UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

Volume 9, Number 2, page 9 June, 2005 ISSN 1481-7179

In this issue:

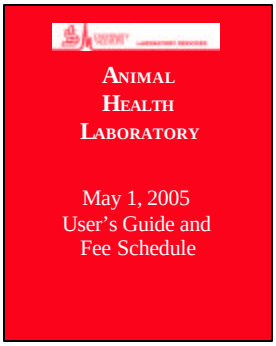
May 1, 2005, AHL User's Guide and Fee Schedule

We hope that this comprehensive source of information will save you time and help you make the most of our services. The Guide/Schedule is also available on the Web at **http://www.uoguelph.ca/ahl/FeeSchedule/AHLFees.html**

We have adjusted some of our fees to reflect increasing costs of materials. We have added some new tests to the test menu and have dropped some tests that have been used infrequently or that have become outmoded. This is part of our ongoing effort to make the best use of testing resources to bring you services that are current and meet today's needs.

Significant changes in the May 1, 2005, Fee Schedule include:

- Update of our professional staff contact list to include:
  - Dr. Jim Fairles, Client Services Veterinarian
  - Dr. Durda Slavic, Bacteriologist
- Added:
  - information about ownership of specimens and isolates
  - new AHL e-mail contact: **info@ahl.uoguelph.ca**
  - note about discouraging return of carcasses
  - reference intervals for cavity fluids
  - testosterone and progesterone interpretation information
  - index of tests by species, as well as tests by discipline
  - new molecular-based (PCR) tests
  - canine and feline 'heartworm profiles' to include CBC and biochemistry profile



For clients in specialty practice, we would be happy to provide the Fee Schedule for you sorted by test name, method, etc., within your species of primary interest. Please contact Helen Oliver at (519) 824-4120, ext 54538.

We will continue to strive to provide the most cost-effective testing strategies to help you deal with animal health challenges, and will keep you posted on changes in test availability and fees through this quarterly AHL Newsletter - also available on the Web at **http://www.uoguelph.ca/ahl/NewsletterLink/ahlnewstable2.html .** *AHL*

Tony van Dreumel retires

After 38 years of service to the University of Guelph and the Ontario Ministry of Agriculture and Food, Dr. Tony van Dreumel retired April 30, 2005. A 1963 graduate of the Ontario Veterinary College, Tony worked in the provincial diagnostic lab in Winnipeg for 3 years, earned an MSc at the University of Manitoba in 1967, and became board certified in anatomic pathology by the American College of Veterinary Pathologists in 1969. From 1970 to 1997, he was Scientific Coordinator of the Veterinary Laboratory Services Branch in OMAFRA and a part-time mammalian pathologist. He served as the President of the World Association of Veterinary Laboratory Diagnosticians, and as a site visit auditor for the American AVLD.

Since 1997, he has served as a mammalian pathologist in the AHL-Guelph lab, and until recently supervised the Mycoplasmology lab as well. Tony has had a long and distinguished career in veterinary pathology, has numerous publications to his credit, and has been an invited speaker at national and international meetings.

Your colleagues wish you a long and happy retirement Tony. *AHL*

Biosafety and sample submission

Jim Fairles, Linda McCaig

The packaging of sample submissions sent to the Animal Health Laboratory is, for the most part, excellent. We do occasionally receive samples that have been broken, damaged or leaking. It is imperative that we realize the biosafety aspects of such submissions. With the wide array of zoonotic agents (e.g., *Salmonella, Coxiella, Chlamydophila*) that practitioners as well as the laboratory must deal with, protection from exposure to these agents is very important. By the time the sample gets to the testing areas in our laboratory, many people have handled it, including your staff, the transportation company, and our staff. Until an agent is identified or a diagnosis is made, we do not know the agent involved, so all samples must be treated with respect.

Occasionally samples arrive that are contaminated with fecal material, have fecal material in the packing material, leaking jars, leaking plastic bags, etc. Our standard operating procedures indicate that we must continue the unpacking of these submissions in a biosafety cabinet using protective gear. Besides exposing staff to the agents, this delays the sample arriving at the lab area for testing. As well, it raises concerns about contamination of other samples in the package, and the possibility of false-positive results.

If you suspect a zoonotic agent, please indicate this on the submission form and place the form in a plastic bag where it is easily accessible on opening the outer packaging. The staff in Specimen Reception can then be alerted of any potential concerns. For example, any submissions from ovine abortions are automatically opened in a biosafety cabinet due to the potential presence of *Coxiella* or *Chlamydophila*.

Thanks in advance for your continued attention to packaging of submissions. To serve you better, we will be contacting you if samples are received that raise biosafety concerns. *AHL*

Bacterial counts of colostrum and bedding

Jim Fairles, Durda Slavic

Several articles and presentations lately have discussed the correlation of the contamination of the environment and the potential for disease. For example, calf scours and colostral bacterial load, and stall bedding and mastitis. These correlations are difficult to make and will require much further research.

Intuitively, a clean environment would logically seem to reduce the spread of disease. Protocols have been developed for sample selection and quantifying bacterial load. **The AHL is now offering colostrum and bedding bacterial counts**. Please contact us for the protocols, sample submission requirements, and price. *AHL*

AHL Newsletter
June 2005 - Volume 9, Number 2
Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistant: **Ms. Helen Oliver**

The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

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

Mailing address & contact information:
(**please return all undeliverable Canadian addresses to**:)
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
Email: *holiver@lsd.uoguelph.ca*

ISSN 1481-7179
Canada Post Publications number - 40064673

Contributors to this issue:
From the Animal Health Laboratory:
Patricia Bell-Rogers, BSc, MSc
Brian Binnington, DVM, Dip Path, Diplomate ACVP
Hugh Cai, DVM, MSc
Susy Carman, DVM, Dip SA Med, PhD
Josepha DeLay, DVM, DVSc, Diplomate ACVP
Jim Fairles, DVM, MBA
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Brent Hoff, DVM, DVSc
Gaylan Josephson, DVM, Dip Path
Emily Martin, DVM, MSc, Diplomate ACPV
Linda McCaig, MLT
Beverly McEwen, DVM, MSc, PhD, Diplomate ACVP
Davor Ojkic, DVM, MSc, PhD
Lois Parker, BSc
Janet Shapiro, DVM, Dip Path, Dip Eq Surg
Durda Slavic, DVM, MSc, PhD
Other contributors:
Nancy Carr, MD, Elginburg, ON
Hilary Burgess DVM, John Prescott VetMB PhD, Kris Ruotsalo DVM DVSc Diplomate ACVP, Pathobiology, OVC
Beth Hanselman DVM, VTH, OVC
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.

RUMINANTS

Bovine viral diarrhea virus (BVDV) in alpaca – clinical illness, early pregnancy loss, abortion and persistent infection

Susy Carman, Nancy Carr, Josepha DeLay, Murray Hazlett

A chronically ill 3-month-old, low-birth-weight alpaca cria, with intermittent pneumonia and nasal discharge, and his dam were added to a herd of 52 alpaca in eastern Ontario. The cria died 5 months later. Necropsy examination was not performed. The dam had been on 3 different farms during her pregnancy, two in Alberta and a different farm in Ontario, where the cria was born. In the same year, both Alberta farms experienced reproductive losses, including abortion and stillbirth, and all 3 farms found animals with antibody to BVDV. None of the 4 farms had known contact with sheep or cattle.

Three months after arrival of the cria at the farm in eastern Ontario, 9 alpacas experienced clinical illness over a 6-wk period, with sore mouth, anorexia and lethargy. None had diarrhea. Subsequently, one animal died of hepatic lipidosis following a period of anorexia, 2 alpaca had early pregnancy loss, and 2 aborted. Seventeen of 19 alpaca with various histories of clinical illness, early pregnancy loss, abortion, delivery of live born cria, or clinically unaffected were found to have antibody to BVDV type 1 and 2, with titers highest to BVDV type 1.

One alpaca fetus was aborted at 5.5 mo gestation, 3 mo after the time of clinical illness. Immunohistochemistry (IHC) for BVDV was positive for fetal tissues. Noncytopathic (ncp) BVDV was isolated from the skin of this fetus, but not from other fetal tissues. The other aborted fetus was not tested. Twelve cria were later born uninfected. A single BVDV-infected male cria was born 9 mo after the clinical illness in the herd. His dam had been at 2 mo gestation at the time of clinical illness in the herd, and she was later found to be BVDV antibody positive. The cria had a low birth weight for the Ontario farm. Although he had chronic diarrhea and episodic lacrimation, he continued to gain weight well until euthanasia. His fleece looked slightly less dense than that of other cria of the same age (Figure 1), with longer straight hair-like fibers from primary follicles extending past wool-like fibers from secondary follicles. Ncp BVDV was isolated from the placenta at birth and at 3 and 26 d of age from buffy coat cells. Nested reverse transcription PCR (nRT-PCR) was positive for BVDV type 1 for EDTA blood and plasma for both 3 and 26 d. The cria continued to be positive for BVDV at euthanasia at 46 d by virus isolation on plasma, serum and multiple tissues, nRT-PCR on plasma and serum, antigen detection ELISA on serum and ear notch, and IHC on multiple tissues, including the cerebral cortex, retina, and pinna. The cria remained BVDV antibody negative to BVDV type 1 and type 2. All ncp BVDV isolates from the aborted fetus and the PI cria were typed as BVDV type 1 using nRT-PCR. **A diagnosis of persistent infection (PI) with BVDV was made.** This is the first confirmed report of a BVDV PI alpaca cria. However, it can be speculated that the chronically ill cria that came to the farm prior to the outbreak of clinical illness was an unrecognized PI cria.

Figure 1. The persistently infected alpaca cria at 4 wk of age, with longer straight hair-like fibers from primary hair follicles extending above the wool-like fibers from secondary hair follicles.

This report confirms that diagnostic tests designed for the identification of BVDV infection in cattle can be used effectively to identify BVDV in alpaca. Abortions due to BVDV have been previously reported in llama and in alpaca. Belknap et al. recommend that BVDV infection should be considered in llamas with abortion, diarrhea, unthriftiness and weight loss. This recommendation should now be extended to alpaca. **Persistently infected alpaca cria are an important source of BVDV infection for the alpaca industry**, where dams and their cria are commonly sent to different farms for breeding. These PI cria have great potential to infect naïve alpaca herds. *AHL*

References

1. Belknap EB, et al. Bovine viral diarrhea in New World camelids. J Vet Diagn Invest 2000;12:568-570.
2. Goyal SM, et al. Isolation of bovine viral diarrhea virus from an alpaca. J Vet Diagn Invest 2002;14: 523-525.

Milk *Mycoplasma bovis* culture and real-time PCR

Hugh Cai, Lois Parker, Patricia Bell-Rogers

The AHL now offers a real-time PCR assay for the detection of *Mycoplasma bovis* in bovine milk and lung tissue samples. We validated the assay by testing 165 individual mastitic milk samples and 53 pneumonic lung samples in parallel with isolation in culture. The sensitivity and specificity were 100% and 99.3% for milk, and 96.6% and 100% for lung tissue samples. It has detection limits similar to culture isolation, but results are available in 1-3 days instead of 5-10 days. **The fee for the real-time PCR is $24/sample.**

The fee for individual milk ***Mycoplasma* culture isolation is $10/sample**, as of May 2005. To achieve the best culture isolation results, please submit fresh milk samples - freezing and thawing can reduce the viable numbers of *Mycoplasma* in milk.

For further information please contact Hugh Cai (ext. 54316) or the Mycoplasmology lab (ext. 54541). *AHL*

Reference

Biddle MK, et al. Effects of storage time and thawing methods on the recovery of Mycoplasma species in milk samples from cows

AHL scrapie resistance PrP genotyping

Hugh Cai

As of May 2005, the fee for real-time PCR genotyping will be **$28/sample for three codons** (136, 154 and 171). It is worthwhile to have all three codons analyzed. A survey on 14,000 British sheep (1) showed that all three codons, especially 136 and 171 are related to the susceptibility to scrapie (Table 1).

Please collect and submit a 3-5 mL EDTA blood sample from each sheep to be tested. For further information please contact Dr. Hugh Cai at ext. 54316 or the Molecular Biology/Mycoplasmology lab at ext. 54541. *AHL*

Table 1. Genotype resistance to scrapie

Genotype (136, 154, 171 /136, 154, 171)	Cases of scrapie per year per million sheep
ARR/ARR	0.0
ARR/ARH	0.0
AHQ/ARH	0.0
ARR/AHQ	0.3
ARR/ARQ	0.4
ARH/ARH	2.0
AGQ/AHQ	5.0
ARQ/ARH	5.2
ARR/VRQ	6.3
ARQ/AHQ	8.7
ARQ/ARQ	36.9
ARQ/VRQ	225.4
ARH/VRQ	405.0
VRQ/VRQ	554.5

Reference

1. Baylis M. Risk of scrapie in British sheep of different prion protein genotype. J Gen Virol 2004;85(Pt 9):2735-2740.

Haptoglobin in routine analysis of bovine serum

Brent Hoff

Haptoglobin (Hp) has proven to be a very valuable aid in the diagnosis of many bovine diseases. **Hp serum levels are increased postpartum, especially in cows that develop retained placenta, mastitis, or displaced abomasum** (DA). Increased haptoglobin is part of the acute phase reaction to inflammation, resulting from the release of cytokines from leukocytes at the site of injury, especially in diseases with acute inflammation, such as pneumonia, mastitis, enteritis, and endometritis. With very high levels of Hp, it is likely that these specific organs are involved. Hp levels correlate well with total serum protein levels and negatively with albumin and AST activity.

Fuerll et al. found the highest Hp concentrations in cows and calves with pneumonia (range 1.6-4.8 g/L), followed by cows with DA (range 1.0-3.5 g/L), as well as cows with mastitis and endometritis (range 0.5-2.8 g/L) (reference interval 0.0-0.4 g/L). Cattle ill with DA plus an additional problem had the following Hp concentrations (g/L median): enteritis 1.10, bronchitis 0.95, nephritis 0.90, and laminitis 0.22. Interestingly, the leukocyte count did not correlate with the Hp concentration. These workers found that Hp was useful acutely, but had limited prognostic value, as the animals with infection recovered with treatment.

In routine testing of cattle, **elevated serum Hp indicates serious inflammation** (pneumonia, mastitis, enteritis). Note that Hp increases during acute parturient stress, and should not be used as an indicator of inflammation in the first week after calving. Remember that Hp is affected by hemolysis, and samples must be handled carefully. *AHL*

Reference

Fuerll M, et al. 5th International Colloquium on Animal Acute Phase Proteins. Dublin, 2005.

AVIAN

Pet bird pathology submissions - tips for veterinarians

Emily Martin, Brian Binnington, Jan Shapiro

The Animal Health Laboratory has necropsy and histopathology services available for pet birds. These procedures are valuable in supporting the veterinarian's clinical evaluation. Samples submitted can include tissue biopsies, and tissues or the whole carcass of a deceased bird. In cases where a necropsy is required, you can submit the entire carcass for a full post-mortem examination, or you can perform the necropsy yourself and submit tissue samples to the AHL.

Biopsies

If you are investigating a feather abnormality, it is important to submit a sample of skin that includes a pulp feather. Sending a feather alone does not provide enough tissue to evaluate histologically. If you have a suspect case of proventricular dilation disease (PDD) you can submit a crop biopsy. Please be aware that only about 50% of crop biopsies are considered diagnostic for PDD.

Samples submitted can include tissue biopsies, and tissues or the whole carcass of a deceased bird

Post-mortem examinations

If you decide to submit a bird for post-mortem examination, we recommend that, as soon as the bird dies, you dampen it with cold water and place it in a refrigerator to promptly chill the carcass. This will offset the normal high body temperature in these species that contributes to early post mortem change. If you cannot submit the carcass shortly after death, or if there is a prolonged wait for the owner to decide if they want further testing done, then the carcass can be frozen. However, please note that this is not the ideal situation as this will cause freeze/thaw artifacts in the tissues and make histologic evaluation more difficult.

If the bird is very small (e.g., finch), you can retract the keel, observe for gross lesions, take samples of a few fresh tissues to hold frozen at your clinic (see list below), open the skull to expose the brain, and then place the entire bird in the formalin jar. (If necessary, the tail feathers and wings can be removed.) This allows us access to all of the tissue we may need for histologic evaluation. When shipping whole carcasses to the AHL, please ship them on frozen ice packs soon after death. Ontario practitioners can ship to the AHL at no charge by Purolator (use Purolator account no. 0966901).

If you decide to do the necropsy yourself, please submit a wide selection of tissues for histologic evaluation. As histologic results may indicate the need for additional testing (e.g., bacteriology, virology, toxicology, *Chlamydophila* assays) we suggest that you also save tissues frozen at your clinic (freeze in a chest freezer).

Depending on what you see grossly, here are a few suggestions for **tissues to submit for histology**:

- from all birds (minimum) - brain, heart, trachea, lung, liver, spleen, kidney, crop, proventriculus, ventriculus, pancreas, and intestines, plus any other affected tissues detected at necropsy.

In addition to the minimum:

- from juvenile birds - bursa, or cloaca with bursa attached.
- from birds that are suspicious for PDD - crop, proventriculus/gizzard (whole, combined), 3-4 pieces of small intestine, adrenal glands (cranial portion of the kidney), and nervous system tissues as listed below.
- from neurologic cases - brain (in situ), spinal cord (in situ), and peripheral nerves (brachial plexus, sciatic).

Tissues to hold frozen at your clinic can include liver, kidney, intestines and lung.

The cost of a case is based on the number of birds submitted (if a full necropsy case) and/or the number of slides cut. You can submit more than one histology jar to ensure the proper ratio of 10 parts formalin to 1 part tissue.

Fees are as follows:

- post mortem examination - $60 per pet bird;
- histology - $45 on a necropsy case for 1-5 slides, then $5/additional slide;
- histology - $45 on a mail-in case for 1-3 slides, then $5/additional slide.

If you have any questions regarding sample submission, please call the laboratory and ask to speak to one of our avian pathologists. *AHL*

Need submission forms?

Revised AHL submission forms were distributed in January. The new forms are available on the Web in pdf format at http://www.uoguelph.ca/ahl/Forms/FormsIndex.htm , and may be printed. Alternatively, fax your request to us at (519) 821-8072, and we will customize the forms that you require with your clinic default information.

SWINE

PRRSV outbreak continues in southwestern Ontario

Susy Carman, Beverly McEwen, Gaylan Josephson, Jim Fairles

In the Dec/04 AHL Newsletter, we documented the fall 2004 PRRSV outbreak in swine herds in southwestern Ontario. Figure 1 is an updated epidemic curve, showing the outbreak continuing into the winter of 2005. Numbers of cases submitted to the AHL for PRRSV diagnosis using PCR increased from 201 in the Oct-Dec 2004 interval to 323 for the Jan-March 2005 interval, with the total number of cases found to be positive increasing from 108 to 172. Even though the overall number of positive cases increased, there was a very small decrease in the 3-month average percent PRRSV-positive cases from 55% to 53%. These data do not include monitoring cases, in which semen was the sample submitted. Gene sequence analysis of the ORF5 envelope gene for PRRSV for 100 strains identified from 2003 to 2005 **shows the outbreak not to be from a single source (except for integrated loops), with viruses distributed over 19 phylogenetic branches, including 2 vaccine-like groups.** *AHL*

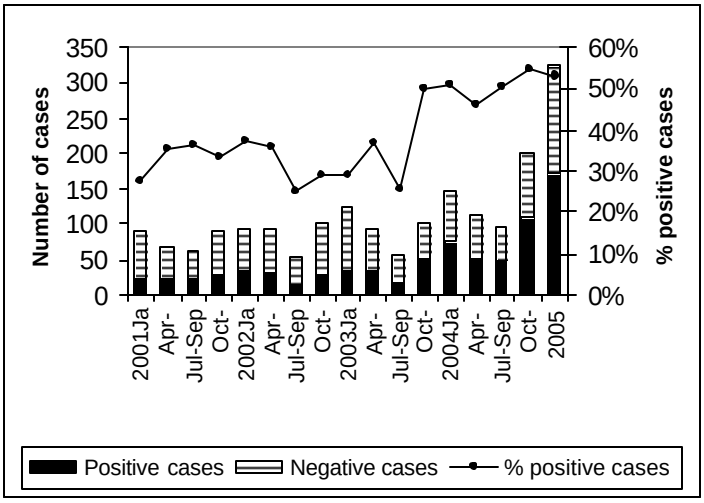


Figure 1. PRRSV PCR submissions tested by the AHL, Jan 1, 2001 - Mar 31, 2005, with semen monitoring removed.

HORSES

Equine viral neurologic disease surveillance 2004

Josepha DeLay, Beverly McEwen

A surveillance project to identify Ontario horses infected with eastern equine encephalitis virus (EEEV) or West Nile virus (WNV) was carried out from Aug-Dec 2004, funded jointly by the Ontario Ministry of Agriculture and Food (OMAF) and the Ontario Ministry of Health and Long-Term Care, with the cooperation of CFIA. The 87 equine neurologic cases submitted to the AHL consisted of 64 live horses for which serology was performed for WNV and EEEV, and 23 dead horses from which either carcasses or tissues were submitted for necropsy, histopathology, and EEEV and WNV PCR testing on brain. Immunohistochemistry (IHC) for viral antigen was performed on selected cases if fresh or frozen tissue was not available for PCR testing.

Our interim report in the Dec 2004 AHL Newsletter described **10 Ontario horses acutely infected with WNV** between Aug-Oct, as identified by positive WNV IgM ELISA and compatible clinical signs. The majority of these horses originated from the North Bay / Sturgeon Falls / Bracebridge area, with other cases scattered throughout southwestern Ontario, and none of the horses had a known history of recent travel. Of the 10 horses diagnosed in 2004, 7 were not vaccinated against WNV, 1 had an unknown vaccination history, 1 had received primary but not booster vaccination, and 1 had been vaccinated less than 2 weeks prior to the onset of clinical signs. Three of the WNV-infected horses died or were euthanized, and 6 survived the acute infection. One case was lost to follow-up. No additional WNV cases were identified in Nov-Dec 2004. In 2003, the number of WNV-infected horses in Ontario was similar, with 8 animals identified. **The relatively low numbers of cases in 2003 and 2004 are in sharp contrast to the 70 WNV-positive clinical cases diagnosed in Ontario in 2002.**

Following predictions, the number of EEEV-infected horses in Ontario this year was much lower than the 10 confirmed and suspected cases identified in 2003. In 2004, **2 horses were diagnosed with acute EEEV infection,** and both animals were euthanized or died. One horse, originating from eastern Ontario and with no history of EEEV vaccination, was identified with acute EEEV infection based on positive EEEV IgM ELISA. In Nov 2004, an additional horse originating from southwestern Ontario was identified with acute EEEV infection based on IHC identification of EEEV antigen within suspicious histologic lesions. This horse also had histologic lesions consistent with concurrent wobbler syndrome, which complicated the clinical presentation; the vaccination history of this horse was unknown. *AHL*

COMPANION ANIMALS

Canine leptospirosis in Ontario – more cases in 2004

Beverly McEwen, Davor Ojkic, John Prescott

Increased serological submissions for *Leptospira spp*. (Table 1) reflect the concerns of our clients about canine leptospirosis.

Table 1. Percent canine serological diagnoses, Ontario dogs, by year, for all *Leptospira* serovars.

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

* titer $\leq$ 1:40, ** titer 1:80 to 1:160, *** titer $\geq$ 1:320

There were more seropositive dogs in 2004 than in previous years, which may in part be the result of submissions that doubled over 2003, since the frequency of leptospirosis has remained at 34%. However, the mild, wet fall of the last 2 years and 2000 may be associated with the relative increase of leptospirosis and the data suggest that leptospirosis continues to increase in dogs in Ontario. For the past few years, leptospirosis has occurred throughout the year, although most cases occur in the fall. The largest number of positive cases (n = 44, 16%) occurred in November 2004. Most cases (63%) in 2004 occurred between August and December, with fewer cases (21%) between April and July, and occasional cases (16%) between January to March. 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 one serovar occurred in 180 of 267 (67%) positive cases, compared to 61% in 2003. One dog was positive to all serovars, and 4 dogs were positive to all serovars except for *L. hardjo*. **A consistent pattern of seropositivity to individual serovars was not evident**, so it is difficult to implicate specific serovars from the data**.** The broad seropositivity observed in these sera is probably a reflection of the involvement of several different serovars in early canine leptospirosis as well as the broad cross-reactivity of IgM antibodies. IgM is the dominant immunoglobulin in the early humoral immune response. Nevertheless, the consensus is that resurging leptospirosis in dogs is the result in most cases of serovar *grippotyphosa*, acquired from raccoons, and to a lesser extent serovar *pomona* acquired from skunks, and serovar *bratislava* acquired from dogs (mostly), horses, pigs and possibly other sources. 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. The high frequency of both *autumnalis* and *bratislava* seropositivity may be because these serovars are not currently included in the canine vaccines and therefore may be proportionately increasing as causes of canine leptospirosis.

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.* Clients are reminded that a single serum sample, if negative, is inadequate to rule out leptospirosis since serum obtained early in the course of disease may be negative for antibodies, as is true of any acute infection.

Although not as dramatic as the surge of canine leptospirosis in 2000, **2004 data show that canine leptospirosis caused by several serovars has now become well established throughout Ontario and, perhaps in part because of increased awareness, has become a more common diagnosis**. Leptospirosis in animals is of public health concern and is an infection that can be controlled by vaccination. Certain vaccine manufacturers may be in process of including serovar *bratislava* in the canine vaccine. *AHL*

Table 2. Percent seropositivity of canine sera to various *Leptospira spp*. serovars, 1998 – 2004.

Serovar	1998	1999	2000	2001	2002	2003	2004	
	%	%	%	%	%	%	%	range*
L. autumnalis	4.8	3.7	30.7	11.7	20.1	28.3	25.7	320-20,480
L. bratislava	16.7	7.4	24.8	10.8	12.0	17.2	19.1	320-20,480
L. grippotyphosa	14.3	1.9	15.0	9.4	6.7	13.9	13.1	320-20,480
L. pomona	16.7	3.7	26.1	6.1	4.8	14.2	8.7	320-20,480
L. icterohaemorrhagiae	0	5.6	13.7	3.8	5.3	10.1	7.4	320-10,240
L. canicola	0	1.9	0	1.4	1.9	13.0	6.9	320-10,240
L. hardjo	-	-	-	-	-	0.7	0.5**	320-1,280

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

Ehrlichia canis identified in a canine neurology case

Hilary Burgess, Brent Hoff, Kris Ruotsalo, Beth Hanselman

In February 2005, a young adult, castrated male Pug was referred to the Ontario Veterinary College because of ataxia. The owners had acquired the dog just over a year prior to presentation. He had a history of intermittent ataxia with progression of clinical signs over the previous 2 wk. The patient's travel history was extensive, with the first 18 mo of his life spent in Costa Rica, followed by a trip to Arizona 1 mo prior to referral. On presentation he had polyuria, polydipsia, decreased appetite, mild enlargement of submandibular lymph nodes, and bilateral vestibular ataxia.

Hematology revealed moderate to marked non-regenerative anemia, marked thrombocytopenia, moderate increase in band neutrophils, mild lymphopenia, and mild monocytosis. On serum biochemistry, there was moderate hyperglobulinemia, moderate hypoalbuminemia, mildly increased bilirubins, a moderate increase in alkaline phosphatase, and a marked increase in alanine aminotransferase. Both pre- and post-prandial bile acid levels were elevated (pre: 14 µmol/L, reference interval 0-6; post: 92 µmol/L, RI 0-20). CSF analysis revealed markedly increased protein concentration, and moderate predominantly nonsuppurative inflammation. **An *Ehrlichia sp.* morula was noted in a single neutrophil during cytological evaluation of the CSF** (Fig. 1). Inclusions were also noted in neutrophils, monocytes and platelets on a buffy coat preparation of a second blood sample. The patient's erythroid and megakaryocytic lines were hypoplastic on bone marrow evaluation. An *Ehrlichia canis* ELISA (Snap 3Dx assay, IDEXX Laboratories) was positive. PCR was positive for *E. canis* and negative for *E. ewingii, Anaplasma phagocytophila* and *Anaplasma platys.* While occasional platelets contained inclusions resembling *A. platys*, this diagnosis was not supported by PCR analysis, and at this time the ability of *E. canis* to infect platelets has not been documented.

Ehrlichiae are gram-negative, pleomorphic cocci that infect blood cells and replicate within membrane-bound vacuoles. *Ehrlichia canis* is transmitted by *Rhipicephalus sanguineus* (the brown dog tick), requiring 24-48 hr of tick attachment before successful transmission. *E. canis* has a worldwide distribution, therefore it is difficult to identify the location of original infection in this case. Because ehrlichiosis is a multisystemic disease, the clinical signs can vary greatly. Ehrlichiae can be present during the acute, subclinical or chronic phases of disease. Mild, vague illness usually occurs within 1-3 wk of infection. At this point, the most commonly noted change in the clinical pathology is thrombocytopenia resulting from platelet consumption in the face of vascular inflammation, immune-mediated platelet destruction, and splenic sequestration of platelets. After the acute phase of infection, the infection either resolves or enters a subclinical phase lasting from weeks to years. Diagnosis of the disease is most commonly made during the chronic phase of infection. As noted in our case, **clinical signs and clinical pathology changes tend to be non-specific in the chronic phase and include thrombocytopenia, mild to moderate non-regenerative anemia, hyperglobulinemia, hypoalbuminemia, and increases in ALP and ALT.** Bone marrow aspirates revealing hypoplasia, as in our case, generally suggest chronic infection. Clinical signs of ehrlichiosis can include multiple neurologic deficits including vestibular ataxia, as well as the nonspecific signs of lethargy, anorexia and weight loss. The neurological signs in this disease are secondary to vasculitis of the meningeal vessels and/or hemorrhage into the CNS.

A definitive diagnosis can be achieved by visualization of intracellular *Ehrlichia* morulae. This occurs more frequently in acute infection than in the subclinical or chronic phases. While the agent responsible for infection can often be presumed based on the cell type infected, this case is an excellent example of when cell tropism is not absolute - morulae were noted within both mononuclear cells and neutrophils. While *E. canis* generally infects mononuclear cells, the PCR analysis ruled out the common agents that infect neutrophils, therefore the morulae within the neutrophils were likely also *E. canis*.

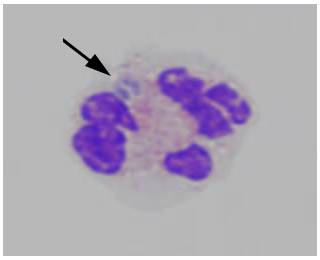


Figure 1. Single *Ehrlichia* morula in a neutrophil in a CSF sample.

Serology is an additional test that is commonly used for the diagnosis of ehrlichiosis. However, it should be noted that a positive titer only indicates exposure, not necessarily active infection. In addition, a negative titer should not rule out ehrlichiosis as clinical signs can appear prior to a detectable antibody response.

Newsletter Figures are posted in full color in the Web version of the Newsletter - http://ahl.uoguelph.ca