of theileriosis characterized by

stasis of infected lymphoid cells in blood vessels of the brain, resulting in thrombosis and infarction. Turning sickness occurs sporadically in young cattle, usually under the age of 3 years. The aetiology is considered to be *T. parva parva* in East Africa, but in South Africa there is epidemiological and serological evidence that the disease is caused by *T. taurotragi.* The specific identification of *Theileria* species may be achieved by direct detection of the parasite by small subunit RNA (sr RNA) or hybridization after specific amplification of parasite sr RNA genes (PCR technique) using species specific sr RNA probes.

Multiplication and stasis of parasitized lymphoid cells occurs in the blood vessels of the brain, meninges, spinal cord, and sometimes in the spleen where they lead to thrombosis and infarction. Other organs rarely become affected. Although the mechanisms that lead to cerebral theileriosis are poorly understood, stress, intercurrent infections and massive re-infestation of animals with partial immunity may be important predisposing factors.

AFIP Diagnosis: Brainstem: Meningoencephalitis, nonsuppurative, multifocal, moderate, with atypical intravascular lymphoid cells, intralymphocytic schizonts, and rare intraerythrocytic piroplasms, breed not specified, bovine.

Conference Comment: *Theileria* are Apicomplexa organisms of the family Theileriidae, are transmitted by ticks, and infect many wild and domestic ungulates. In general, *Bos indicus* breeds have a natural resistance to disease while *Bos taurus* breeds have high morbidity and mortality rates following infection. Severity of disease is dependent on the specific *Theileria* sp. involved and relates to the degree of lymphocytic and erythrocytic infection. Corresponding sequelae include lymphocyte proliferation, lymphocytolysis, and anemia. *T. parva* and *T. annulata* are often associated with severe disease; *T. mutans*, *T. taurotragi*, and *T. buffeli* with moderate disease; and *T. velifera* with little or no disease.

Infectious sporozoites, inoculated into the host by feeding ticks, enter lymphocytes and develop into schizonts. The parasite transforms the lymphocyte and induces an uncontrolled proliferation that is independent of antigenic stimulation or growth factors. Through parasite induced degradation of the inhibitor of NF-kB, NF-kB becomes persistently activated, protecting the cell from spontaneous apoptosis. NF-kB also causes upregulation of IL-2 and IL-2R, which is mitogenic for T-cells. Infected cells continuously divide in synchrony as the schizont divides, resulting in infection of successive daughter cells. This process, involving the persistent activation of NF-kB, which abates following successful treatment, is termed "parasite-induced reversible transformation". Within 14 days of infection, schizonts develop into merozoites, are released from the lymphocytes, infect erythrocytes, and transform into piroplasms. The piroplasm stage is ingested by a feeding tick where it develops into a sporozoite, completing the cycle.

As multiple species of Theileria can be recognized in a specific geographical region, and schizont and piroplasm morphology are similar among species, identification can be difficult microscopically. The gross and clinical differential diagnosis can include Heartwater (*Cowdria ruminantium*), Trypanosomiasis, Babesiosis, Anaplasmosis, and Malignant catarrhal fever (Herpesviridae).

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CASE IV – 822/2001 (AFIP 2798269)

Signalment: 100 + day-old, female, silky chicken (*Gallus domesticus*), avian

History: There were increased chicken mortalities in a retail live poultry market in Hong Kong with over 230 birds found dead in this market overnight in May 2001. Fifteen birds were submitted for necropsy and virology testing.

Gross Pathology: The consistent change in all birds was severely edematous and congested lungs with increased free fluid in the thorax and the abdominal viscera appeared to have mild edema. Most had a congested moist trachea and some splenomegaly with irregular petechial or ecchymotic haemorrhages. Some had air sacs that were cloudy, thickened and congested with some petechia and yellow necrotic debris. Some birds had hemorrhagic foci in the proventriculus and small or large intestinal mucosa. The caecal tonsils in some were also congested and had focal haemorrhage. There were occasional small haemorrhages in muscle or fascia and in the bursa of Fabricius. Individual chickens had marked subcutaneous edema of the head and upper neck.

Laboratory Results: Influenza A antigen capture ELISA (Directigen®) was positive on pooled cloacal and tracheal swab. Highly pathogenic H5N1 avian influenza virus was isolated from pooled tracheal and cloacal swabs.

Contributor's Morphologic Diagnosis: Lung, necrotizing parabronchitis and pneumonia, acute, multifocal to diffuse, severe, silky chicken, avian.

Contributor's Comment: A feature of these cases was the marked pulmonary edema observed grossly and microscopically. Changes in other tissues from this bird included severe necrotizing tracheitis, fibrinonecrotic to purulent air sacculitis with congestion and oedema, splenic congestion and haemorrhage with multifocal areas of necrosis, congestion with focal necrotic areas in caecal tonsils, proventriculus and pancreas and severe congestion in liver and kidneys. Highly pathogenic avian influenza (HPAI) virus infections are characterized histologically by vascular disturbances leading to edema and haemorrhages because the virus replicates in vascular endothelial cells causing cell death. The longer the bird survives, the necrosis and apoptosis seen in the acute cases becomes less prominent and inflammation, haemorrhage, and edema become more prominent.

These cases did not show the severe cyanosis, subcutaneous haemorrhages and oedema of combs and wattles seen in some cases of HPAI. Apart from some watery discharge from nares and conjunctiva and the occasional bird with some subcutaneous ecchymotic haemorrhage in the legs, there were minimal signs to suggest HPAI externally.

The viruses isolated from these birds consistently produced embryo death within 48 hours of inoculation of 9-11 day old SPF chicken embryos. On further characterization they were shown to be H5N1 avian influenza viruses possessing the characteristic genomic sequence for polybasic amino acid residues at the

hemagglutinin cleavage site and were classed as HPAI on their intravenous pathogenicity index (IVPI) in pathotyping tests. These 2001 viruses were genetically distinct from the H5N1 avian influenza viruses isolated in Hong Kong in 1997 that caused 18 human cases of H5N1 influenza with 6 mortalities. However, the 2001 viruses had evidence of recent reassortment of gene segments and as the other precursor viruses (H9N2 and H6N1) for the 1997 H5N1 were still circulating in the retail markets the decision was made to depopulate the live poultry markets in May 2001.

AFIP Diagnosis: Lung: Pneumonia, necrotizing, acute, multifocal, moderate, with septal and interstitial edema, silky chicken (*Gallus domesticus*), avian.

Conference Comment: Avian influenza (Orthomyxoviridae) is a type A influenza virus. The type designation is based on envelope matrix (M) antigens and the nucleoprotein (NP) within the virus. Avian, swine, equine, and most significant human influenza are type A viruses. Subtypes are described based on envelope glycoproteins. Fifteen hemagglutinin (HA) and nine neuraminidase (NA) antigens are currently used. Standard nomenclature for naming viruses includes virus type, host of origin, geographic origin, strain number, year of isolation, and subtype designation.

Classically, highly pathogenic avian influenza was associated with the H7 hemagglutinin antigen. As it became apparent that some highly virulent strains contained hemagglutinin antigens other than H7, the definition of highly pathogenic strains needed redefining. Current guidelines for identification of HPAI are based on specific virulence criteria as determined in a laboratory (intravenous pathogenicity) as well as molecular characteristics (e.g. cleavage site amino acid sequences and cell growth characteristics).

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