Toxicology

TOXICOLOGICAL TESTING IN DOGS AND CATS – GENERAL GUIDELINES

Veterinary clinics are often confronted with a dog or cat with clinical signs suggesting exposure to a toxic substance. Clinical signs of many poisonings are similar and may be unable to be reliably differentiated on clinical grounds alone. A careful clinical history can often narrow the differentials. In some cases, owners are concerned that their animal has been deliberately poisoned and are seeking testing to confirm or rule out this possibility. Owners will often request a "toxin screen" in cases of suspected intoxication. Client communication and education is vital so that expectations of testing are not unrealistic. Toxin testing requires a specific toxin to be nominated as there is no suite of tests that covers all possibilities. Toxin testing is inherently expensive, requires specific sample types and false negatives can occur; for instance the toxin may have been eliminated from the body or be undetectable, but clinical signs may persist.

Gribbles Veterinary can offer specific testing for a range of toxic substances, however it is important to consider the specific sample requirements and testing limitations for each toxin when advising your clients. Many tests are referred to external laboratories and may have extended turnaround times.

Specific toxin testing is NOT available for the following compounds: Fe-EDTA molluscicides, cholecalciferol based rodenticides, tick paralysis toxin, tetrodotoxin, lily toxin, amatoxin (mushrooms). Please contact the laboratory if you need testing for a specific toxin not listed here; we can often source unusual tests as needed from our network of referral laboratories.

General guidelines for sampling where toxin type is uncertain should aim to provide a wide range of samples for potential testing. Fresh tissue samples should be chilled or frozen for transportation to the laboratory.

Pre mortem sampling:

- Suspected intoxicant (food, bait, water, medication)
- Vomitus
- Urine
- Faeces

Post mortem sampling:

- Fresh liver, kidney (ideally enough to fill a yellow-top pot, or the whole organ minus a small histology sample for smaller animals)
- Urine (yellow top pot)
- Stomach contents, small intestinal contents (yellow top pot)
- Representative histological samples (the most important organs are liver and kidney, upper GIT). Remember formalin to tissue ratio should be at least 10:1 to allow adequate fixation.

Clinicians should also consider syndromes which may mimic intoxication such as hypocalcaemia, hypoglycaemia, hepatic encephalopathy, peripheral neuropathies and primary CNS diseases.

If litigation is threatened then you will need to:

- Have a detailed record of all findings
- Record the identity of the animal(s)
- Collect and label specimens
- Seal specimen containers
- Maintain continuity of possession
- Obtain a receipt of specimens.

Examples of intoxicants that can be tested are provided below.

Biological control agents

- Carbamates
- Metaldehyde
- 1080 (sodium fluoroacetate)
- Strychnine
- Synthetic pyrethroids
- Organophosphates
- Organochlorines
- Anticoagulant rodenticides (warfarin, pindone, coumetetryl, bromadiolone, difenacoum, brodifacoum)

Heavy metals

- Arsenic
- Lead

Human medicinals

- Paracetamol
- Aspirin
- Drugs of addiction (opiates, sympathetic amines, benzodiazepines, cannabinoids, barbiturates, cocaine, methadone)
- Antidepressants (Amitryptyline, tricyclic antidepressants)
- Phenobaritone, pentobarbitone

Biological toxins

- Cyanobacteria
- Botulism
- Mycotoxins

TOXICOLOGICAL TESTING OF PRODUCTION ANIMALS – GENERAL GUIDELINES

Possible toxic causes of production animal ill health and death are numerous and out of the scope of this publication. When investigating suspected poisonings in animals it is important to be methodical:

Take history, examine the animals (clinically or necropsy), examine the environment, collect samples for laboratory examination and record all the findings. You may need to consult with your local laboratory for advice on appropriate samples, sample volumes and handling details.

A useful reference is “Veterinary Clinical Toxicology Third edition.” Parton,K. Bruère, A.N. Chambers, J.P. 2006 Foundation for Continuing Education Publication No 249.

If litigation is threatened then you will need to:

- Have a detailed record of all findings
- Record the identity of the animal(s)
- Collect and label specimens
- Seal specimen containers
- Maintain continuity of possession
- Obtain a receipt of specimens.

Examples of more common intoxicants that can be tested are provided below.

Biological control agents

- 1080 (Sodium fluoroacetate)
- Strychnine
- Synthetic pyrethroids
- Organophosphates

- Organochlorines
- Carbamates
- Metaldehyde
- Anticoagulant rodenticides (warfarin, pindone, coumetetryl, bromadiolone, difenacoum, brodifacoum)

Heavy metals

- Arsenic
- Lead
- Copper
- Selenium
- Zinc

Biological toxins (non-plant origin)

- Cyanobacteria
- *Clostridium botulinum* toxin
- Mycotoxins (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Urea

Biological toxins (plant origin)

- Mycotoxins (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Nitrate/nitrite
- Cyanide
- Oxalate
- Ergot alkaloids
- Pyrrolizidine alkaloids
- Indospicine
- Ptaquiliosides
- Pimelea toxin

TOXICOLOGICAL TESTS KNOWN TO BE AVAILABLE

- 1080 testing
- Antidepressant drugs (amitriptyline, tricyclic antidepressants)
- Arsenic
- Aspirin
- Carbamates
- *Clostridium botulinum* toxin by ELISA
- *Clostridium perfringens* epsilon testing by ELISA
- Copper
- Cyanide
- Cyanobacteria
- Drugs of addiction screen (opiates, sympathetic amines, benzodiazepenes, cannabinoids, barbiturates, cocaine, methadone
- Ergot alkaloids
- Indospicine
- Lead
- Metaldehyde testing
- Mycotoxin testing (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Nitrate/nitrite (Qualitative)
- Nivalenol
- N-methylcarbamate pesiticides
- Organochlorines
- Organophoshate pesticides
- Paracetamol

- Phenobarbitone
- Ptaquiliosides
- Pimelea toxin
- Pyrrolizidine alkaloids
- Rodenticide testing (Warfarin, pindone, coumetetryl, bromadiolone, difenacoum, brodifacoum rodenticides)
- Selenium
- Synthetic pyrethroids
- Urea
- Zearalenone
- Zinc

MYCOTOXICOLOGY

SPORIDESMIN TOXICITY (FACIAL ECZEMA)

Managing this disease involves consideration of the four factors below:

DETECTING CASES OF LIVER DAMAGE

Species: Sheep, cattle, camelids, deer

Specimen: Serum for GGT and GLDH

Container: Red top or gel tube

General information: Serum GGT and GLDH rise quickly following sporidesmin injury and are almost always high when signs of liver injury (e.g. photosensitivity and jaundice) appear. At 2-4 weeks following ingestion of sporidesmin, there is a good relationship between the GGT level and degree of liver damage. Cases can be graded as mild, moderate or severe at this stage but this has not yet been proven to have prognostic potential. Testing for GLDH helps differentiate other causes of liver disease. In cases of facial eczema, GLDH levels are usually lower than GGT levels.

DETERMINING IF ZINC INTAKES ARE ADEQUATE FOR PROTECTION

Species: Sheep, cattle, camelids, deer

Specimen: Trough water, drench, feed

Container: Yellow top pot

General information: Determination of zinc levels in drinking (trough) water or drenches can detect whether levels are toxic or adequate for facial eczema protection. Zinc drenches can also be tested for proper mixing of the zinc additive. Zinc in feed can also be tested.

Serum zinc levels for facial eczema prevention (see Minerals section of Handbook) are general guidelines as clinical and subclinical sporidesmin toxicity can still occur with serum zinc values within this range depending on level of toxin challenge, other trace element intake e.g. Copper and other host or environment factors.

Serum zinc assay is however useful in cases of suspected zinc over-dosage/toxicity - see below.

DIAGNOSING ZINC TOXICITY

Species: Sheep, cattle, camelids, deer

Specimen: Live animal: Serum in plain (red top) or lithium heparin tube for zinc and GGT levels, EDTA for a full CBC if there is anaemia; Dead animal: Fixed abomasum, liver and pancreas for histopathology. Fresh liver and kidney in yellow top pot for zinc determination.

General information: Diarrhoea, weight loss, and metabolic disease in cattle are the main clinical signs of zinc toxicity. A few will also develop a haemolytic anaemia. In the live animal, measuring serum zinc concentrations is a valid way to check for possible zinc toxicity. In those animals that appear anaemic or have red urine, a CBC is also recommended. Overdosing zinc can suppress copper and iron absorption.

At necropsy, the lesions to look for are abomasal ulceration and pancreatic atrophy and fibrosis. Abomasal ulceration occurs when zinc sulphate is used for drenching, as this triggers closure of the oesophageal groove, diverting the drench directly into the abomasum. For this reason, zinc sulphate drenches are not recommended. Pancreatic changes will occur with poisoning due to any form of zinc salt but are a chronic change and often hard to detect. The more common lesion is hepatobiliary necrosis due to sporidesmin toxicity. It is worth stressing that most cases of suspected zinc toxicity turn out to be sporidesmin toxicity due to under dosing with zinc.

SPORE COUNTING FOR FACIAL ECZEMA (FE)

Pasture spore counting

Specimen: Pasture from at least 5 locations

Container: Paper bag

General information: Selecting the sampling site: This depends on the type of farm and the management policy. If all the animals are in one group, e.g. a dairy farm, only the paddock the animals will graze the next day need be sampled. If set stocking is practised, then it is necessary to sample the dangerous paddocks.

Tips to help predict the most susceptible paddocks for spore counting:

a) Spore counts on north and west facing slopes are usually higher than east and south facing slopes.

b) Flats generally have lower counts than slopes above them as cool air flows downward at night.

c) Paddocks with a lot of pasture litter and those that are well sheltered often have higher counts.

A useful system is to use a warm slope as an indicator site and to sample it regularly, at least three times a week. When spore numbers on it start to rise, the other paddocks should be checked to define the spore pattern over the farm. Using this system, a bank of information will accrue and susceptible paddocks will be identified. However, it is not safe to assume only the same paddocks will be susceptible every year.

Method for obtaining a pasture sample for spore counting:

- Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10 m apart, and submit in paper bags not plastic bags so as to avoid " plant sweating".
- Avoid parts of paddocks, which are sheltered by trees or hedges. Take separate samples if you need to know spore numbers under hedges.
- Sample from an area of even slope.
- If you sample the same site regularly, follow the same route across it.
- Take samples at least 3 times a week and more often if the weather favours spore production. Spore numbers will rise in the absence of rain if the weather is humid, particularly late in the season. Numbers do not always rise immediately after rain and the peak may occur up to a week after the last fall.

Faecal spore counting

Faecal spore counting represents more accurately what the animals are ingesting and therefore more accurately reflects FE risk. Faecal samples are also easier to collect. Faecal spore numbers are approximately double the pasture levels but this is very dependent on grazing pressure. The ratio may be less than 2:1 in cattle because of their lower faecal dry matter. As a tentative recommendation levels should be considered dangerous when faecal spore counts approach 75-100,000 spores per gram of faeces.

As you are interested in the spore intake of the herd or flock rather than individual animals, pooling of 5-10 samples is recommended. These are best pooled at the laboratory. Fresh faeces may be removed from the pasture, yards or from the rectum for this purpose.

MYCOTOXIN SCREENING

The Mycotoxin Screen tests for a range of mycotoxins.

Toxins tested: Aflatoxins B1, B2, G1, G2, Fumonisins B1, B2, Deoxynivalenol, Nivalenol, HT2, T2, Ochratoxin A, Zearalanone and Vomitoxin.

Species: Sheep, cattle, horses, dogs, pigs, chickens

Specimen: Silage, grain in yellow top pottle. Pasture in paper bags

Special handling for pasture: Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level from at least five places, which are at least 10 m apart, and submit in paper bags not plastic bags so as to avoid "plant sweating".

Collection Protocol: The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring.

General information about the diseases:

Zearalenone is produced by several species of Fusarium fungi. It is an oestrogenic mycotoxin that causes infertility in sheep and pigs and has been incriminated as a cause of infertility in cattle. Most Fusarium growth occurs in late summer and autumn at the base of pasture on dead leaf litter and in poorly stored grain. Green and growing grasses are less affected.

Tricothecenes are thought to be involved in some cases of ruminant ill thrift. The toxins are produced by Fusarium and other fungal species.

NIV and DON usually infect grain, especially maize, and may cause a problem in pigs fed such maize. The effects are dose related:

a) < 5mg/kg is associated with feed refusal and decreased weight gain.

b) > 5 mg/kg can cause vomiting, diarrhoea, abortion, nervous signs and death.

T2 toxin T_2 toxicity is predominantly associated with mouldy maize and is mostly a problem in pigs and poultry. In pigs, besides ill thrift this toxin can also cause an irritant contact dermatitis.

Aflatoxin is a member of the bisfuranocoumarin group of compounds, produced as metabolites mainly by *Aspergillus flavus, Aspergillus parasiticus* and *Penicillium puberulum*. Sheep and adult cattle are quite resistant to the toxin, horses moderately resistant whereas dogs, pigs and calves are sensitive and may be fatally intoxicated at a dose rate of <1 mg/kg body weight. Colonisation and toxin production can occur in grains such as maize, cottonseed and peanuts in all phases from growth through harvest. They can be produced in peanuts, soybeans and other small grains mainly during storage. Toxin production is encouraged when warm, moist ambient conditions are combined with crop damage (drought or storm).

ENDOPHYTE TOXINS

ERGOT ALKALOIDS

Species: Sheep, cattle, pigs

Specimen: Pasture in paper bag, grain in yellow top pot

Special handling/shipping requirements: The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring.

Collection protocol: Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10 m apart, and submit in paper bags not plastic bags so as to avoid " plant sweating".

General information about the disease: Ergot Alkaloids are mainly involved in heat stress, gangrene of the extremities and animal ill thrift. It is found in 'wild-type' ryegrass and fescue. Many newer releases of pasture cultivars have been bred to reduce the level of these toxins.

LOLITREM B AND ERGOVALINE

Species: sheep, cattle, horses, deer, camelids

Specimen: Pasture

Container: Paper bag

Special handling/shipping requirements: The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring.

Collection protocol:

Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10 m apart, and submit in paper bags not plastic bags so as to avoid " plant sweating".

General information about the disease:

Lolitrem B, a potent tremorgen, is the predominant alkaloid involved in rye-grass staggers. Ergovaline, a vasoconstrictor, is also present. If you have ryegrass, both ergovaline and lolitrem B can be present. If you want to have only one test done, a test for lolitrem B is recommended. If you have fescue, an ergovaline test is recommended. Other problems reported include ill-thrift, heat stress, scours, infertility and lowered milk production.

OTHER MYCOTOXINS *(contact the laboratory for information on availability and cost of testing)*

- Lolitrem B for Perennial Ryegrass can also be tested in fat and faeces as an indication of exposure.
- Ergotamine / ergocristine / ergocryptine / ergocornine / ergosine arising from mould infestation in pasture and grain
- Peramine in Perennial Ryegrass
- Lolines in fescue and other pastures
- Formononetin / genistein / diadzein / biochanin A / coumarin / coumestrol in clovers and pastures
- Volatile acids and lactic acid in water and silages
- Tyramines / Tryptamines in phalaris (in development)