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LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Post mortem services and fees at the AHL

Jim Fairles

When consulting with clients about diagnostics, it is always important to have an accurate quote. With submissions for necropsy at the Guelph and Kemptville sites of the AHL, the quote becomes more difficult to determine accurately, as the pathologist may need to use ancillary tests beyond the gross PM and histopathology to reach a diagnosis. This is especially true in abortion cases, where ancillary tests must almost always used. OMAFRA supports the AHL in reducing our agricultural animal fees as much as possible. Below are examples of 2009 AHL fees associated with bovine and equine abortion cases.

Ruminant abortion

Necropsy; Histopathology - routine; IHC BVDV; Bacteriology – abortion culture only, e.g. 2 samples; Mycoplasmology - isolation; Virology - isolation. **Total ~$225**

Equine abortion

Necropsy; Histopathology - routine; Equid herpesvirus PCR; Bacteriology - abortion culture only, e.g. 2 samples; Virology - isolation. **Total ~$350**

In complex cases and multiple submissions, diagnostic costs can reach $500-$750. At the same time, for specific diagnoses, e.g., neosporosis, gross and histopathology may be all that are needed, and costs will be less.

If you have any concerns with costing maximums for diagnostics, please indicate this in the history on the Post Mortem submission form. Please feel free to call and discuss pricing and diagnostic concerns with our pathologists.

In instances where there is a human health concern or a potential outbreak situation and there is unwillingness or inability to proceed with further testing, limited funds are available through the OMAFRA-funded Animal Health Strategic Investment for disease diagnostics, decided on a case-by-case basis, if certain criteria are met. You may discuss this with the OMAFRA extension veterinarian or the pathologist or myself at the AHL. *AHL*

AHL Continuing Education Session Open House held May 2, 2009

Durda Slavic, Kris Ruotsalo, Jim Fairles, Josie Given

An enthusiastic group of practitioners and animal health care technicians gathered bright and early on this Saturday morning to participate in our Continuing Education Open House. Participants attended a variety of sessions focused on the practical aspects and application of diagnostics within the fields of bacteriology, toxicology, post mortem examination, molecular technology/ PCR, immunohistochemistry, as well as cytology and hematology.

This was a great opportunity to share information about the AHL in general, discuss our particular areas of interest and expertise, and to meet new people.

Feedback from this meeting was very positive. **We will use these excellent suggestions to plan more of these events in the future.**

Thanks to all who participated! *AHL*

Do you recognize this toxic plant?
(answer and article on p 23)

Don't be the missing link

Bruce McNab

Every veterinarian knows that an animal's age, type, stage-of-production, location, and clinical history all provide important information. With such information, an experienced clinician can identify most likely rule-outs. Then they can confirm the diagnosis using evidence from a clinical examination, strategically selected tests, and response to treatment.

Just like the clinician, laboratory personnel need case-history and demographic information to do their job. That information is often vital to the pathologist for their selection and interpretation of tests and pathology; and ultimately the service they provide to the submitting veterinarian.

History, animal location (e.g., barn postal code), demographic information, and test results (positive and negative), are all important to the overall surveillance of animal health. Ontario's ability to provide quantitative data demonstrating surveillance coverage, including normal and abnormal patterns and trends, is becoming increasingly important to support trade and program funding.

Having been in practice, I can appreciate how difficult it can be for a busy practitioner to ensure forms are completed fully. But I have also sat-in on rounds at the laboratory, where I have seen pathologists short-changed by the lack of information on many submissions. And as an epidemiologist, I have seen Ontario short-changed by incomplete data on animal age and history, counting towards BSE surveillance.

There are at least 3 excellent reasons for a veterinarian to ensure all animal demographic and case-history information is clearly recorded on laboratory submission forms, including:

- the information helps laboratory diagnosticians provide better service to the submitting veterinarian,
- the information 'counts' towards surveillance in support of the industry and private veterinarians, and
- it automatically ensures the existence of more complete medical records for the submitting veterinarian (including off-site data-backup).

These are 3 excellent reasons to **not** be the missing link. *AHL*

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Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends.

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

New Animal Health Strategic Investment projects approved!

Jane Gaviller-Fortune, Grant Maxie

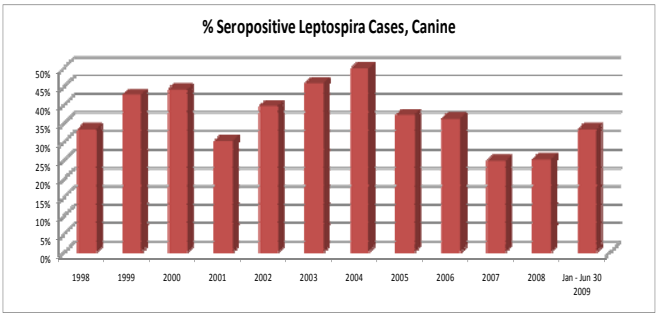
A second call for proposals went out for Animal Health Strategic Investment (AHSI) projects in June 2009, and in July 2009, 9 new projects were approved for funding. The following table outlines the newly funded research proposals:

Project ID	Project lead Project team members	Project title	Project term
09-14	Dr. John Barta, OVC Dr. Hugh Cai, Julie Cobean, Dr. Robert Hanner, Mr. Joseph Ogedengbe, Mosun Ogedengbe	DNA bar-coding of coccidial parasites of production animals: development and field testing of a LightCycler-based quantitative test	2 yr
09-15	Dr. Hugh Cai, AHL Dr. Janet MacInnes, Dr. Durda Slavic, Patricia Bell-Rogers, Rita Rebelo, Rebeccah Travis	Rapid detection and typing of *Haemophilus parasuis*	2 yr
09-16	Dr. Bob Friendship, OVC Dr. Jeonghwa Park	Is the treatment of exudative epidermitis in pigs becoming problematic because of the emergence of antimicrobial resistance?	8 mo
09-17	Dr. Murray Hazlett, AHL Dr. Hugh Cai, Dr. Josepha DeLay, Dr. Beverly McEwen	Development of in-situ hybridization (ISH) for diagnosis of *Coxiella burnetii* and *Toxoplasma gondii* in small ruminants	1 yr
09-18	Dr. Ken Leslie, OVC Dr. Todd Duffield, Dr. Brent Hoff, Dr. Brian McBride, Brian Nelson, Dr. Andrew Peregrine	Associations between the incidence of *Cryptosporidium parvum* and selenium status in neonatal dairy calves in Ontario	2 yr
09-19	Dr. Davor Ojkic, AHL Cheryl Firby, Dr. Scott Gillingham, Doug McGhee, Dr. Rachel Ouckama, Dr. Cynthia Philippe, Dr. Babak Sanei	Surveillance for inclusion body hepatitis in broiler breeders	2 yr
09-20	Dr. David Pearl, OVC Dr. Agnes Agunos, Dr. Jim Fairles, Shiona Glass, Dr. David Léger, Dr. Beverly McEwen, Dr. Scott McEwen, Dr. Jane Parmley, Dr. Richard Reid Smith, Dr. Durda Slavic	The development and design of a surveillance system for antimicrobial resistance in agriculture animal pathogens in Ontario	3 yr
09-21	Dr. Crawford Revie and Dr. Javier Sanchez, UPEI Dr. David Kelton, Dr. Bruce McNabb, UPEI MSc epidemiology grad student	Developing and implementing techniques to harvest additional surveillance information from existing AHL diagnostic laboratory data	3 yr
09-22	Dr. Durda Slavic and Dr. Michele Guerin, AHL and OVC Dr. Marina Brash, Dr. Hugh Cai, Leanne Cooley, Scott Houghton, Dr. Babak Sanei, Dr. Lloyd Weber	Optimization and validation of real-time PCR to determine the prevalence of pathogenic and non-pathogenic *Brachyspira* spp. in Ontario layer flocks and to identify risk factors associated with dirty egg syndrome	2 yr

Fast facts: canine leptospirosis

Beverly McEwen, Davor Ojkic, John Prescott

A slight increase in the percent of microscopic agglutination test (MAT) seropositive canine *Leptospira* sp. cases occurred in the first 6 months of 2009 compared to the previous 2 years.



Seropositive dogs are usually positive to several serovars and because there is cross reactivity amongst serovars, deducing the infective serovar from the MAT is problematic. It is generally thought that most canine leptospirosis in Ontario is caused by serovars grippotyphosa and pomona. A paper by Alton GD, et al. (Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1999-2006. Can J Vet Res 2009;73:167-175) that appears to document a recent decline in incidence will soon be available for free download from PubMed. *AHL*

AHL Lab Reports

RUMINANTS

Acute selenium toxicosis in lambs

Brian Binnington, Brent Hoff, Murray Hazlett, Heather Murray, Beverly McEwen

Selenium deficiency is a problem in Ontario sheep and goats due to the low selenium levels in the soil. Skeletal and myocardial degeneration compatible with white muscle disease is the most frequent condition associated with deficient tissue levels of selenium. Since 2000 at the AHL, there have been 28 cases in sheep and 16 cases in goats where *selenium deficiency* was considered to be associated with the death of the animals.

Selenium injection of young animals is practiced in Ontario to help reduce the incidence of deficiency, however, excessive selenium by injection or feeding can result in toxicity. Cases in which *selenium toxicity* was suspected in sheep and goats are uncommon at the AHL, with only 4 cases since 2000. Two of these cases highlight the potential for toxicity.

Case A: In February of 2009, three 2- to 3-day-old lambs weighing 3.8 to 4.5 kg were submitted for necropsy. The owner reported that he had given a 1 mL injection of a selenium and vitamin E product that has 3 mg of selenium/mL as sodium selenite to the lambs at 24 hours of age. One to 2 days later, several lambs had difficulty walking and appeared to be short of breath.

Necropsy findings were similar in the 3 lambs, with excessive clear yellow fluid in the thoracic cavity and congested wet lungs with scattered petechial hemorrhages. Histopathology demonstrated diffuse alveolar capillary dilation and congestion with eosinophilic fluid in alveoli, bronchioles, septa and pleura. Intra-alveolar hemorrhages were scattered throughout the lobules. Liver selenium levels using spectrofluorophotometry in 2 lambs were 8.6 and 5.1 ug/g (adequate reference interval 0.25 to 1.5 ug/g).

Case B: A case in 2000 had multiple deaths of lambs at 36 to 84 hours following injection with a double-strength selenium (as sodium selenite) and vitamin E product that had 6 mg of selenium/mL. At necropsy and on histopathology, the lungs were congested and edematous with multifocal hemorrhages. Interstitial edema of the myocardium with peracute myocardial necrosis was also seen. The liver selenium level was very elevated in the one lamb tested at 68 ug/g levels (adequate reference intervals 0.25 to 1.5 ug/g).

Clinical signs reported with acute selenium toxicity in lambs include weakness, staggering, respiratory distress or convulsions prior to death. If present, lesions consist primarily of congestion, edema, hemorrhage in various tissues with lung congestion, edema, hemorrhage and fluid effusions most frequently described.

A case of acute selenium toxicosis in sheep reported in the Veterinary Record gave liver levels in acutely poisoned sheep of 7.1 to 10.8 mg/kg (ug/g). In Veterinary Toxicology, the IM LD_{50} for sodium selenite in lambs is reported to be 0.445 mg/kg and an intramuscular injection of 5 mg of selenium can be lethal for lambs. In Mineral Levels in Animal Health – Diagnostic Data, it is stated that levels of 3.6 -18.0 ppm (ug/g) wet weight occurred in lambs with suspected overdose of sodium selenite.

For young lambs and goat kids, the margin of safety for sodium selenite injection is narrow, and strict adherence to recommended dosage is essential

For young lambs and goat kids, the margin of safety for sodium selenite injection is narrow, and strict adherence to recommended dosage is essential. Mistakes can occur in selecting the wrong product such as the double-strength selenium product for older animals that provided 6 mg/mL. The use of too large a syringe, such as a 10 mL syringe, can result in overdosing when trying to measure the small amount (0.5 ml) of injectable selenium product recommended for newborn lambs. *AHL*

References

Clarke ML, et al. Mineral or Inorganic Substances In: Clarke ML ed. Veterinary Toxicology 2nd ed. London. Bailliere Tindall 1981:71.

Kyle R, Allen WM. Accidental selenium poisoning of a flock of sheep. Vet Rec 1990;126:601.

Puls R. Mineral Levels in Animal Health – Diagnostic Data. 2nd ed, Clearbrook BC. Sherpa International 1994:251.

AVIAN/FUR/EXOTIC SPECIES

First report in Ontario of *Coxiella*-like infection in a Pionus parrot

Margaret Stalker, Jennifer Hopper, Marina Brash, Kim Chan, Rebeccah Travis, Hugh Cai

A 7-month-old male blue-headed Pionus parrot was submitted for necropsy after a brief illness characterized by a 3-month history of weight loss, head-bobbing, green diarrhea, followed by sudden death. Postmortem examination revealed a thin bird with intense dark yellow discoloration of the skin and subcutaneous tissues. There was fibrin within the abdominal airsacs; the liver was enlarged and mottled, the spleen was enlarged and meaty, and green urates were present within the cloaca.

Histology revealed generalized lymphoplasmacytic hepatitis with scattered foci of necrosis, and numerous tiny faintly basophilic coccoid-appearing intracytoplasmic organisms within hepatocytes and Kupffer cells, which stained only faintly with modified acid fast stains (Figure 1). Similar organisms were visible within macrophages in the interstitium of the kidney.

The working diagnosis was *Chlamydophila psittaci* infection, however, further testing for *Chlamydophila*, including antigen ELISA, PCR and immunohistochemistry, was uniformly negative. Examination of modified acid-fast stained smears from the liver revealed organisms more closely resembling *Coxiella* sp. than *Chlamydophila*, although the PCR for *Coxiella burnetii* was also negative.

In an attempt to reach a definitive diagnosis, PCR amplification of the 16S rRNA gene was performed using universal primers. Sequence analysis and comparison with published GenBank sequences revealed 99% identity to a novel *Coxiella* sp. only recently described in a case series of 7 psittacine birds and a toucan from the California Animal Health and Food Safety Laboratory System. Affected birds in this study exhibited nonspecific clinical signs including anorexia, lethargy, weight loss and respiratory distress prior to death, and on necropsy all had hepatosplenomegaly with multifocal hepatic necrosis and mixed inflammation, with *Coxiella* organisms in macrophages in liver, as well as in spleen, kidney, lung, adrenal and bone marrow in some birds. **The organism identified in these birds differs from the more familiar *C. burnetii*, which causes abortion and neonatal mortality in mammals.** It is not known how prevalent this infection is in captive birds, nor how affected birds acquire the infection. The close resemblance of this case to avian psittacosis/ornithosis, a disease of public health significance, underscores the utility and importance of having advanced laboratory testing capabilities available. *AHL*

Reference

Shivaprasad HL, et al. *Coxiella*-like infection in psittacines and a toucan. Avian Dis 2008; 52:426-432.

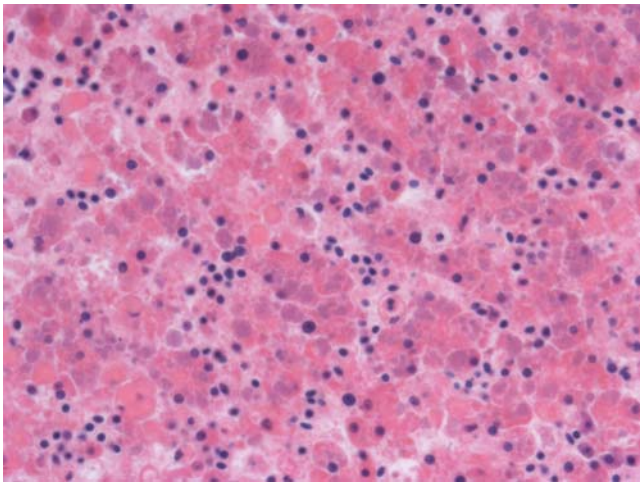


Figure 1: Histology of the liver from a juvenile Pionus parrot, showing myriads of tiny faintly basophilic organisms within the cytoplasm of numerous hepatocytes and Kupffer cells (H&E, 400X).

SWINE

Pseudomelanosis coli in two groups of slaughterhouse pigs.

Murray Hazlett, Josepha DeLay, Bob Friendship

Carcasses from 2 groups of pigs (A, B) were suffering partial condemnations as a result of green to red-brown discoloration of colons and rectums. These by-products are considered specialty foods within various ethnic communities, and are referred to as chitlins or bung.

Losses in the first group of carcasses averaged about 2-3%. Gross lesions in both groups were similar, with diffuse dark green to black discoloration of the mucosa of affected rectums that varied in intensity throughout the length of each section. (Fig 1).

Histologically, pigment-laden macrophages were seen in lamina propria of rectal mucosa and were often present in large numbers. The pigment within macrophages varied in color from golden brown to olive green (Fig 2). Various histochemical and immunohistochemical stains were used in an attempt to determine the composition of the pigmented material, as listed in Table 1.

In humans, pseudomelanosis coli is characterized by abnormal brown discoloration of the colonic mucosa caused by accumulation of pigment in macrophages in lamina propria and submucosa, and is not regarded as clinically significant. The pigmented debris is often identified as lipofuscin and reflects increased cell turnover associated with chronic use of anthraquinone laxatives. Because the brown pigmentation has been shown not to be due to melanin, the condition is termed 'pseudomelanosis'. Similar lesions have been identified in ileum and jejunum of humans and the pigment in these sites is most frequently identified as aluminum, silicon, and titanium compounds associated with food additives, or hemosiderin or iron sulfate associated with gastrointestinal ulceration and hemorrhage.

The cause of this condition in swine is unknown. There was a degree of inflammation and fibrosis in rectal mucosa and submucosa of some of the cases submitted to the AHL, and some of the iron-containing pigment identified may have been due to associated hemorrhage. Dietary factors may be a cause, as indicated in humans, and this is currently under investigation in our cases. *Porcine circovirus 2* is associated with colitis in pigs, however significant PCV-2 antigen could not be demonstrated in these cases. *AHL*

Table 1. Staining characteristics of pseudomelanosis coli.

Stain	Group A	Group B
Iron (Perle's)	+	+
PAS	+	
Bile	-	-
Mineral	+	
Acid-fast	-	
PCV2	questionable	-ve
Melanin		-
Copper		- (stain negative, EM +ve)
Lipofuscin		-

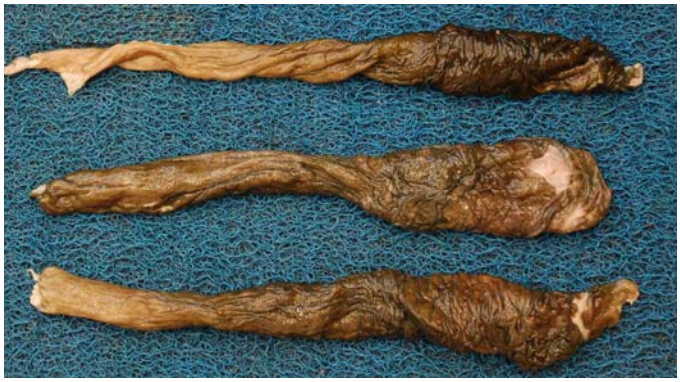


Figure 1. Pig rectums with pseudomelanosis coli.

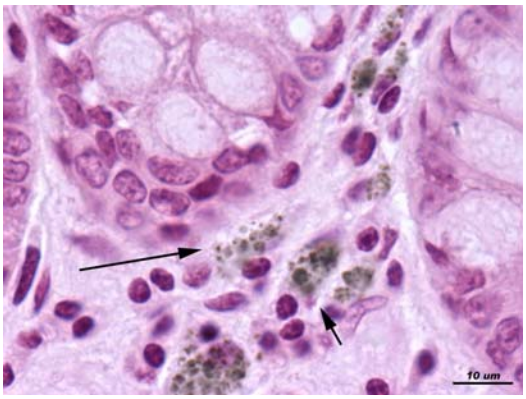


Figure 2. Brown-green pigment in macrophages in lamina propria of colon (arrows) H&E.

HORSES

Ontario Racing Commission Death Registry 2008

Josepha DeLay

The Ontario Racing Commission Death Registry has been in place since 2003 and continues to provide excellent data regarding the causes of morbidity and mortality in racehorses in this province. Fifty-one (51) horses were necropsied at the Animal Health Laboratory in 2008, consisting of approximately equal numbers of Standardbreds and Thoroughbreds.

The distribution of 2008 ORC necropsy diagnoses by body system is similar to that in previous years, with **most cases due to fractures involving limbs (31%) or other sites (10%).** Other predominant diagnoses involved the respiratory (18%), gastrointestinal (10%), non-fracture musculoskeletal (10%), and cardiovascular (8%) systems. The cause of death remained undetermined in 10% of cases in 2008, in contrast to 0- 4% of cases in the preceding 5 years. *AHL*

From the Toxic Garden:

Houndstongue (*Cynoglossum officinale*) causes pyrrolizidine alkaloid toxicosis

Margaret Stalker, Brent Hoff

Discovery of a flourishing specimen of this toxic pasture weed growing beside the Animal Science and Nutrition building here at the University of Guelph prompted us to write this brief note. Houndstongue is a biennial member of the Boraginaceae, the borage or forget-me-not family, which includes other notable toxic plants including the Australian pasture weed, Patterson's Curse (aka Salvation Jane). A European native inadvertently introduced into North America in the late 1800s, houndstongue now inhabits pastures, roadsides and other disturbed areas across the continent.

The mature plant is 0.5 to 1.2 m in height, with a basal rosette of elongate 8-cm wide leaves with a hairy surface, said to resemble a hound's tongue. Upper leaves partially clasp the stem, and are narrower, with a curled appearance. Small, inconspicuous red-purple flowers form in early summer in the upper portions of the plant along stems borne in leaf axils (Figure 1). Each flower produces 4 green burlike nutlets that turn brown as they mature. These seeds cling to fur or clothing, and are dispersed widely by this method.

Houndstongue contains several pyrrolizidine alkaloids, which are metabolized by the liver to reactive pyrroles, forming adducts with hepatic proteins and nucleic acids and causing the liver injury typical of this class of compounds. Lesions include hepatocellular necrosis at high doses, and characteristic megalocytosis with biliary proliferation and periportal fibrosis with exposure at lower doses over a longer time period. Although not particularly palatable, the plant is readily eaten when included in dried or pelleted forages.

While cattle are susceptible to intoxication, houndstongue is also highly toxic to horses; as little as 1 plant per day ingested over a 2-week period can cause clinical disease. Clinical signs include lethargy, anorexia, icterus and photosensitivity. A specific assay is not readily available in diagnostic labs, and diagnosis is based on evidence of exposure combined with compatible clinical signs and histologic lesions. *AHL*

Reference

Stegelmeier BL, et al. Pyrrole detection and the pathologic progression of *Cynoglossum officinale* (houndstongue) poisoning in horses. J Vet Diagn Invest 1996;8:81-90.

Figure 1: Houndstongue (*Cynoglossum officinale*)

COMPANION ANIMALS

AHL feline pathology toxicologic diagnoses, 1998 to June 2009

Josepha DeLay, Beverly McEwen, Brent Hoff

Various toxicoses were confirmed or suspected as the cause of death in 41 cats submitted to the AHL from 1998 through June 2009. Of these cases, the diagnosis was confirmed by analysis and identification of specific toxic compounds in 35 cats (Table 1). Histologic lesions consistent with toxicosis were evident in 6 cats, but the exact cause was not confirmed in these cases due to lack of suitable material for testing. **Ethylene glycol was the most frequently diagnosed toxicity in this time period** (21 cases, 51%).

The mean age of cats with toxicologic diagnoses during this time was 5.6 years, with ages ranging from 6 months to 16 years. The highest numbers of confirmed cases of intoxication were submitted during August (23%), April (21%), and May (15%), presumably reflecting times of increased outdoor activity and increased potential of exposure to toxic substances.

Diagnosis of toxicoses can be difficult due to multiple factors including lack of specific clinical signs in some cases and sudden death with no premonitory signs, as well as technical constraints and cost of analysis for possible toxins. Toxicologic analysis typically consists of screening tests to identify the presence of a potentially toxic compound, followed by confirmatory testing and possible quantification to determine if sufficient toxin was present to have resulted in the clinical scenario described. Analytical methods commonly used for identification of toxic compounds include:

- gas and gas-liquid chromatography,
- thin-layer chromatography,
- mass spectrometry, and
- Immunoassays, such as ELISA.

The chemical composition of various toxic substances determines the analytical method most suitable for detection of the substance. As a result, the consideration of history, clinical signs, and necropsy and histologic findings to narrow the potential list of toxicants can allow selection of tests more specific for these compounds and increase the chance of identifying the toxic substance involved.

Samples typically collected for toxicologic analysis include blood, urine, stomach content, vitreous humor, liver, kidney, and brain. The duration of clinical signs and the suspected intoxicant will influence the type of sample used for analysis. For example, stomach content may be useful in suspected cases of recently ingested toxins without extensive vomition, whereas urine or tissue samples may be more helpful in subacute toxicities. When performing in-clinic necropsies, all of the above samples can be frozen and held at the clinic for future toxicologic testing, if suggested on the basis of gross and histologic lesions. *AHL*

Table 1. Feline pathology toxicologic diagnoses, 1998 - 2009

Toxin	Number of cases
Ethylene glycol	21 (51%)*
Melamine (cyanuric acid)	6 (15%)**
Carbamate / organophosphate	5 (12%)
Carbon monoxide	2 (5%)
Anticoagulant rodenticide	1 (2%)*
Iron	1 (2%)
Lily (suspected)	1 (2%)
Acetaminophen (suspected)	1 (2%)
Bromethalin (suspected)	1 (2%)
Unknown	3 (7%)

*combined ethylene glycol and anticoagulant toxicity in 1 case
**melamine (cyanuric acid) toxicity identified in an additional 9 cases with no pathology submission