Fall 2000

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

Fall is well upon us and no doubt winter will be close behind, but from the standpoint of weather, we have been most fortunate. We have found relatively few major disease problems this summer and fall, hope that it keeps up. With the diagnosis of TB in wild deer in Michigan, we have examined a number of deer from Indiana looking for evidence of the disease. We have found no evidence of TB in deer or any other Indiana animal. That is good as our state has been TB free since 1983. We have also examined a number of captive elk for lesions of Chronic Wasting Disease. To date, we have found no lesions of CWD. We have received only a few samples of corn from the 2000 harvest for testing so far this year. As of now, we have had one sample with a trace amount of fumonisin, other mycotoxins have not been found in the samples examined. Some reports of significant levels of aflatoxin have been reported from adjacent states.

The faculty and staff of the ADDL hope you have an enjoyable and meaningful Thanksgiving. If there are things we should be doing but aren't, please let us know and, if there are things we're doing that you feel we shouldn't, let us know that also.

HAPPY THANKSGIVING

FINAL DIAGNOSIS

Hepatic coccidiosis

In each issue, we will feature a case submitted to ADDL that we hope will be of interest to you.

Case History

This case involved a meat rabbit farm with some 1500 animals. A female rabbit, approximately 7-11 weeks of age, was presented alive to the Animal Disease Diagnostic Laboratory at Purdue University for euthanasia and necropsy. The reported history was that the animals are asymptomatic for approximately 5-6 weeks at which time they begin to develop diarrhea. At this time, the owners also report sudden deaths in many rabbits.

Gross Necropsy Findings:

The rabbit was thin (weighing 2 pounds, 4 ounces) with reduced fat stores and muscle development. The hair coat was rough and fecal material was adhered to hair at the perineum. Small intestinal loops were swollen and gray. Gray-green, semi-solid digesta filled the small intestines. Formed fecal pellets were in the colon. A direct examination of the fecal material revealed numerous oval, colorless, thin-walled oocysts, each with a single, round sporont (morphologically consistent with coccidian oocysts).

Histopathologic Findings:

Hepatic lobules had extensive biliary hyperplasia with numerous intralesional coccidia. Bile ducts were markedly dilated and lined by hyperplastic columnar epithelial cells thrown into multiple papillary fronds. Numerous protozoal developmental stages (including undifferentiated gamonts, micro- and macrogametocytes and developing oocysts) ranging in size from 25-50 microns in diameter were within lining epithelial cells. Ductal lumens were filled with numerous, thin-walled, approximately 50 microns in length, ovoid oocysts (based on location and morphologic characteristics, these organisms were consistent with Eimeria stiedae). The hyperplastic bile ducts were surrounded by large amounts of fibrous connective tissue with lymphohistiocytic inflammatory infiltrates.

Morphologic diagnosis:

Liver; Marked chronic proliferative cholangitis with intraepithelial coccidial organisms and hepatic atrophy

Etiology:

Eimeria stiedae

Discussion

Eimeria species are coccidian parasites of the phylum Apicomplexa. They are parasites of the epithelial cells of the gut mucosa and those lining certain ducts. The life cycle of each species is host specific and direct. Eimeria species can be a serious problem in lagomorphs (especially in the young), where as many as 14 species of coccidian parasites have been described, all but one being found in the small intestine, cecum or colon. E. stiedae is an inhabitant of the epithelial cells of the bile ducts and is the cause of severe liver damage in rabbits. The rabbit is infected by ingestion of sporulated oocysts. Sporozoites penetrate the mucosa of the small intestine and pass via the hepatic portal system to the liver and the mesenteric lymph nodes. In the liver, they enter the epithelial cells of the bile duct and occasionally the liver parenchymal cells, where they become schizonts. The schizonts produce merozoites, but the number of asexual generations is unknown. Oocysts pass through the bile and appear in the feces 18 days after ingestion; sporulation occurs in 3 days. Light infections are often inapparent. Heavy infections are characterized by anorexia, a distended abdomen and weight loss and, occasionally, diarrhea and icterus. The liver is greatly enlarged, the bile ducts are dilated and appear on the surface of the liver as white nodules of variable size and contain creamy fluid packed with oocysts. Microscopically, there is destruction and regeneration of the bile ducts epithelium with extensive hyperplasia of the ductular epithelium. Developmental forms of the parasite are seen in the bile duct epithelial cells and

oocysts appear in the lumen. Biliary outflow may be obstructed by oocysts, resulting in a distended bile duct. The liver parenchyma may be destroyed by the pressure from the expanding biliary ducts and gradually replaced by fibrous connective tissue. A diagnosis of E. stiedae infection can be made by the demonstration of very large numbers of oocysts in the feces, but since intestinal coccidian are frequently present in rabbits and may be present in large numbers without any serious clinical signs, the most significant diagnosis is made by examination of the liver. Control of infection requires strict attention to sanitation and husbandry. Avoidance of stress, frequent disinfection of cages, hutches, transport carriers, and litter pans with 10% ammonia solution may reduce or prevent clinical disease. The most effective chemotherapeutic agents used to treat hepatic and intestinal coccidiosis are the sulfonamides. Their major role may be to control the organism until natural immunity develops.

- by Jan Lacey, DVM,
ADDL Graduate Student

ON THE ROAD

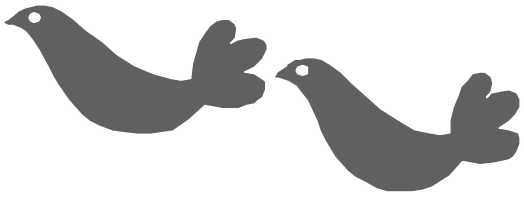
Dr. Tsang Long Lin, Avian Pathologist, presented his research on Turkey Corona Virus at the World Poultry Congress 2000 in Montreal, Canada in July, 2000.

Drs. Greg Stevenson, Chuck Kanitz and Suresh Mittal (VPB) presented their research on porcine circovirus at the International Pig Veterinarian Society annual meeting in Melbourne, Australia, September, 2000.

Drs. Leon Thacker, Ching Ching Wu, Greg Stevenson, Chuck Kanitz, Steve Hooser, Duane Murphy, Robert Everson, Bill Van Alstine, Randy White, Matt Renninger, Tariq Qureshi (VPB), and Alan Bunning, Linda Yankovich, Christina Wilson attended the American Association of Veterinary Diagnosticians annual meeting in Birmingham, Alabama, October, 2000.

Dr. Tsang Long Lin presented his research findings on a DNA vaccine against Infectious Bursal Disease of Poultry at the World Congress of Vaccines meeting in Leige, Belgium, August, 2000.

Visit our web page at:
http://www.addl.purdue.edu



Coccidiosis in Chukars

Several dead and dying chukars were referred to the Heeke ADDL by the practitioner due to limited response to treatment. Initially, the birds received a 4-day treatment of Baytril, and sulfadimethoxine for 1 day. Mortality was reduced, but lab findings included coccidiosis. Resumption of coccidiosis treatment for 4 days resolved the problem. In addition, the use of a gram positive antibacterial reduced secondary losses to clostridial enteritis. For game bird raisers, the dilemma of raising birds on wire with little disease (but primary feather damage) or raising birds on litter with enteric bacteria and protozoa without cleared pharmaceuticals remains quite real. The local practitioner can be a real help in meeting the needs of this clientele.

-by Tom Bryan, DVM, MS, Avian Diagnostician, Heeke ADDL

Yew (Japanese or English Yew) Ingestion is Deadly to Livestock!

Yew (Taxus sp.), also called Japanese or English Yew, is a highly toxic evergreen ornamental bush which is commonly used in household landscaping throughout Indiana and the Midwest.

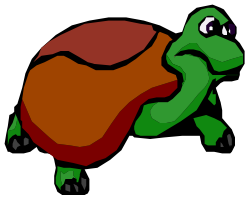
Numerous, preventable deaths in livestock (primarily cattle, horses, sheep, and goats) due to Taxus ingestion occur every year in Indiana. This usually happens when yew bush trimmings are thrown to livestock to eat. As little as one half to two pounds of yew leaves can be fatal to a 500 pound animal. The most common clinical sign reported to the ADDL is sudden death within 24 hours. Occasionally, respiratory difficulty and weakness are reported prior to death. Diagnosis is made by finding yew leaves in the stomach/rumen contents at necropsy or by finding taxine alkaloids in samples of stomach/rumen contents submitted frozen to the ADDL. There is no specific antidote for yew poisoning, therefore prevention is important. If livestock producers are uncertain as to what yew looks like, direct them to any store which sells bushes and shrubs. These stores are almost certain to carry this very common landscaping plant.

-by Stephen Hooser, DVM, Toxicologist
Robert Everson, PhD, Analytical Chemist
Christina Wilson, B.S., Asst. Chemist
Regina Bedel, BS, Technician

Purdue ADDL and Heeke ADDL will be closed on the following dates.

November 23-24,2000	Thanksgiving
December 22-26, 2000	Christmas
January 1, 2001	New Years Day
January 15, 2000	Martin Luther King Day

Please plan accordingly



Septicemic Cutaneous Ulcerative Disease of Chelonians

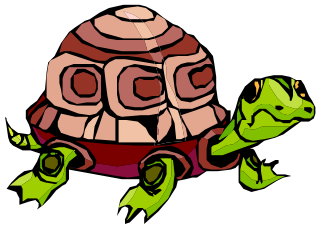
Septicemic cutaneous ulcerative disease of chelonians was originally described by Kaplan in 1957 and is commonly referred to by its acronym SCUD. The original causative agent was identified as the bacterium *Escherichia freundii* and the disease was referred to as Escherichiosis. This organism has been renamed *Citrobacter freundii.*

This syndrome, which most often affects turtles in the families *Trionychidae* and *Emydidae*, is no longer attributed to a single bacterial species. Today, SCUD is viewed more as a syndrome with many bacteria such as *Citrobacter freundii, Serratia anolium, Beneckea chitonovora* and other gram negative bacteria acting together with poor husbandry, poor water quality, abrasions and invertebrate predation to culminate in SCUD. It has been hypothesized that the proteolytic and lipolytic actions of *Serratia* upon the plastron and carapace allowed the entry of *Citrobacter freundii*, a gram negative rod normally found in soil, water and the intestinal tract of various animals, including chelonians and man. New research has found infections devoid of *Serratia* and/or *Citrobacter* presenting with similar lesions and signs leading to the investigation of other contributing factors.

The pathogenesis of SCUD involves a cutaneous insult either from an abrasion, enzyme degradation from *Serratia*, invertebrate predation, shell rot from *Benekea chitonovora* or some other form of injury, allowing the entry of a gram negative bacteria, most commonly *Citrobacter freundii*. This infection develops into irregular, caseated, crateriform ulcers on the plastron, carapace and skin. From this stage, the infection can become septicemic, causing multifocal hepatic and other visceral organ necrosis, hemolysis, limb paralysis, loss of digits or claws, cutaneous vasodilation and hemorrhage. The animal presents with signs of lethargy, anorexia, reduced muscle tone, cutaneous ulcerations or death. Erythrocytes may be vacuolated and contain numerous bacteria.

The bacteria can be cultured on eosin-methylene blue agar from samples taken from cutaneous sores, blood, and visceral necrotic foci. Spontaneous recovery has been reported but prognosis is poor if not treated. Treatment entails debridement of ulcers and abscesses, use of antibiotics and shell support with fiberglass and resin if destruction is extensive. Chloramphenicol has reportedly been effective at an initial dose of 8mg per 100g of body weight, IM or IP, followed by 4mg per 100g of body weight, IM or IP, twice a day for 7 days. Gentamicin drops have also been applied topically along with Gentamicin IM, at 10mg/kg every 48 hours for 10 doses. Shell lesions can take 1-2 years to heal.

- By Frank Ridgley, Class of 2001
- Edited by Kaori Sakamoto, DVM, ADDL Graduate Student



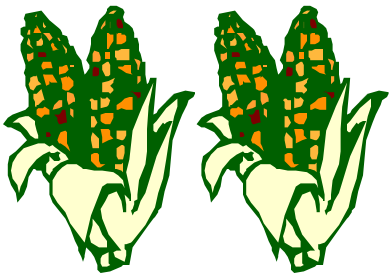
Donations to ADDL

Some of you have asked how to make donations to the ADDL. The gift account is administered by the Purdue Research Foundation. Should you wish to donate, please make the check payable to the Purdue Research Foundation with a memo that it be directed to ADDL's account. The mailing address is

Purdue Research Foundation
PTC – 3000 Kent Avenue
West Lafayette IN 47906

Faxing Laboratory Reports

Because of the increasing volume of reports we are faxing to veterinarians, we strongly suggest a dedicated fax line for your offices. It is difficult for us to phone each time a fax does not complete its transmission. Our new computer system has automatic faxing capability and we are planning to use that feature in the near future so to ensure that you receive the information you need in a timely manner, please consider a dedicated phone line for your fax machines.



Equine Leukoencephalomalacia: Clinical Features, Diagnosis, andTreatment

Equine leukoencephalomalacia (ELEM), commonly called "Moldy Corn Poisoning", is a disease of the central nervous system that affects horses, mules, and donkeys. It is commonly associated with feeding of moldy corn over several days to weeks. The fungus that is most commonly isolated is *Fusarium moniliforme* which produces three toxins, fumonison $B_{1,}$ $B_{2,}$ and $B_{3.}$ Fumonisin B_1 and B_2 appear to have similar toxicity, while fumonisin B_3 is mostly nontoxic. The toxin fumonisin has been associated with both central nervous system and liver damage. *Fusarium proliferatum* has also been implicated as another possible agent in this disease. The toxin has been described as causing damage to the vascular endothelium of the central nervous system and, in some cases, hepatocellular necrosis and vacuolization. The incidence of finding corn infected with *F. moniliforme* increases after a long, dry summer, followed by a wet harvest. Outbreaks of ELEM occur sporadically from late fall to early spring.

The clinical signs associated with the neurologic form of ELEM in horses include apathy, drowsiness, pharyngeal paralysis, blindness, circling, difficulty backing, staggering, hyperexcitability, seizures and eventual recumbency. However, in some cases, sudden death may be the only clinical sign observed. Once animals show the neurological signs, death usually occurs within 48-72 hours. If an animal survives the acute syndrome, neurological deficits are observed. A recovered horse is sometimes referred to as a "dummy" because of its loss of intelligence.

The signs associated with the hepatic form include petechial or ecchymotic hemorrhages of the mucous membranes, icterus, edema of the head and neck, decreased appetite, depression, lingual paralysis, clonic convulsions, and coma.

On postmortem examination, the classic finding is gray to brown areas of malacia and cavitation of white matter of the cerebral hemisphere. It is usually unilateral, but may be asymmetrically bilateral. Histologically, there is marked, multifocal, liquefactive necrosis, multifocal vascular congestion, and perivascular hemorrhage throughout the white matter of the cerebrum. A cellular inflammatory response is generally absent.

In the hepatic form, the liver may appear normal or may appear small and firm. Histologically, there may be diffuse vacuolization of hepatocytes, fatty degeneration, centrilobular necrosis with inflammatory cell infiltrate, bile duct proliferation, bile stasis, increased mitotic figures within the hepatocytes, or periportal fibrosis.

There is no specific therapy for ELEM. By the time clinical signs are noted, it is usually too late in the course of the disease. Removal of the contaminated feed from susceptible animals is very important. Avoidance of the mycotoxins is the only way to prevent the disease. If the animal is acting delirious or agitated, sedation is necessary. The majority of the therapy is symptomatic. These include gastrointestinal protectants such as activated charcoal and laxatives to aid elimination of the toxin, fluids, and dextrose for hydration and energy. If the clinical signs have progressed to recumbency, euthanasia may be needed for humane reasons.

- by Ryan Rothenbuhler, Class of 2001
- edited by Marlon Rebelatto, DVM, ADDL Graduate Student

ADDL STAFF NEWS

Congratulations to Dr. Brad Njaa, ADDL pathologist, Dr. Lavun Anothayanontha, ADDL graduate student, and former graduate students Drs. Cindy Fishman and Matti Kuipel for successfully completing the American College of Veterinary Pathologists board examination and earning the distinction of Diplomate, ACVP.

The Bacteriology labs at both Purdue ADDL and Heeke ADDL have passed the Johne's culture and PCR check tests.
Congratulations particularly to Bonnie Vera, Cecelia Santrich and Tom Hooper who routinely perform these tests for ADDL.

Dr. Matti Kuipel won the graduate student poster award at the American Association of Veterinary Laboratory Diagnosticians meeting in Birmingham, Alabama, October 2000.. His poster was titled "Coronavirus Associated Epizootic Catarrhal Enteritis (ECE) of Ferrets.

Dr. Randy White was named Chairman of the Aquaculture Committee of the American Association of Veterinary Laboratory Diagnosticians.

Screwworm

We realize that this is likely an inappropriate time of the year to publish an article on Screwworm. But because it was recently in the news that positive and false positive cases were reported in the U.S., we will print the following article which was plagiarized from a USDA/APHIS article found on the world wide web as a reminder of its potential for entrance. The U.S. has spent millions of dollars in successfully eradicating *Cochliomyia hominovirax*; if they are reintroduced, it could well be a veterinarian who would make the first identification. The diagnosis of this screwworm occurrence is classified as an exotic myiasis and, therefore, a reportable disease. Being aware that it would be rare to find this parasite in Indiana, we are also cognizant of the importance of identifying it early if it should arrive here so that immediate eradication actions can be taken.

A screwworm infestation is caused by larvae of the fly *Cochliomyia homino-virax*. These larvae can infest wounds of any warm-blooded animal, including human beings. ***The screwworm fly is about twice the size of a regular house fly*** and can be distinguished by its greenish-blue color and its large reddish-orange eyes. Infestations can occur in any open wound, including cuts, castration wounds, navels of newborn animals, and tick bites. The wounds often contain a dark, foul-smelling discharge. ***Screwworm larvae distinguish themselves from other species by feeding only on the living flesh,*** never dead tissue. Once a wound is infested, the screwworm can eventually kill the animal or human, literally eating it alive. After mating, the female screwworm fly fly lays her eggs in open wounds. One screwworm fly can lay up to 400 eggs at a time and they can hatch into larvae in as little as 12 hours. A single adult female can lay as many as 2800 eggs during its 31-day lifespan. The screwworm larva ***grows inside the wound to greater than one-half inch within 5 to 7 days*** of entering the wound. The full-grown larva then drops from the wound, tunnels into the soil, and forms an immobile protective case that houses the pupa. An adult screwworm fly emerges from the pupa.

Screwworms are eradicated through a form of biological control. Millions of sterile screwworm flies are raised in a production plant located in Tuxtla Gutierrez in the southern Mexican State of Chiapas near the Isthmus of Tehuantepec. During the pupal stage of the fly's life cycle, the pupae are subjected to gamma radiation. The level of radiation is designed to leave the fly perfectly normal in all respects but one: it will be sexually sterile. Thus, when the artificially raised flies are released into the wild to mate with native fly populations, no offspring will result from the matings. These unsuccessful matings lead to the gradual reduction of native fly populations. With fewer fertile mates available in each succeeding generation, the fly, in essence, breeds itself out of existence. The screwworm fly can travel up to 180 miles in several days and under warm, favorable conditions can complete a life cycle in as few as 3 weeks. ***Left untreated, screwworm-infested wounds lead to death. Multiple infestations can kill a grown steer in 5-7 days.***

As early as 1825, western States reported serious screwworm problems. Infestations spread to the Southeast by the 1930's. Losses to livestock producers exceeded $400 million annually.

A plan for eradicating the pest began in the early 1950's, when USDA's Agricultural Research Service developed a new control method. Under this method, laboratory-raised flies sterilized by gamma rays are spread by aircraft over infested areas. As millions of sterile flies flood an area, the sterile males mate with fertile female flies. The resulting eggs do not hatch. This sterile insect technique was tested in a field trial on the Dutch island of Curacao in 1954 and then used operationally in Florida by 1957. By 1959, screwworms had been eradicated from the Southeast.

The sterile insect technique was next applied to the more extensively infested Southwest starting in 1962. Self-sustaining screwworm populations were eliminated from the United States by 1966. A barrier zone of sterile flies was set up along the 2,000-mile-long U.S.-Mexican border to prevent reinfestation from Mexico. However, constant reinfestation from migrating flies or larvae carried by animals, which are then transported by people, remained a problem. The United States-Mexico Joint Commission was formed in 1972 between Mexico and the United States with the goal of eliminating the pest from Mexico and pushing the barrier to the Isthmus of Tehuantepec, just north of Guatemala. A new sterile screwworm plant at Tuxtla Gueierrez, Chiapas, Mexico, was dedicated in 1976. With a production capacity of more than 500 million sterile flies per week, it replaced the former production plant in Mission, Texas, which was closed in January 1981. APHIS also is cooperating with Central American countries and Panama in efforts to eradicate screwworms from those countries and establish and maintain a barrier of sterile flies at the Darien Gap between Panama and Colombia.

As a result of these cooperative efforts, Mexico was officially declared free of screwworms in 1991, Belize and Guatemala in 1994, and El Salvador in 1995. In addition, Honduras is considered technically free, with no pest detections since January 1995. Currently, screwworm program officials are focusing their efforts on eradicating the pest from Nicaragua and Costa Rica. APHIS hopes to begin eradication activities in Panama, the final frontier of the program, in 1997. Eradication activities include regulation of cattle movement, wound treatment, and the release of sterile flies. To date, the program has been very successful.

The screwworm program's resources were strained by introduced outbreaks that occurred in Mexico in 1992 and 1993. Of the 66 cases that were identified, the closest one was only 121 miles from the U.S. border. While these outbreaks were eliminated, the resources that APHIS spent in eradicating them pushed back the entire Central American program by 12 to 18 months.

As part of the screwworm program's overall strategy, a new sterile fly rearing facility in Panama will be established to replace the existing one in Mexico. The Panamanian government has agreed to provide land for the new facility. Establishing the new facility well outside the area where screwworms have been eradicated will reduce the risk of reinfestation through an accidental release of fertile flies.