

Animal Health Laboratory



AHL Newsletter

Volume 10, Number 3, page 17

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September, 2006

We're very pleased to announce that Dr. Brian Binnington has accepted the position of **Veterinary Pathologist**, **AHL-Kemptville**, and will transfer there in September.

Brian is an OVC '73 graduate, with a Diploma in Pathology; he was board certified as an anatomic pathologist by the American College of Veterinary Pathologists in 1986. Brian has a long history as a pathologist with UofG/VLSB/AHL - 18 yrs in the VLSB-Brighton lab, and 8 yrs at AHL-Guelph.

Brian looks forward to using his extensive laboratory experience to serve our animal health clients in eastern Ontario, in all species, with emphasis on poultry and pet birds.

Dr. Marina Brash rejoins the AHL

We're delighted to announce that Dr. Marina Brash was the successful candidate in our recent competition for the position of **Veterinary Pathologist, Avian and Fur-Bearing Species**, and started August 14, 2006.

Dr. Brash received her DVM from OVC in 1981, was in mixed animal practice for 5 years in Seaforth, and completed a DVSc at OVC in 1991. She worked as a pathologist in VLSB and the AHL from 1990 to 1998, and was board certified as an anatomic pathologist by the ACVP in 1992.

We look forward to Marina using her strong client focus and expertise in diagnostic and consultative work regarding poultry, pet bird, fur-bearing, and exotic species. Welcome back Marina!



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News in companion animal endocrinology Kris Ruotsalo

LABORATORY SERVICES

Free T4 Determination of free T4 by equilibrium dialysis is currently unavailable due to the discontinued production of the assay kit by the manufacturer. Free T4 in **dogs** will now be determined by the **Diasorin 2-step method** (code FT42S). This radioimmunoassay has been endorsed by the OFA (Orthopedic Foundation for Animals), and is a component of the profile required for certification of dogs in the OFA thyroid registry.

As before, only a single serum sample is required. The assay will be run Thursdays and can be ordered individually as **fT4 2-step** or in combination with thyroid panels:

Thyroid profile 2 (TT4, fT4.2-step, cTSH) Thyroid profile 3 (fT4.2-step, TgAA, cTSH) Until we complete validation of the Diasorin 2step method for use in **cats and horses**, we encourage clinicians to investigate suspected thyroid problems using serum total T4.

Endogenous ACTH We have added a new chemiluminescent endogenous ACTH assay for use in **dogs and horses** - available Monday thru Friday. Sample requirements remain the same: 1 mL of EDTA plasma, frozen in a plastic tube, and shipped on ice.

Canine TLI We have also added a new chemiluminescent method for canine TLI - available Monday thru Friday, requires 1mL of serum, obtained after a minimum 6-hr fast.

Surgical biopsies: What to expect from the pathology report

Josepha DeLay

The goal of most veterinarians when submitting surgical biopsies of suspected neoplasms for histopathology is straightforward – is it neoplasia and, if so, what is this tumor and did we get all of it? As practitioners, we cannot adequately deal with a disease entity for which we don't have a specific diagnosis.

In the AHL histopathology lab, we examine submitted biopsies grossly, and record size, type (excisional, incisional, punch, wedge), and pertinent features. We mark the surgical margins of the biopsy with ink prior to sectioning, so that we can accurately identify these margins on the slides. Representative sections are taken, including sections perpendicular to the surgical margins and the deep margin.

The pathologist describes the histologic appearance of the lesion; for neoplasms, this will include the cytologic features of the neoplastic cells and growth habit of the tumor, which allow (in most cases) categorization as a specific tumor. The inked (labeled) surgical margins of the biopsy are examined to determine if neoplastic cells are present at or approaching the site of the incision, or if, subjectively, an adequately wide zone of normal tissue separates the tumor from the incision.

For neoplasms that do not fit neatly into a specific tumor category, we attempt to correlate the available evidence, such as cytologic variation among the neoplastic cells, their mitotic rate, and presence of infiltrative growth, with the potential malignancy of the neoplasm. For poorly differentiated neoplasms, we may recommend histochemical or immunohistochemical stains, or electron microscopy, to provide further information regarding the tissue of origin of the neoplastic cells, as this information may aid in prognostication.

Practical tips to increase the information provided by surgical biopsies:

- **Describe the appearance and growth habit** of the mass identified at surgery discrete mass? obvious infiltration and involvement of surrounding tissue? The pathologist will attempt to correlate your description with the microscopic appearance of the mass.
- If you are concerned about the completeness of excision of the mass at any specific margins of the biopsy, **mark that margin** with a suture or provide some other type of orienting feature so that we can identify that region specifically when we are trimming the tissue.
- For biopsies from more than one anatomic location, **use a separate, labeled sample jar for each biopsy**. Multiple masses in a single animal will not necessarily have the same diagnoses, and this will allow us to tell you which of the anatomic sites could require additional intervention.
- Ensure adequate fixation of your biopsy so that histologic features are preserved. The ratio of formalin to tissue in a sample jar should be 10:1, which is easily accomplished for small samples and punch biopsies. For larger samples, you will need to use a correspondingly larger plastic container and the biopsy should be partially sectioned to allow formalin exposure of the center of the biopsy. It is best to keep the margins of even large biopsies intact - exposure can be accomplished by slicing through the central area of the biopsy. *AHL*

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Summer brings three 'herd outbreaks' of toxicities

Brent Hoff, Gary Thomson, Margaret Stalker, Robert Walsh, Kirsten Graham, Marcia R S Ilha

1. Dogs - Cyanobacteria (blue-green algae) toxicity

Three dogs (2 Labrador retrievers and a Weimaraner) in a group of 11 dogs at a dog sitter's farm died suddenly and unexpectedly within 1 hr. The dogs had access to a pond and several of the dogs had been observed swimming and eating rotting vegetation and horse feces at the pond edge. The dogs spent 5 min at the pond and went for a supervised walk around the farm for about 30 min. When the dogs arrived back at the house after 35 min, 2 dogs became weak and collapsed with shallow breathing. The third dog developed similar signs within a few minutes. Death from respiratory arrest occurred within 1 hr in all three dogs. A fourth dog (a Labrador retriever cross) subsequently developed similar signs, but survived.

The clinical pathology data revealed no significant abnormalities in 2 dogs from which antemortem blood was collected. One dog was submitted for postmortem examination. No gross or microscopic lesions were detected in tissues. Gastric content was negative for organophosphates, carbamates, strychnine and the mycotoxins penitrem A and roquefortine. Brain cholinesterase activity was reduced at $0.6 \,\mu mol/g/min$ (ref. 3.2 ± 1.6). Pond water was negative for microcystins; but positive for anatoxin-a.

The most likely etiology in the death of these dogs is therefore cyanobacteria (blue-green algae) intoxication. Not all algal species produce toxins. Neurotoxic cyanobacteria intoxication is most commonly associated with ingestion of water with excessive growth of Anabaena spp., Aphanizomenon spp. and Oscillatoria spp., which may produce the neurotoxins anatoxin-a (a nicotinic depolarizing alkaloid) and anatoxin-a(s) (an irreversible acetylcholinesterase inhibitor). Other cyanobacterial toxins include the hepatotoxic microcystins, produced by Microcystis spp, Anabaena spp., and Oscillatoria spp. Most reports of intoxication involve algal blooms during periods of warm sunny weather. Cyanobacteria ingested with water can be rapidly broken down in the acidic environment of the stomach, releasing toxins. Free toxin can be rapidly absorbed from the small intestine.

Animals that exhibit clinical signs of intoxication have a **poor to grave prognosis**. The important control measure is to limit or eliminate animal exposure to water bodies containing mats of algae along the shoreline.

2. Cattle - Tansy ragwort toxicity

In early June, 2 beef cows in a pastured herd of 30 animals were noted to be dull and slow prior to death. A 5year-old bull on the same farm was lethargic with bloody diarrhea, and was euthanized. At necropsy, these 3 animals had consistent findings of hepatic fibrosis without icterus, markedly distended gall bladders and marked edema of abdominal viscera, particularly the abomasal wall. On histologic examination, all had various degrees of hepatic fibrosis, hepatocellular megalocytosis and proliferation of bile ductules in portal areas.

The histopathology report from cow 1 and the death of cow 2 prompted a visit to the farm; a large amount of tansy ragwort (Senecio jacobaea) was identified in the pasture (Figures 1, 2). Tansy ragwort is an invasive weed that can quickly spread, reducing production on pasture. This perennial herbaceous plant is toxic to most animals (including humans), and is potentially lethal to cattle and horses, which can be poisoned by eating only 2-8% of their body weight. The plant remains toxic after it is cut. The poisonous principles are **pyrrolizidine alkaloids** that cause hepatocellular necrosis, mitotic arrest and hepatic fibrosis.

Clinical signs of toxicity are variable and nonspecific and can include diarrhea, tenesmus, bloody feces, rectal prolapse, poor appetite, weight loss, paresis and central nervous signs. The disease, in our experience, is sporadic in Ontario.



Figure 2. Tansy ragwort (Senecio jacobaea) close-up (foreground).

3. Horses - Japanese yew toxicity (continued on p.23)

Sudden unexpected death of 3 horses.

AHL Lab Reports

CATTLE

Bovine abortion update, 2000 - 2006

Beverly McEwen, Susy Carman, Durda Slavic

Neospora spp. continues to be the single pathogen most frequently identified in bovine fetuses submitted to the AHL for gross and/or histological examination (Table 1).

The number of bovine abortions submitted for examination has decreased since 2000/2001. The ability to determine an etiologic diagnosis depends upon the number of submissions from an affected herd and the quality and type of specimens submitted. Submission of an entire

fetus and placenta increases the diagnostic rate.

Detailed information on appropriate sample submission is available in the 2006 AHL User's Guide & Fee Schedule and AHL Newsletter "Bovine abortion diagnostics", March 2002. *AHL*

Reference

DeLay, J. Bovine abortion diagnostics: Maximum benefit for your client's dollar. AHL Newsletter, 2002;6:4.

Table 1. Bovine abortion cases submitted to the Animal Health Laboratory, 2000-2006, fiscal years

Selected etiologic diagnoses # cases /(%) ¹	00/01	01/02	02/03	03/04	04/05	05/06
Neospora spp.	47 (19%)	36 (12.5%)	17 (8.3%)	21 (10.9%)	20 (15.3%)	12 (10.2%)
Placentitis, etiology not identified ²	63 (25.4%)	43 (15%)	31 (15.2%)	21 (10.9%)	17 (13.0%)	10 (8.5%)
Arcanobacterium pyogenes	9 (3.6%)	8 (2.1%)	4 (2.0%)	13 (6.7%)	3 (2.3%)	8 (6.8%)
Bacterial abortion, other ³	44 (13.4%)	24 (8.2%)	28 (14.0%)	12 (6.1%)	10 (7.6%)	8 (6.8%)
Mycotic abortion	4 (1.6%)	7 (2.4%)	7 (3.4%)	9 (4.7%)	3 (2.3%)	4 (3.4%)
Bovine viral diarrhea virus (BVDV)	8 (3.2%)	5 (1.7%)	6 (2.9%)	8 (4.1%)	3 (2.3%)	4 (3.4%)
Bovine herpesvirus 1 (IBRV)	9 (3.6%)	7 (2.4%)	9 (4.4%)	6 (3.1%)	2 (1.5%)	6 (5.1%)
Bacillus licheniformis	5 (2%)	4 (1.4%)	4 (2.0%)	6 (3.1%)	1 (0.8%)	1 (0.8%)
Listeria monocytogenes	2 (0.8%)	6 (2.1%)	1 (0.5%)	3 (1.6%)	4 (3.1%)	1 (0.8%)
Ureaplasma spp.	12 (4.8%)	10 (3.5%)	14 (6.9%)	2 (1.0%)	0	3 (2.5%)
Leptospira spp.	1 (0.4%)	1 (0.3%)	1 (0.5%)	2 (1.0%)	1 (0.8%)	1 (0.8%)
Coxiella burnetii	0	0	2 (1.0%)	0	0	0
Mycotic - Candida sp./yeast ⁴	5 (2.0%)	0	0	0	1 (0.8%)	1 (0.8%)
No significant lesions, etiology not identified	104 (41.9%)	131 (45.6%)	70 (34.3%)	74 (38.3%)	66 (50.3%)	59 (50%)
Total abortions submitted	248	292	203	196	131	118

¹Pathology cases. Numbers represent diagnoses; more than one diagnosis may be present for a single abortion.

² Previously included with idiopathic abortions. Etiology possibly not identified as not all submissions requested bacterial, *Mycoplasma spp.*, or virus culture.

³ Includes Escherichia coli, Salmonella spp., Staphylococcus spp., Histophilus somni, Streptococcus spp., Pasteurella spp., Acinetobacter spp., Providencia spp., Actinobacillus spp., and those with lesions compatible with bacterial abortion.

⁴ Previously included with mycotic abortion.

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POULTRY - FUR-BEARING SPP.

A preliminary study of inclusion body hepatitis (IBH) cases in Ontario

Brian Binnington, Davor Ojkic, Babak Sanei

A 2-year epidemiological study of Ontario IBH cases, funded by the Poultry Industry Council, will commence in the fall of 2006 to clarify some of the poorly understood aspects of the disease. Complete details will be sent to participating hatcheries and poultry practitioners. Poultry producers may contact their poultry practitioner, their hatchery, or Dr. Babak Sanei for more information.

In the **screening phase** of the project, breeder flocks will be tested for FAdV antibodies and serotypes prior

to the onset of production.

In the **outbreak investigation phase**, IBH field cases will be identified in broiler flocks through veterinary practitioners and participating hatcheries; epidemiological data will be captured, and samples submitted to the AHL for histological examination and virus isolation attempts. Field isolates of virus will be genotyped, and serological testing will help to establish the movement of FAdV serotypes from breeder flock to progeny. *AHL*

Dermatophytosis ("ringworm") in juvenile mink

Brian Binnington, Katie Welch, Bruce Hunter, Durda Slavic

This summer, the AHL has received submissions of young mink with skin and fur problems from 3 different ranches. Bruce Hunter has talked to other ranchers who have also experienced, compared to previous years, an increased number of litters with skin disease that was thought to be ringworm. Although mortalities have been low and the fur does grow back, there has been a significant decrease of pelt quality in affected mink.

The skin lesions have affected litters of juvenile mink without apparently causing problems in adult mink. The necropsy and laboratory findings were similar for the 3 submissions. There were multiple, variably sized areas of hair loss (alopecia), thinning and scaling of fur over the face, body and legs. The skin in more severely affected areas was thickened, rugose and covered by crusts with matted hairs. Microscopic examination of skin sections demonstrated epidermal thickening and hyperkeratosis with multiple serocellular crusts. Localized epidermal vesiculation and ulceration was present in some sections. The ostia of hair follicles contained keratin and cell debris. Most follicles had numerous fungal arthrospores in the hair sheath and within the hair shaft. Accumulations of neutrophils and mononuclear cells were present around hair follicles and in the dermis, especially around blood vessels. A few follicles had ruptured and there was a granulomatous inflammatory reaction with multinucleated giant cells in the adjacent dermis. The histologic diagnosis was hyperplastic dermatitis with folliculitis and furunculosis due to dermatophytes.

Samples of skin and hair were submitted for bacteriological and mycological evaluation. Cultures of skin and hairs on Mycosel agar yielded growth of fungi that morphologically were compatible with *Trichophyton mentagrophytes*. Bacterial cultures of skin and subcutaneous swabs grew a few to moderate numbers of *Staphylococcus sp.* in one case and no bacterial pathogens in the other 2 cases. The staphylococci were considered to be secondary opportunists.

The affected ranches were not in direct contact with each other and the reason for the increased incidence of ringworm this year was not determined. *Trichophyton mentagrophytes* infections have been associated with exposure to rodents or their immediate environment. The infectious spores of dermatophytes can be spread amongst mink by cage materials, equipment, or the hands and clothing of workers.

Trichophyton mentagrophytes can infect people, so caution, good hygiene and the use of gloves are necessary when working with infected animals. *AHL*



Figure 1. Multiple areas of alopecia and skin thickening on the ventral surface of the body of a juvenile mink with dermatophytosis (ringworm).

SWINE

PRRSV – Laboratory diagnosis in partnership with the AHL

Susy Carman, Jim Fairles

Diagnosis of PRRSV infection in a swine herd can benefit from the use of a range of testing methodologies. As your partner in the swine industry, the AHL strives to offer accurate reliable virus and antibody detection tests to aid in the diagnosis of PRRSV infection in diverse situations. Our gel-based RT-PCR test for ORF7 PRRSV identifies both North American and European strains of PRRSV in the same assay. This is very important with the increasing frequency of European strains in North America. We offer standard next business day testing of serum, blood swabs and herd-based test to use for serological monitoring of swine semen for \$22 per test, same day testing for rush samples that arrive by 8:00 AM for \$28 per test (please call in advance), and testing for tissues at \$24 per test. We also offer stat testing outside normal business hours (please make arrangements in advance). For monitoring of boar studs, we encourage the submission of serum or blood swabs, rather than semen, to establish an earlier result. The AHL has 2 flows for PRRSV-PCR testing, with one flow restricted for PRRSV-negative herds and boar studs. To ensure that we triage your samples into the appropriate stream, we ask you to advise us on the submission form that this is a "PRRSVnegative herd". To monitor our PCR methodology, we are regular participants in PRRSV proficiency panel testing.

Restriction fragment polymorphism (**RFLP**) typing and sequence analysis of ORF5 are both available to aid in the tracking of PRRSV strains in swine herds. To enhance our diagnostic services, we have evaluated many real-time PCR tests for PRRSV. The majority have been determined

to be unacceptable for our use. As per our ISO validation standard, we are continuing with the analytical and field validation of a real-time PCR test that will identify both North American and European strains of PRRSV. The AHL also offers postmortem, histopathology, antigen detection immunofluorescent tests for PRRSV on frozen tissue, and immunohistochemical (IHC) tests for PRRSV on formalin fixed tissue.

We consider the **IDEXX ELISA** to be the best herds for PRRSV antibody. Since no test is 100% specific, we also offer indirect immunofluorescence (IFA) tests for IgM and IgG for North American strains of PRRSV to evaluate IDEXX PRRSV singleton positive reactors in PRRSVnegative swine herds. On request, we can provide similar IFA testing for the European strains of PRRSV.

Since laboratory test results do not stand on their own and no test is 100% specific, appropriate sample selection, proper shipping and handling, selection of appropriate tests, testing by validated methods, and valid interpretation of results are all important in reaching a diagnosis. The herd history and clinical signs must always be considered when interpreting laboratory test results. If you have questions about any of our test results, please contact us as soon as possible so we can work with you to determine other avenues of investigation using our many established tests for PRRSV diagnosis. AHL

SMALL RUMINANTS

Anthelmintic resistance on the rise in sheep parasites?

Paula Menzies, Andrew Peregrine

Losses due to gastrointestinal parasitism in sheep appear to be on the rise in Ontario. While this may be due to the hot and wet summer - perfect conditions for L3 larvae on pasture – there are also reports that these losses come within a couple of weeks of having dewormed the flock. This raises the issue of whether we are seeing anthelmintic resistance or if producers are not properly administering medication. Under-dosing can occur because of underestimating the animal's weight, improper calibration of the drench gun, or poor administration technique. Clinical signs of GI parasitism include diarrhea, ill thrift, bottle jaw and pale mucous membranes. In severe hemonchosis, the presentation is often sudden, with death due to profound anemia.

If you suspect anthelmintic resistance, perform a fecal egg count reduction test. This involves selecting

infected lambs (ideally not treated for at least 8 weeks previously), weighing them for accurate dosing, and randomly assigning them to a treatment or control group (15 animals per group). Fecal samples are taken individually from 10 animals from each group at day 0 (time of treatment) and 10-14 days later. After treatment, lambs are returned to pasture. As quantitative counts are required (eggs per gram), the refrigerated fecal samples can be sent to the AHL for analysis please call the lab for details 519-824-4120, ext 54522. Results can then be analyzed statistically. If the anthelmintic fails to reduce egg counts by at least 95%, then anthelmintic resistance is likely. AHL

Reference

Eamens GJ, ed. Laboratory Specimen Submission Manual, 8th ed. Veterinary Laboratory Service, NSW Agriculture. 1995:50-52.

HORSES

Summer brings three 'herd outbreaks' of toxicities (continued from p. 19)

3. Horses - Japanese yew toxicity

Two Quarter Horses were unexpectedly found dead on pasture. No signs of thrashing or convulsions were evident in the area surrounding the dead animals. A third horse dropped dead while being walked from the pasture. No ab-

normalities were apparent to the owners when the animals were turned out to pasture the previous evening.

The clinical pathology findings on the third animal were within normal reference intervals for mature horses. The necropsy findings on the 2 horses submitted for postmortem examination

were marked intestinal bloating with no displacement or entrapment and a few dried brown lancet-shaped leaves consistent with and identified as Japanese yew (*Taxus cuspidata*) in the stomach content of 1 horse.

The practitioner confirmed the presence of dried yew trimmings in the pasture, present on pasture for a few days after a 6-week period of drying in the front yard.

Yew (*Taxus spp.*) is highly toxic to all livestock species, as well as humans and dogs, although monogastric animals such as horses are considered more susceptible. *T*.

cuspidata (Japanese yew) and *T. baccata* (English yew) are common introduced shelter, shade and ornamental plants in Ontario. All parts of the plants are considered toxic and contain **taxine alkaloids**, a complex mixture of cardiotoxins. As little as 100 g of yew leaves is potentially lethal to a 500 kg

> horse. In horses, death due to yew poisoning often occurs with a few premonitory clinical signs and can occur <15 min post ingestion. Cases have been recorded where horses have suddenly and unexpectedly collapsed "as if they had been shot".

> > *Taxus spp.* should not be

planted on or near livestock properties. Trimmings from these plants should be promptly burned or buried in inaccessible locations. Yew plants or their trimmings should not be located near hay or other feed storage facilities because of feed contamination risk. *AHL*

Acknowledgment - Our thanks to Carole Ann Lacroix (OAC Herbarium) and Jack Alex for their role in plant identification. Reference

Hazlett M, et al. *Taxus* spp. toxicosis in a herd of cattle. AHL Newsletter 2005;9:28.

CFIA update on EIA-ELISA testing

Further to our note in the June 2006 AHL Newsletter that we had switched to ELISA for EIAV antibody testing, below are excerpts from a July 11/06 CFIA note to equine practitioners and horse owners (boldfacing added for emphasis):

"As of March 31, 2006, EIA approved laboratories are no longer testing equines using agar-gel immunodiffusion (AGID) test, also known as the Coggins test. **The only test in use in EIA approved laboratories is the ELISA test**. Under the current testing protocol, when an EIA approved laboratory reports a negative EIA-ELISA test result, the horse is deemed negative and no further testing is required. However, when an approved laboratory obtains a **positive or atypical EIA-ELISA result**, the approved laboratory reports this finding as *ELISA inconclusive - to be confirmed by RCE* and the serum sample is forwarded to the CFIA's RCE located in St -Hyacinthe, Quebec, where it is retested first by EIA-ELISA and, if positive, the results are confirmed by EIA-AGID tests. If the confirmatory testing is AGID positive or atypical, the horse's EIA status is reported accordingly and the CFIA applies control measures as per the current EIA policy. In situations in which test results are reported as ELISA negative or ELISA positive /AGID negative, the animal is considered EIA negative and no further testing or field action is required.

"Both AGID and ELISA are simple, accurate, reliable tests for the demonstration of EIA virus infection. However, although the ELISA detects antibodies somewhat earlier and at lower concentrations than the AGID test, **some falsepositive results have been noted.** Therefore, according to the *World Organisation for Animal Health (OIE)*, a positive test result by ELISA requires confirmatory retesting using AGID. Horse owners and private veterinary practitioners should be aware of this requirement and allow the time required for EIA testing, so that in the event of a positive ELISA result, there is a sufficient margin allowed for confirmatory testing by AGID.

"For more information on the disease and/or the CFIA's EIA control program, please refer to the following Web site: <u>http://www.inspection.gc.ca/english/anima/heasan/disemala/equianem/equianeme.shtm</u> or contact the CFIA's local offices.

"Any concerns or questions regarding this change of policy should be directed to Dr. Les Kumor at: <u>lkumor@inspection.gc.ca</u> or Dr. Carole Simard at: <u>Simardc@inspection.gc.ca</u> " *AHL*

Yew (*Taxus spp.*) is highly toxic to all livestock species, as well as humans and dogs

COMPANION ANIMALS

Canine leptospirosis update

Beverly McEwen, Davor Ojkic, John Prescott

Recent publications have documented a resurgence of canine leptospirosis in Canada and the USA. Increased serological submissions for *Leptospira spp*. (Table 1) reflect the concerns of our clients about this disease.

Table 1. AHL canine serological diagnoses, by year, for all *Leptospira* serovars

Year	Number of submissions	Positive n (%)	Suspicious n (%)	Negative n (%)
1998	42	11 (26)	6 (14)	25 (59)
1999	54	8 (14)	10 (18)	36 (66)
2000	153	63 (41)	20 (13)	70 (45)
2001	213	37 (17)	75 (35)	101 (47)
2002	209	49 (23)	91 (43)	69 (33)
2003	424	143 (34)	158 (37)	123 (29)
2004	795	267 (34)	282 (35)	246 (31)
2005*	1136	251 (22)	404 (36)	481 (42)

*Data now include all canine serological testing for *Leptospira spp*. submitted to AHL including Ontario and other provinces.

There were fewer seropositive dogs in 2005 than in 2004, despite increased submissions from a broader geographic area, resulting in an apparent decreased frequency of leptospirosis. For the past few years, leptospirosis has occurred throughout the year, although most cases occur in the fall. **Seropositive dogs were from all regions in Ontario**.

The frequency of seropositivity to *L. autumnalis* and *L. bratislava* have been consistently greater than other serovars since 2000 (Table 2). Seropositivity to more than 1 serovar occurred in 143 of 251 (57%) positive cases, compared to 67% in 2004. Five dogs were positive to all se-

rovars except for L. hardjo. A consistent pattern of seropositivity to various serovars was not evident. The broad seropositivity observed in these sera is probably a reflection of the involvement of several different serovars in canine leptospirosis as well as the broad cross-reactivity of IgM antibodies. It seems most likely that it is a particularly crossreactive serovar, with a tendency to be linked to pomona seropositivity. IgM is the dominant immunoglobulin in the early humoral immune response. It has still not been resolved by isolation studies whether the high frequency of autumnalis seropositives represents genuine infection with this serovar or is the result of cross-reacting antibodies. Interpretation of titers is also affected by immunization; dogs immunized with serovars canicola and icterohaemorrhagiae may show titers up to 320 or 640 in the first months after immunization (when of course they would be protected against these serovars). Similar relatively low serological responses are likely also to occur in dogs immunized with the newer vaccines containing serovars L. grippotyphosa and L. pomona.

Although not as dramatic as the surge of canine leptospirosis in 2000, **2005 data show that canine leptospirosis caused by several serovars has become established throughout Ontario in the last few years and, perhaps because of increased awareness, has become a more common diagnosis.** *AHL*

References

Prescott J, et al. Resurgence of leptospirosis in dogs in Ontario: recent findings. Can Vet J 2002;43:955-961.

Ward MP. Clustering of reported cases of leptospirosis among dogs in the United States and Canada. Prev Vet Med 2002;56:215-226.

	1998	1999	2000	2001	2002	2003	2004		2005
	%	%	%	%	%	%	%	%	range*
L. autumnalis	4.8	3.7	30.7	11.7	20.1	28.3	25.7	14.4	320->20,480
L. bratislava	16.7	7.4	24.8	10.8	12.0	17.2	19.1	12.9	320-20,480
L. grippotyphosa	14.3	1.9	15.0	9.4	6.7	13.9	13.1	9.4	320->20,480
L. pomona	16.7	3.7	26.1	6.1	4.8	14.2	8.7	6.1	320-20,480
L. icterohaemorrhagiae	0	5.6	13.7	3.8	5.3	10.1	7.4	3.5	320-5,120
L. canicola	0	1.9	0	1.4	1.9	13.0	6.9	3.8	320-2,560
L. hardjo	-**	-	-	-	-	0.7	0.5^{***}	0.4	320-640

Table 2. Percent seropositivity of canine sera to various Leptospira spp. serovars, 1998 - 2005.

* values are the reciprocal of the titer; ** serology not done; *** fewer cases tested for L. hardjo (n=559)

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