CONTROL ID: 2021212

TITLE: A Novel Nephrotoxic Mycotoxin: Pathology and Etiological Diagnosis

ABSTRACT BODY:

Narrative: We hereby describe a novel mycotoxin which should be considered in differential diagnosis of acute and chronic nephrotoxic insults in animals. Orellanine is a bipyridyl toxin which bears striking structural resemblance to the herbicide Paraquat. It is produced by mushrooms of the genus *Cortinarius* which grow throughout North America and Europe. Although clinical cases of orellanine poisoning in animals are yet to be reported, numerous human cases have been reported. In human cases, orellanine causes a slowly-developing severe oliguric renal failure in the course of 1-2 weeks post ingestion. Survivors develop chronic renal failure requiring hemodialysis for life. We believe that lack of awareness of this novel toxin as a danger to animals, coupled with lack of diagnostic tests, are reasons why intoxication cases in animals are yet to be identified. Using a mouse model, our lab has characterized renal lesions associated with orellanine toxicosis. In mice dosed with 10 mg/kg bw 2 X subcutaneously 2 hours apart, renal lesions consisted of acute tubular necrosis. For etiological confirmation, we have developed an analytical procedure utilizing LC/MS/MS method for detection and quantitation of orellanine in kidneys. Extraction of 0.1 gram of kidney in 0.4ml methanol:3M HCl, (10:1) LOQ is 5ppm LOD is 0.5ppm. The method can detect orellanine in suspect mushrooms or in fresh renal tissue such as a renal biopsy. Additional work is ongoing to develop tests based on less invasive specimens such as serum and urine. In conclusion, procedures based on pathology and analytical chemistry for diagnosis of a potent novel nephrotoxic mycotoxin orellanine have been developed. The LC/MS/MS-based procedure is available for etiological confirmation of suspected cases of orellanine-induced renal failure.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS:

Trainee Letter:

CONTROL ID: 2019856

TITLE: Phosphorus Toxicosis Following Fireworks Ingestion

ABSTRACT BODY:

Narrative: A four month old male Yorkshire terrier consumed Slider fireworks on July 1, 2012. The dog vomited a large amount of stomach contents the following day (July 2), was weak and also had diarrhea. It was taken to an emergency clinic and initial blood chemistry results demonstrated hypoglycemia while the alkaline phosphatase and alanine aminotransferase levels were within the normal range. Fluid therapy was initiated and the dog remained recumbent.
An abdominal radiograph on July 3 revealed a small area of gas distended small intestine and ascites. No unusual masses were observed. An abdominocentesis procedure was unable to obtain a peritoneal fluid sample. The condition of the dog worsened. Exploratory abdominal surgery was attempted, but the dog died.
The veterinary practitioner described a yellowish tan liver with specks of brown to red coloring. There was mild hepatomegaly and the liver was friable. Early gastric mucosal necrosis was suspected. The stomach contents were dark, brown fluid. The gastric mucosa near the greater curvature was purple and hemorrhagic.
Formalin fixed liver and a fireworks sample were submitted to the Animal Disease Research

and Diagnostic Laboratory at South Dakota State University, Brookings, SD. Microscopic examination of the liver revealed severe acute diffuse hepatocyte degeneration and necrosis. Centrilobular veins were surrounded by necrotic hepatocytes and hemorrhage. Hepatocytes near portal triads were swollen and vacuolated.
The fireworks (Sliders) were analyzed using ICP-OES and contained 46, 154 ppm (4.6%) phosphorus, 62, 710 ppm (6.2%) potassium and 19,429 ppm (1.9%) magnesium on a dry basis.
Fireworks typically contain potassium nitrate, potassium chloride, sodium nitrate and potassium perchlorate. Phosphorus, in the form of white phosphorus has been used in explosives. Clinical signs following ingestion of excess phosphorus include gastroenteritis with vomiting and diarrhea. After several days and animal can develop a secondary phase of severe liver damage with renal damage also.
This case is compatible with phosphorus toxicosis caused by consumption of the Slider fireworks.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS:

Trainee Letter:

CONTROL ID: 2031422

TITLE: Hemolytic Crisis in Horses from Exposure to *Pistacia* spp.

ABSTRACT BODY:
Narrative: Three adult horses were submitted to the California Animal Health and Food Safety Laboratory System for necropsy in October 2013. The first horse had anorexia, fever and pale mucus membranes 24 hours prior to death. Another horse on the premises had similar symptoms with mild ataxia and died the next day. A third horse died two days later. Microscopically, all three horses had hemoglobinuric nephrosis; one of which had severe, diffuse, centrilobular to midzonal, bridging hepatocellular necrosis with bile stasis. The horses had been on dry pasture with a pistachio orchard, supplemented with *Panicum* and alfalfa hay. Trimmings from the pistachio trees (small leaf and large leaf pistachio; *Pistacia atlantica*, *P. terebinthus*, respectively) were piled in the enclosure and clinical signs began after horses were seen consuming the clippings. Other plants identified within the enclosure included: coffee berry, coyote brush, penny royal, *Pistacia chinesis*, grape, oak and toyon.
Fluorescent antibody test results for *Leptospira* spp. on the kidneys and in the urine were negative in all three horses and PCR test results were negative for *Leptospira* spp. in one horse. Silver stain on the kidneys from the third horse demonstrated spirochetes, however. Antibody titers in this horse to *Leptospira interrogans* serovars Pomona, Bratislava and Icterohaemorrhagiae were 1:6400, 1:6400 and 1:3200, respectively. Antibody titers to *Leptospira interrogans* in the first horse were 1:400 for serovars Canicola and Bratislava and 1:800 for Icterohaemorrhagiae, and in the second horse were 1:400 for Bratislava, Hardjo, Icterohemorrhagiae and Pomona.
Pyrogallol was identified in the kidneys of 2/3 horses; *Pistacia terebinthus* and *P. atlanticus* contained 2.3 % and 6.6% gallic acid, respectively; all considered toxic levels. Extract from the pistachio inoculated on washed equine erythrocytes produced hemolysis whereas extractions of hay (leaves and seeds) did not demonstrating that the pistachio trees have oxidative properties (pyrogallol) which cause hemolysis and hemoglobinuria.
The cause of the intravascular hemolysis seen in these horses was likely due to a newly

recognized plant toxicosis (*Pistachia* sp.). This case also demonstrates the importance of evaluating more than one animal in an outbreak with multiple mortalities in a herd.

CURRENT CATEGORY/DISCIPLINE: Toxicology | Pathology

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AWARDS:

Trainee Letter:

CONTROL ID: 2018965

TITLE: Intralaboratory Development and Validation of an Analytical Method for Determination of Aflatoxin M1 and B1 in Liver

ABSTRACT BODY:
Narrative: Aflatoxins are potent mycotoxins which negatively impact animal and human health. Among other effects, aflatoxins are especially hepatotoxic and immunosuppressive. The etiological diagnosis of aflatoxicosis which is based on analysis of contaminated feed matrices has disadvantages and is not quite confirmatory. Currently there are no tissue based methods for etiological diagnosis and confirmation of aflatoxicosis. A high performance liquid chromatographic method with fluorimetric detection and pre-column derivatization for the determination of aflatoxin B1 and M1 in bovine liver has been developed and validated. The selectivity, recovery, precision, matrix effect, limit of detection, limit of quantification, and linearity have been validated. Current method demonstrates good selectivity for both aflatoxins against bovine liver matrix. The limit of detection is 0.01 and 0.02 ng g-1 and limit of quantification is 0.03 and 0.07 ng g-1 for aflatoxin M1 and B1, respectively. The calibration curves show good linearity (R2 is 0.9999 and 0.9991 for aflatoxin M1 and B1, respectively) from 0.2 ~ 10.3 ng g-1. The mean recoveries calculated at three levels of fortification (0.2, 2 and 10 ng g-1) is 55% and 59% for aflatoxin M1 and B1, respectively, and the maximum relative standard deviation value for the intra-lab repeatability is 18% and 15% for aflatoxin M1 and B1, respectively. The comparison of post-extraction fortified liver at 2.0 ng g-1 with aflatoxin standard solution indicates the absence of liver matrix effect. These results indicate that the proposed method is suitable for the determination of aflatoxin B1 and M1 in bovine liver. The next step is interlaboratory validation of this test before it can be implemented for the routine analysis.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS: Trainee Travel Award

Trainee Letter: 2014 AAVLD Trainee Travel Award Application Dahai Shao.pdf

CONTROL ID: 2021977

TITLE: A LC-MS Method for the Analysis of Nitrate and Nitrite in Serum

ABSTRACT BODY:
Narrative: Current methods for nitrate and nitrite quantification in biological samples

generally rely on either direct analysis by specialized equipment such as ion chromatographs with electrochemical detectors or by reduction (in the case of nitrate) followed by derivatization and quantification by relatively non-selective techniques such as fluorescence or ultraviolet-visible light spectroscopy. Here, we propose a novel method for the analysis of nitrate and nitrite in serum obtained from livestock using reduction (in the case of nitrate) followed by derivatization and analysis by liquid chromatography-mass spectrometry (LC-MS).
Samples are enriched with 15N-nitrite, treated with a 20% sulfosalicylic acid solution to precipitate proteins, and reacted with an acidified solution of sulfanilamide and N-(1-napthyl)ethyelenediamine (i.e. Griess Reagent). Derivatized nitrite and 15N-nitrite are resolved from interferences on a 2.0 x 50 mm, 4 µm Polar-RP column (Phenomenex) with a 10 minute gradient of water and acetonitrile, both modified with 0.1% formic acid. Analytes are detected on a Quattro Micro (Waters) triple-quadrupole with positive mode electrospray ionization and multi reaction monitoring. On this system the method has a linear range of 1.0–50µM (0.07–3.5 ppm) nitrite in water and standard additions demonstrated 85-100% recovery in serum.
These results indicate that the method is a highly selective screen for nitrite in serum using equipment commonly available in veterinary diagnostic laboratories across the country, and is comparable in sensitivity with ion chromatographic methods. We are currently evaluating the application of the method to other matrices such as aqueous humor. For nitrate quantification, the inclusion of 15N-nitrate will further allow correction for nitrate reduction efficiency. Additionally, as artifacts created during derivatization with the Griess reagent are separated from the nitrite adduct by chromatography, there is no risk of interferences affecting the quantification of nitrite in the sample, as is common with spectroscopic quantification. Additionally, the method is very amenable to batch processing and the total cost of reagents used during the assay are approximately $1/sample. Thus, the assay offers laboratories that do not possess specialized equipment a cheap, fast and sensitive method for the quantification of nitrate and nitrite in serum with possible application to other biological fluids using equipment already in their possession.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS: Trainee Travel Award

Trainee Letter: Agrawal Letter AAVLD 2014.pdf

CONTROL ID: 2021299

TITLE: A New Approach to the Rapid Analysis of Toxins and Toxicants Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

ABSTRACT BODY:
Narrative: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been a commanding tool for the high-throughput analysis of biomolecules such as proteins, polymers, nucleic acids and bacterial cells/cultures. However, using MALDI mass spectrometry to analyze low-molecular weight analytes has historically been challenging. This is due to several factors which include the abundant presence of several matrix ions which can interfere with detection of analytes < 500 Da and limitations in instrument sensitivity. However, exploiting advances in the sensitivity of new MALDI instrumentation, and through matrix manipulations, a procedure has been adapted for the detection of toxins and toxicants that are of interest in diagnostic veterinary toxicology. Using a Bruker microflex™ LRF high performance bench-top MALDI-TOF-MS, which is equipped with an additional gridless reflectron, provides the superior resolution and mass accuracy

needed to accommodate detection of low molecular weight analytes. Several toxins and toxicants, including microcystins, brodifacoum, strychnine, and ractopamine, have been analyzed using this MALDI-TOF-MS. Methods adapted for the analysis of these compounds included: external calibration of the instrument using α-cyanohydroxycinnamic acid matrix ions in both positive and negative ion mode, a reflector voltage set at 19.99 kV, a detector scan range of 0 to 1,000 Da, and approximately 1,000 laser shots of data summed per sample. Prior to analysis, the samples are co-crystallized with α-cyanohydroxycinnamic acid matrix in a ratio of 1:1. Using this method, microcystin-LR (995 m/z), microcystin-LA (910 m/z), microcystin-RR (1038 m/z), and microcystin-YR (1045 m/z) can be detected at concentrations as low as 0.01 ppm. Additionally, rodenticides such as brodifacoum (523/525 m/z), strychnine (334 m/z), and ractopamine (301 m/z) are also detectable with estimated detection limits ranging from 0.1 ppm to 1 ppm. Although some sample preparation is involved in using this method, the MALDI-TOF-MS is a high-throughput format technique that can prove to be a rapid reliable tool for detection and confirmation of a variety of toxins and toxicants for diagnostic veterinary toxicology.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS:

Trainee Letter:

CONTROL ID: 2019833

TITLE: Comparison of Two Extraction mMethods for Monensin in Feed by LCMSMS

ABSTRACT BODY:

Narrative: Monensin, Lasalocid, Salinomycin, and Narasin also known as polyether antibiotics are mainly used as coccidiostats in animal feed. These compounds possess unique structural properties which allow them to act as cation carriers across biological membranes. Thus they have microbiological activity against gram positive bacteria. Also ionophores can act as growth promoters in animals. Due to the potential of these compounds to be toxic above recommended levels or their interaction with certain drugs to potentiate their effects monitoring levels in feed is essential. This study compares two extraction methods for the determination of Monensin using LCMSMS as the output. AAFCO feeds at various concentrations (ppm) ie., low,(20), medium (300), and high (1200) were used in the study. Method 1 uses hexane:ethyl acetate (90:10) 5g/40ml and Method 2 uses methanol:water 5g/20ml (90:10) for extraction of the feeds. It was found that both extraction levels worked well for the low and medium levels falling within the range of standard deviation values given by the AAFCO. However at the high level (1200 ppm) it was found that extraction Method 2 with standard addition method of 500 and 1000 ppm worked best for accurate results. The LCMSMS method has an LOQ of 1ppm and an LOD of 0.1ppm for Monensin. In future for high samples gram to solvent ratio will be investigated to see if this can improve performance.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021161

TITLE: The Effect of *Foot-and-Mouth Disease Virus* Inactivation on the Status of Serum Mineral and Vitamin Concentrations

ABSTRACT BODY:
Narrative: The effect on serum trace mineral and vitamin concentration of three USDA APHIS approved treatments for the inactivation of *Foot-and-Mouth Disease virus* (FMDV) from animal specimens were compared. In order to ship animal blood serum from some foreign countries into the United State the samples must be treated to inactivate the FMDV. Several treatment options for inactivation of FMDV are approved by the FDA, of which three seem the most appropriate for serum. The three options are to expose the specimen to heat (72 C for at least 30 minutes), to make the specimen acidic (pH <= 5.5 for at least 30 minutes), and/or to make the specimen basic (pH >= 10 for at least two hours). Previously analyzed serums were pooled to produce a sample sufficiently large enough to compare the various treatment options. Analysis was performed to determine the concentration of Vitamins A and E, as well as trace minerals Ca, Cu, Fe, K, Mg, Mn, Mo, P, Se, and Zn. When compared to regularly used methods for analysis of trace minerals and vitamins in serum the preliminary data indicates that making samples acidic works best for trace mineral analysis and making samples basic works best for analysis of vitamins. The average recovery of the trace minerals was 91% for the acid treatment as compared to our routine analysis procedure. Additionally, analysis of two reference serums using the acid treatment produced results in the acceptable range. For Vitamins A and E the average recovery was 100% and 74%. Further investigation into the effect of the treatments on the stability of the vitamins and minerals on storage is ongoing.

CURRENT CATEGORY/DISCIPLINE: Toxicology

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AWARDS: AAVLD Laboratory Staff Travel Award

Trainee Letter: