Spring 1998

FROM THE ASSISTANT DIRECTOR

By the time you receive this Newsletter, I will be attending the State Veterinary Foreign Animal Disease Diagnostician (FADD) Training Course in Plum Island, New York. The purpose of this course is to orient veterinarians in the diagnosis of foreign animal diseases of livestock and poultry. I was one of two persons from the State of Indiana selected to attend this course, and I am excited about being able to attend and learn more about foreign animal diseases. I am certain that this training will be beneficial to me as well as to you in helping all of us at ADDL continuously monitor for foreign animal diseases. (I should also add that four other of our veterinary pathologists have already received this training at some time during their career.)

As I am sure you are aware, there have been several new cases of Pseudorabies in swine diagnosed in the last few months. This disease issue has been closely watched by swine producers, veterinary practitioners, and state animal health officials as well as by the public through journalism media. We are continuing to do all that we can to work with you as practitioners as well as the State Veterinarian's Office in dealing with this disease. We greatly appreciate your understanding and your patience as we all work together on this disease problem.

Hopefully, by now, you have received a new copy of our 1998 ADDL User's Guide. We hope you will take a few moments to familiarize yourself with this new guide and we hope it will be helpful as you prepare to send diagnostic samples to this laboratory.

As always, I hope you enjoy this Newsletter. We have attempted to fill it with interesting and informative articles regarding animal diseases and diagnostics. If you have any comments regarding this Newsletter, please let us hear from you.

Naturally Occurring Tyzzer's Disease in a Foal

Tyzzer's disease, an acute multifocal bacterial hepatitis caused by *Bacillus piliformis*, is a fatal disease of young animals that has been reported in a number of domestic, wild and laboratory animals. This article describes the finding of Tyzzer's disease in a foal.

Case Report: The reported history was as follows: An 8 day old, male foal was born seven days premature to a 5 year old maiden mare. A few days after birth, the foal was sluggish and had a temperature of 96° F. The foal was treated with intravenous dextrose, Lactated Ringer's Solution® (LRS), Banamine®, and Gentocin®. The foal appeared to respond well to treatment. The temperature returned to 100ºF and the foal was up and nursing. The following morning the foal became weak and died upon transport to the veterinary clinic.

The gross examination was performed by the referring veterinarian. The only gross lesion observed was severe icterus.

Multiple tissue samples including liver, kidney, heart, spleen, and lung from this foal were submitted to the Animal Disease Diagnostic Laboratory. Histologically, the liver contained multiple coalescing foci of coagulative hepatocellular necrosis scattered throughout the parenchyma. The centers of these foci were composed of pyknotic nuclei, karyorrhectic nuclei, pink fibrillar material, and rare erythrocytes. The periphery of the necrotic foci was infiltrated by moderate to large numbers of degenerate neutrophils. Hepa-tocytes bordering these necrotic foci contained intracytoplasmic basophilic filamentous bacterial rods, which stained faintly with hematoxylin and eosin (H&E). Giemsa and Warthin Starry stains revealed numerous filamentous bacilli arranged in parallel and interlacing bundles within the cytoplasm of hepatocytes. In addition to the hepatic lesions, moderate suppurative subendocardial inflam-mation was observed. The lung and kidney were microscopically unremarkable.

Discussion: The microscopic lesions and identification of filamentous bacilli (*Bacillus piliformis*) with H&E, Giemsa and Warthin Starry stains were consistent with a diagnosis of Tyzzer's disease.

Tyzzer's disease is not common in horses but has been reported in foals between 7 and 40 days of age. The pathogenesis is not well understood. The organisms are most likely picked up by the foal from the environment. The proposed pathogenesis involves an oral route of infection resulting in a primary enteric infection and subsequent dissemination via the portal circulation to other tissues, especially the liver. Rodents, rabbits, cats, and non-clinically infected horses may serve as reservoirs. Because the dam may act as the source of the infection, subsequent foals should be monitored carefully and treated appropriately. In conclusion, the disease is sporadic, and as such, specific control and preventative measures are not commonly indicated.

- by Lavun Anothayanontha, DVM
- edited by M. Randy White, DVM, PhD

Circovirus Associated Disease in Weaned Pigs in Indiana

Circoviruses are very small non-enveloped icosahedral viruses with a single stranded circular DNA genome and have been described in pigs (porcine circovirus, PCV), chickens (chicken anemia virua, ChAV), psittacines (psittacine beak and feather disease virus, PBFD) and pigeons. Circoviruses from pigs, psittacines and pigeons are 16.5-17nm in diameter, whereas ChAV is slightly larger at 24nm. ChAV, PBFDV and pigeon circovirus are all associated with disease and mortality. In virus-infected cells in psittacines and pigeons, circoviruses cause characteristic intracyto-plasmic inclusions that ultrastructurally are composed of paracrystalline arrays of small 17nm icosahedral virions.

Based on serology, infection of swine by PCV is common in Germany, the UK, Canada, New Zealand and the United States. Although infection with PCV is common, inoculation studies in pigs have not demonstrated

clinical disease. Recently, PCV has been suggested as a cause of disease in weaned pigs in California and Canada.

Over the last year, a porcine circovirus has been identified associated with disease in weaned pigs from 6 Indiana swine farms. The clinical history of circovirus associated disease in all investigated cases manifested as poor growth in 5-15% of weaned pigs with wasting and death in some. Affected pigs were mildly dyspneic and had cutaneous hyperemia of extremities following exercise and were lethargic. No icteric pigs as described in Western Canada have been found to date.

The main post-mortem lesions of affected pigs consisted of enlarged lymph nodes up to three times normal, which were firm, white and homogeneous on cross section. The lungs were heavy, non-collapsing, and rubbery and had multiple randomly distributed firm dark-red lobules. All other organs appeared usually unremarkable.

Microscopic lesions were found in lymphoid tissues, lung, liver and kidney. The most unique lesion was multifocal granulomatous inflammation affecting multiple lymph nodes, spleen, tonsil and thymus, characterized by epitheloid macrophages and multinucleate giant cells that contained variable numbers of intracytoplasmic magenta-to-basophilic inclusion bodies. Lymph nodes had depletion and coagulative necrosis of follicular centers with extensive karyorrhexis of necrotic cells. Epitheloid macrophages, multinucleate giant cells, and fewer lymphocytes and eosinophils surrounded necrotic areas. Cells with inclusion bodies were multifocally distributed in clusters within areas of granulomatous inflammation. Intracytoplasmic inclusions were round, homogeneous, magenta-to-basophilic, varied in size (5-25 µm) and were single or formed botryoid clusters. Inclusions were positive for DNA with the Feulgen stain and were much more easily visualized with Feulgen stain than with H & E. Acid-fast stains on areas of granulomatous inflammation in multiple tissues did not reveal any acid-fast bacteria.

No inclusions were observed in non-lymphoid tissues. The lungs had multifocal to diffuse interstitial pneumonia. In the kidneys there was multifocal, lympho-histiocytic, interstitial nephritis and pyelitis. The livers had moderate hepatitis characterized by periportal and multifocal lympho-histiocytic aggregates, bile duct hyperplasia and scattered necrosis of individual hepatocytes. Results of other diagnostic tests (bacteriology and virology) did not demonstrate any other consistent infectious agent(s).

Electron microscopic examination of the intracytoplasmic inclusions in macrophages from a selected pig revealed that they were electron dense and round to ovoid with sharp margins. The matrix was heterogeneous, with different areas being granular, crystalline in a herringbone pattern or crystalline in cross-sectional arrays of non-enveloped, small, icosahedral, viral particles, approximately 17 nm in diameter. No intranuclear virus particles were observed.

Circoviral antigen was detected by immunohistochemistry within inflammatory lesions in lymph nodes, spleen, kidney, lung and liver in a selected pig. The used polyclonal rabbit serum was raised against purified porcine circovirus that was isolated from a diseased pig from Western Canada.

The light and electron microscopic characteristics of the inclusions in these pigs were consistent with those described for circoviruses in psittacines and racing pigeons and similar to the description of circoviral inclusions in pigs in Western Canada. The light microscopic appearance of these inclusions is unique among viral inclusions and can be easily misinterpreted as necrotic cellular debris or other foreign substances such as adjuvant in the cytoplasm of macrophages. Positive immunohistochemistry of these inclusions in pigs in Indiana using polyclonal rabbit antiserum raised against purified circovirus that was isolated from diseased pigs in Western Canada demonstrates antigenic cross-reaction between the circovirus isolated in Canada and the virus infecting these Indiana pigs. The described clinical syndrome in weaned pigs in Indiana is similar to that described as post-weaning multisystemic wasting syndrome in Western Canada. In addition, the lesions in lymph nodes, spleen, lung, liver and kidney in pigs from Indiana are very similar to those described in pigs in Western Canada. Taken together, these findings suggest that the circovirus associated post-weaning multisystemic wasting syndrome (PWMS) that is described in pigs in Canada is also present in Indiana. There is no history of introduction of pigs or porcine semen into the affected Indiana swine farms from Canada.

In all pigs, there was a close association of large numbers of circovirus infected cells and viral inclusion bodies with granulomatous lesions in lymphoid tissues. In Indiana,

granulomatous lymphadentitis is an uncommon lesions in pigs seen most often in association with *Salmonella choleraesuis* or *Myco-bacterium avium*. These organisms were not demonstrated in any of these pigs by culture and/or special stains. The close association of viral antigen with the lesion in lymphoid tissues, the consistency of the lesion in all pigs and the lack of other demonstrated causes for the lesion strongly suggest circovirus as the cause. The association of circovirus with lesions in other organs in these pigs is less compelling. The lesions in lung, liver and kidney contained only scattered circovirus positive cells and the lesions were less unique. Interstitial pneumonia, hepatitis and/or nephritis is often seen in weaned pigs in Indiana from a variety of causes. Even so, it should be noticed that in the investigated pigs from Indiana and in those from Canada there is a consistent association between circoviral infection and lesions in lung, liver and kidneys as well as a consistent absence of other common causes for such lesions.

To date, inoculation studies in pigs have not confirmed that circovirus in pigs causes lesions or clinical disease. Until these studies are completed, the significance of circovirus in pigs is unknown. Previous inoculation studies using PK-15 cell-derived PCV have not demonstrated clinical disease. Thorough evaluation of pigs in these studies for lesions was not done. It is possible that PK-15 cell-derived PCV has been attenuated through years of cell passage or is different from circovirus. further research is needed to clarify the identity, pathogenicity and significance of porcine circovirus-like virus.

No routine diagnostic testing for porcine circovirus is available at this point in time and the diagnosis of circoviral disease is based on the clinical syndrome and histopathological findings in absence of other possible causes.

- by M. Kiupel, DVM, G.W. Stevenson DVM, PhD, S.K. Mittal, DVM, PhD, and C.L. Kanitz, DVM, PhD

Nitrite Toxicosis In Freshwater Fish

"Brown Blood Disease"

A common problem in freshwater aquariums and production systems is nitrite poisoning. This typically occurs in newly established tanks systems ("New Tank Syndrome") where the nitrifying bacteria, *Nitrobacter*, has not become established, in tanks that are over crowded and overfed, and after treating the tank with antibodies or chemicals that kill the bacteria. In all of these situations ammonia from metabolic wastes from the fish and from organic matter (uneaten food, dead plants, etc.) builds up. Ammonia is extremely toxic to fish. The bacteria *Nitrosomonas* oxidizes the ammonia to nitrite which is usually further oxidized to nitrate which has a low toxicity for fish. The nitrates are then removed through water changes and uptake by plants. If the *Nitrobacter* bacteria is not established or becomes overwhelmed by the amount of nitrite present, nitrite levels quickly reach toxic levels.

Nitrites are actively transported across the gills and readily oxidize hemoglobin to form methemoglobin. Methemoglobinemia results in hypoxia severe enough to cause sudden death but often the fish will live until they exert themselves. The term "brown blood disease" comes from the appearance of the blood that has high levels of methemoglobin (which is brown). Often, gross lesions are lacking, therefore; the brown appearance of the blood can be a diagnostic tool. Another diagnostic method involves measuring nitrite levels in the water. This may be unrewarding if mortality is high enough to decrease fish density and subsequent nitrite levels. Nitrite levels should not exceed 0.10 mg/1 in channel catfish or 0.50 mg/1 in salmonids. The LC50 for the majority of freshwater fish ranges from 0.60 to 200 mg/1. Saltwater fish have a much higher tolerance for nitrites.

Treatment not only includes decreasing the population to decrease ammonia levels, but also adding a chloride salt (in the form of sodium chloride or calcium chloride) to the water. The level of salt needed to treat (<50 mg/1) is not toxic to freshwater fish. The chloride ion

competes with the nitrite ion at the gills. When the chloride ion is present at least three times and not more than six times the level of the nitrite ion, it is preferentially transported across the gills. Thus transport of the nitrite ion is reduced. Keeping the chloride levels in the water at least 20 mg/1 can prevent nitrite toxicosis. Additional treatments can include emergency water changes to dilute the nitrite problem.
- by Melanie Greeley, DVM
- edited by Tim Muench,

Tylenol (Acetaminophen) Toxicosis in Cats

Acetaminophen is the main ingredient of Tylenol and several other non-aspirin pain relievers. It possesses both analgesic and antipyretic effects. The feline toxic dosage is 50-100 mg/kg. One regular-strength tablet (325 mg) may be toxic to cats, and a second could be lethal. One "extra strength" (500 mg) tablet can result in toxicosis. The most common abnormalities observed upon physical examination of cats are: increased respiratory rate, pale-muddy mucous membranes, hypothermia, and tachycardia. Other signs are CNS depression, anorexia, vomiting, swollen face and paws, salivation, diarrhea, coma and death.

Cyanosis and pale-muddy mucous membranes develop from methemoglobinemia within 3-12 hours after ingestion of a toxic dose of acetaminophen. Hematuria and hemoglobinuria may appear when blood methemoglobin levels exceed 20%. Laboratory findings include anemia with a large number of Heinz bodies. Death can occur within 18 to 36 hours when methemoglobin concentrations exceed 50%.

Acetaminophen is metabolized to its highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) in cells with P450 activity. In most species, excluding cats, a majority of administered acetaminophen is excreted in the urine as glucoronide and sulfate conjugates which are essentially non-toxic metabolites. A small amount of acetaminophen is normally metabolized to highly reactive intermediates which are scavenged by glutathione and excreted. Once glutathione stores are depleted, the reactive intermediates bind to intracellular macromolecules resulting in cell death. Cats are relatively deficient in activity of the enzyme glucuronyl transferase which conjugates acetaminophen to glucuronic acid for excretion. For a given dose of acetaminophen, less than 3% of acetaminophen glucuronide is excreted by cats, while humans and dogs eliminate 50-60% as the glucuronide conjugate. Therefore, in cats a relatively greater proportion of acetaminophen is available and metabolized to reactive intermediate compounds. Cellular stores of glutathione become rapidly depleted in the liver, erythrocytes, as well as in other cells throughout the body. Glutathione depletion leaves the cells unprotected from the oxidizing effect of the toxic acetaminophen metabolite NAPQI.

In the majority of animals, including dogs and humans, acetaminophen toxicity primarily causes damage to the liver. However, while cats can and do have liver damage associated with acetaminophen toxicosis, the primary manifestation of toxicosis is severe methemoglobinemia leading to hemolysis and methemoglobinuria.

Blood from animals with methemoglobinemia is darker and browner than normal. Heinz bodies are formed from the precipitation of damaged hemoglobin within the red blood cell, which leads to increased osmotic fragility of the erythrocyte and hemolysis. Diagnosis is usually based on a history of ingestion, appropriate clinical signs, methemoglobinemia, Heinz Body anemia, hemoglobinuria, and elevated serum activities of liver enzymes.

Treatment for acute acetaminophen toxicosis in cats include:

1) Induction of vomiting followed by activated charcoal and a saline cathartic if ingestion is recent (within 4-6 hours).
2) Oxygen therapy if there is severe cyanosis.
3) Intravenous or oral administration of acetylcysteine (140 mg/kg as a 5% solution initially, followed by 70 mg/kg IV every 4 hours, for a total of 4 to 6 treatments). Acetylcysteine provides cysteine required for increasing synthesis of glutathione.

4) Ascorbic acid (30 mg/kg orally) for treatment of methemoglobinemia. Vitamin C should be given every 6 hours as needed.
5) Fluid therapy for possible acidosis.
- by Darko Mladenovic, ECFVG
- edited by Stephen Hooser, DVM, PhD

AVIAN SUSCEPTIBILITIES

1/97-1/98

	Bacillus spp.	*Escherichia coli*	*Enterobacter spp.*
ANTIMICROBIC	NUMBER %S	NUMBER %S	NUMBER %S
Amikacin	5 100	79 100	3 100
Amoxic/Cluv	4 50	61 89	3 100
Ampicillin	5 60	79 91	3 0
Apramycin	2 100	51 94	1 100
*[a]Ceftiofur	5 40	73 100	2 100
Cephalothin	5 80	79 76	2 100
Clindamycin	4 100	47 0	2 100
*[a]Enrofloxacin	2 100	99 84	3 100
*[a]Erythromycin	5 60	120 6	3 100
Florfenicol	1 100	18 0	2 100
*[a]Gentamicin	5 100	120 77	2 100
Lincomycin	1 100	32 0	2 0
Neomycin	2 100	51 84	1 100
Novobiocin	2 100	51 6	3 0
Oxacillin	5 60	79 3	2 100
*[a]Penicillin	5 20	120 3	3 100
*[a]Sarafloxacin	1 100	59 78	1 0
*[b]Sulfadi/Trime	1 100	33 9	1 100
*[a]Spectinomycin	2 50	51 22	1 0
Sulfachlorpyr	2 100	72 78	2 100
*[a]Tetracycline	5 100	120 35	3 0
Tiamulin	2 100	51 29	3 0
Tilmicosin	2 100	46 0	
Tribuissin	5 80	79 59	
Tylosin	2 100	51 0	

* Compounds listed in Table 1 (Suggested Groupings of Therapeutic Antimicrobial Agents for Systemic Use) of the NCCLS M31T document. These are used for therapeutic treatment of animal diseases. Other compounds with prophyactic, control or prevention indications are not listed except for poultry.
*[a] US-FDA approved.
*[b] US-FDA not approved but are frequently used in an extra-label manner in the indicated animal.
- Prepared by the Bacteriology Laboratory - ADDL - Purdue University.

AVIAN SUSCEPTIBILITIES
1/97-1/98

	Klebsiella spp.	*Pasteurella multocida*	*Proteus spp.*	*Pseudamonas spp.*
ANTIMICROBIC	NUMBER %S	NUMBER %S	NUMBER %S	NUMBER %S

Amikacin	14 100	7 100	4 100	5 100
Amoxic/Cluv	11 100	7 100	3 67	3 0
Ampicillin	14 0	7 100	4 75	5 20
Apramycin	5 60	1 0	1 100	3 100
*[a]Ceftiofur	12 100	7 86	4 100	5 20
Cephalothin	14 86	7 86	4 100	5 0
Clindamycin	13 0	6 50	4 0	4 0
*[a]Enrofloxacin	7 100	5 100	2 50	3 100
*[a]Erythromycin	14 0	7 57	5 0	5 0
Florfenicol	3 0	7 100	1 0	2 0
*[a]Gentamicin	14 93	1 0	5 60	5 100
Lincomycin	1 0	1 100	1 100	1 0
Neomycin	5 80	1 100	1 0	3 100
Novobiocin	5 20	7 71	4 0	3 0
Oxacillin	14 0	7 86	5 40	5 0
*[a]Penicillin	14 0	1 100	2 50	5 0
*[a]Sarafloxacin	3 67	1 0	1 0	2 50
*[b]Sulfadi/Trime	2 0	1 100	2 50	1 0
*[a]Spectinomycin	5 40	7 86	5 0	3 33
Sulfachlorpyr	5 80	1 100	1 0	3 67
*[a]Tetracycline	14 57	1 100	4 25	5 40
Tiamulin	5 20	7 86	1 0	3 0
Tilmicosin	3 0	1 0		2 0
Tribuissin	14 86			5 40
Tylosin	5 0			3 0

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AVIAN SUSCEPTIBILITIES
1/97-1/98

	Serratia spp.	*Salmonella spp.*	*Staphylococcus auerus*	*Enterobacter spp.*
ANTIMICROBIC	NUMBER %S	NUMBER %S	NUMBER %S	NUMBER %S
Amikacin	3 100	6 100	5 100	14 100
Amoxic/Cluv	3 33	4 100	4 100	13 38
Ampicillin	3 33	6 100	5 60	14 36
Apramycin	1 100	6 100	4 100	3 67
*[a]Ceftiofur	3 67	6 100	5 100	14 71
Cephalothin	3 0	6 100	5 100	14 29
Clindamycin	2 0	2 0	2 100	13 0
*[a]Enrofloxacin	1 100	18 94	21 48	3 100
*[a]Erythromycin	3 0	18 0	21 52	14 0
Florfenicol	3 100	2 0	1 0	1 0
*[a]Gentamicin	1 0	18 83	21 24	14 100
Lincomycin	1 100	4 0	3 33	1 0
Neomycin	1 0	6 100	4 100	3 100

Novobiocin	3 0	6 0	4 100	3 0
Oxacillin	3 0	6 0	5 100	14 0
*[a]Penicillin	1 100	18 22	21 52	14 0
*[a]Sarafloxacin	1 0	14 93	17 35	1 100
*[b]Sulfadi/Trime	1 100	4 0	3 33	2 0
*[a]Spectinomycin	3 67	6 33	4 0	3 33
Sulfachlorpyr	1 0	18 44	20 80	3 67
*[a]Tetracycline	1 0	18 61	21 24	14 93
Tiamulin	3 67	6 17	4 100	3 0
Tilmicosin	1 0	6 0	4 100	2 0
Tribuissin		6 33	5 60	14 93
Tylosin		6 0	4 100	3 0

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