LABORATORY SERVICES Newsletter
ANIMAL HEALTH LABORATORY GUELPH KEMPTVILLE RIDGETOWN

Volume 3, Number 2

In this issue:

What's happening at the AHL?

- **Dr. Shelley Newman** began on May 3 in the Guelph lab as a **pathologist, avian and fur-bearing animals**. Shelley is an OVC '90 graduate, was in private practice for 3 yrs, completed a DVSc at OVC, and has just finished a 2-yr contract at the Virginia-Maryland Regional College of Vet Med. She also successfully completed the ACVP board exams last fall. She can be reached by phone at (519) 824-4120, ext. 4523, or by Email at snewman@lsd.uoguelph.ca
- Enclosed with this issue of the Newsletter is a copy of our new **generic submission form**, plus an order form for this and any of our other forms.
- Please note the **confidentiality statement** on the new generic form, and on our other revised forms. Submission of samples to the AHL from food- and fiber-producing animals implies agreement to share test results, in confidence, with the Ontario Ministry of Agriculture, OMAF for the purpose of disease surveillance..
- Please note also that **demographic information** must be supplied on food-and fiber-producing animals to receive the OMAF-supported rate. This information is critical to the success of the Ontario Animal Health Surveillance Network and in turn the Canadian Animal Health Network.
- **Drs. Bob Hampson** and **Gary Thomson** will continue to provide back-fills this summer.

- Please note that the **AHL Purolator 'incoming collect' number has been changed to 0966901.**
- Feedback for the AHL? Please feel free to call, fax, or E-mail us at any of our labs.

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SEROLOGY

Evaluation of rabies virus antibody tests in domestic animals

Dr. Susy Carman, AHL

As part of a three-year collaborative research project to compare the usefulness of three different tests that measure serum antibodies against rabies virus in domestic animals, **the AHL is now accepting sera from dogs, cats, horses and other domestic animals.**

- For this rabies virus antibody testing, please forward **2 mL of sera to the AHL, along with a history of the donor animal, including records of rabies vaccination.**
- The AHL will forward your sera to the **CFIA, Rabies Center for Expertise, Nepean** for testing. Only the results of the OIE-approved **fluorescent antibody virus neutralization test** will be reported to practitioners.
- Using the fluorescent antibody virus neutralization test, sera are compared to a WHO calibrated serum standard. Results are expressed in international units (IU), and reported as either <0.5 IU/mL or >0.5 IU/mL.
- These results will usually be available within one week, unless testing needs to be repeated. The AHL will report these results to practitioners by fax through our regular computerized reporting system, and bill veterinarians for the testing. **The charge will be $40 per test**.

Practitioners should be aware that:

- Neutralizing antibody levels <0.5 IU/mL may not reflect immunity to rabies virus.
- The majority of animals vaccinated with approved vaccines and having antibody levels >0.5 IU/mL are protected from challenge.
- Some animals diagnosed rabid have previously received rabies vaccination(s).
- Vaccine manufacturers do not guarantee duration of immunity beyond what is stated on the label.
- Serology cannot be used to prove exposure or non-exposure to field strains of rabies virus.

For more information about this rabies testing program, please contact Dr. Susy Carman at 519-824-4120 ext 4551.

Maedi/visna and CAE testing

Ms. Linda McCaig, AHL

The regional office of CFIA in Guelph no longer wishes to forward blood samples for maedi/visna and caprine arthritis/encephalitis (CAE) serology and **is requesting that veterinary clinics submit directly to Charlottetown**. The CFIA lab in Charlottetown asks that samples meet the following submission requirements:

1. Samples must be taken by a **CFIA accredited veterinarian** (list available from your District CFIA office).
2. Samples must be accompanied by the **proper requisition** - #4053 for fewer than 10 animals, or

#4054 for more than 10 animals. Please fill out the requisition completely and sign.
3. Clotted samples should be **spun down** and serum poured off into a separate labeled tube (labels should match IDs on the requisition).
4. Ship serum samples chilled on **ice packs**.

The CFIA lab indicated that they will accept all requests for testing but that disease control testing is not high priority. Turnaround time is at least one month or longer for disease control requests. CFIA recommends that the clinic call ahead to schedule testing.

CFIA mailing address and phone number:
CFIA- Center for Animal and Plant Health 93 Mount Edward Road Charlottetown, P.E.I. C1A 5T1
Phone: 902-368-0950, ext 252 Fax: 902-368-0295

An alternative source of testing is **Palliser Labs** in Alberta. However Palliser uses the AGID method which is less sensitive than the ELISA method used by CFIA. The AGID method has a 30-40% false negative rate (according to CFIA). Palliser charges $10/sample for the first 10 samples and $6/ sample for the samples >10. There is also a $12 setup fee for fewer than 20 samples.

SWINE

Summary of porcine abortion diagnoses and diagnostic recommendations

Drs. Gaylan Josephson, Nonie Smart, Beverly McEwen, Murray Hazlett, Susy Carman, Doug Key, Hazel Alexander, AHL

From both a practitioner and a laboratory perspective, diagnosis of porcine abortions can be frustrating. The number of specimens received in the laboratory is often dependent on the producer's perception of the severity of the problem - abortion rates of 1-2% within a breeding herd are regarded as "normal", with an increase often noted in the fall. In many cases, only a small percentage of aborted litters are submitted. The quality of specimens is often not optimal, and the history accompanying the samples may not be complete. In about 50% of porcine abortions, post mortem examination and ancillary tests, including serology, do not identify an infectious cause of abortion. Nevertheless, negative results can be useful in indicating that particular agents are not present.

We reviewed 114 submissions of porcine abortions made by 74 producers to AHL laboratories since October 1997 (Table 1). Samples consisted primarily of aborted fetuses with or without serum samples from aborting dams. Individual tissue samples - fresh, frozen or formalinized - were rarely submitted.

Diagnosis of abortion caused by **porcine parvovirus (PPV) was confirmed by virus isolation or fluorescent antibody (FA) testing of lung tissue from aborted fetuses.** PPV is ubiquitous in swine herds in Ontario, and very few herds do not have natural immunity. However, individual animals, particularly gilts, may be naive, and PPV is still identified as a cause of abortion. Pathognomonic gross and histological lesions are not apparent in the fetus, although meningo-encephalitis can be identified occasionally. Virus isolation from tissues of aborted and/or stillborn pigs is occasionally successful, but we prefer FA testing of lung from fetuses <16 cm CR length.

Table 1. Summary of porcine abortion pathology diagnoses, AHL, October 1997 to April 1999

Diagnosis	No.of diagnoses
Bacterial abortion	32
Parvovirus	15

PRRS	4
Congenital anomaly	1
Goiter	1
Fetal pneumonia, no agent	2
Placentitis, no agent	7
Idiopathic	53
Total	115
Pathology diagnoses	62/115 (54%)

Serum samples from animals aborting due to PPV are not diagnostic, as these animals will be positive, but samples from various animals of different parities will give an indication of the immune status of the herd. Identification of positive sera or fluids from fetuses or from stillborn or pre-suckled pigs will give an indication of in utero infection.

Abortions caused by **porcine reproductive and respiratory syndrome virus** (PRRSV) were usually associated with respiratory signs in the herd and were **confirmed through the finding of elevated ELISA values in aborting sows** (often up to a 3.5 S/P ratio, using the IDEXX test). Fetal examination is usually unrewarding, as gross and/or histological lesions are usually not observed. Fluorescent antibody tests can be performed on fetal tissues, with lung tissue preferred. Virus isolation is difficult, as the virus is rapidly degraded by post-mortem autolysis. In addition, not all of the piglets in the litter are infected at the time of delivery. The submission of several weak-born, live, non-suckled piglets improves the chances of virus isolation, since these piglets are often viremic. It is important that these pigs not ingest sow colostrum, since the antibody content may interfere with virus isolation attempts. Since PCR testing does not rely on the presence of live virus, it offers a better chance of making a diagnosis, but several fetuses must be tested. Since the virus can only be isolated in the serum from adults for 1-2 weeks post-infection, the dams are no longer viremic at the time of abortion or farrowing, and PCR attempts made at this time are usually negative.

Congenital goiter was identified in fetuses from one litter. A total of 15 abortions, mostly in gilts, had occurred in this herd of 150 sows over a period of 10 months.

Bacterial abortions, although not usually associated with abortion storms, are identified via cultural attempts, with the results correlated to the presence of histological lesions (Table 2). Tissues from porcine abortions are often contaminated due to autolysis, so bacterial isolates must be interpreted with caution, especially if pure cultures are not isolated from placenta, lung and stomach contents. Several fetuses and placental tissue should be submitted.

Serum samples from aborting sows, along with fetuses, are necessary to arrive at a diagnosis of **leptospira-induced abortion.** Multifocal, interstitial nephritis may be seen in kidneys of aborted fetuses. Titers in aborting sows will be elevated at the time of abortion. The current AHL panel of *Leptospira* serovars includes *Leptospira pomona, bratislava and grippotyphosa*. The significance of *L. bratislava* is unknown, as elevated titers to this serovar are very infrequent, and when present, are of low value. Testing for other serovars can be done by special request.

One of the more common causes of non-infectious abortion, and one that cannot be identified by laboratory testing, is the so called **"fall" or "cold weather" abortion**. To make this diagnosis, other causes of abortion must be excluded, and combined with an adequate herd history that includes information about sow body condition, decreasing environmental temperatures and decreasing hours of

daylight, without a corresponding increase in feed intake. Since this is not a laboratory diagnosis, abortions relating to this syndrome were not identified in this study.

Table 2. Bacterial isolates from porcine abortions, AHL, Oct 1997 to March 1999

Bacteria/yeast	Number identified
Escherichia coli	15
Streptococcus suis	6
Non-hemolytic Streptococcus sp.	2
Klebsiella sp.	1
Staphylococcus hyicus	1
Campylobacter sp.	1
Streptococcus equisimilis	1
Pasteurella sp.	1
Erysipelothrix sp.	1
Bordetella bronchiseptica	1
Leptospira grippotyphosa, serology	1
Yeast *(Candida spp.)*	3
TOTAL	35

1. Several fetuses, placenta and serum from aborting sows.
2. Alternatively, various tissues for other testing:
- bacteriology
- lung, placenta
- parvovirus FA
- lung
- histopathology - formalin-fixed lung, liver, spleen, thyroid, kidney, placenta, heart, adrenal, jejunum, spiral colon and brain, plus any tissue with gross lesions
- leptospira serology - serum from aborting sows
- PCR for PRRSV - serum, lung

When tissue samples are harvested, they should be individually packaged in leakproof, labeled, whirlpak bags. Unless samples can be kept cool and delivered to the laboratory the following day, they should be frozen and packaged so that they arrive at the laboratory frozen.

Swine influenza surveillance

Drs. Gaylan Josephson, Susy Carman, Beverly McEwen, AHL

The Animal Health Laboratory continues to see an increase in the number of diagnoses of swine influenza. Tentative diagnoses made via clinical signs or suggestive histological findings are confirmed through fluorescent antibody (FA) testing, virus isolation, or through serological profiling.

The numbers of cases positive by either FA testing or virus isolation from 1992 to 1999 are listed below:

							1999

Year	1993	1994	1995	1996	1997	1998	4 mo
SIV cases	2	3	4	5	9	16	8

To date, all swine influenza virus (SIV) isolates have been identified as H1N1 subtypes of influenza A virus, with the exception of one H3N2 isolate made in the spring of 1997 (AHL Newsletter, 1998; 2 (1): 5.). The H3N2 strains recently associated with severe respiratory problems in swine in the USA have not yet been recognized in Ontario.

In Ontario, H1N1 strains have been identified in typical explosive swine influenza outbreaks, as well as in nursery, grower and finisher operations in which the typical respiratory outbreak has not occurred. In these herds, an occasional pig with a "barking" cough has been identified, or the SIV may be part of the porcine respiratory disease complex.

All known influenza subtypes exist in aquatic birds and are shed in large number in feces. When picked up by pigs, which can be infected by both human and avian strains, these viruses can be altered (1). These strains, to which humans have no immunity, can appear suddenly, and can result in serious pandemics worldwide. **Continuous surveillance of pigs is therefore an important part of an early warning system for humans.**

1. Webster RG. Influenza: An emerging disease. Emerging Infectious Diseases 1998; 4(3).

Streptococcus equisimilis in swine

Drs. Nonie Smart, Tony van Dreumel, Beverly McEwen, AHL

Streptococcus equisimilis is occasionally associated with infections in neonatal or young pigs (1). Most commonly it is isolated from swollen or arthritic joints. **Recently, we have isolated this organism in pure culture from internal organs (spleen, liver, kidney, brain) of mature sows in a number of cases.** The clinical history given in these cases was either sudden/ acute death or signs consistent with septicemia. Clinically significant *S. equisimilis* infections in mature animals are very uncommon. The pathogenesis of these infections is unknown as there are very few references available on this bacterium in the literature. S*treptococcus equisimilis* is often carried asymptomatically in tonsil and presumably this colonization may have been the source of infection in the mortalities described above. As compared to other streptococcal diseases of pigs, the association of environmental stressors or health status of pigs with the occurrence of *S. equisimilis* septicemic disease is unknown.

1. Fangman TJ, Fales WH. Multiple *Streptococcus sp.* implicated in lameness and central nervous system sign in piglets and sows. Swine Health Production 1999; 7: 113-115.

SMALL ANIMALS

Zinc toxicosis in a dog that ingested $1.23

Dr. Brent Hoff, Mr. Nick Schrier, AHL; Dr. Lisa Carioto, VTH, OVC; Dr. Jane Burgess and Dr. Peter Sponagle, Kortright Animal Hospital, Guelph

A nine-year-old, female, Pug dog was presented to the Veterinary Teaching Hospital of the Ontario Veterinary College with a history of episodes of syncope, pale mucous membranes and jaundice. The referring veterinarian suspected lead toxicosis and noted several metal objects that resembled coins on radiography. Laboratory analysis revealed severe, regenerative anemia, and leukocytosis with a left

shift. Analysis for blood lead was negative. Further investigation in the AHL toxicology laboratory revealed 18 mg zinc/L in the dog's initial serum sample.

Endoscopy was performed, and, due to the large number of coins present, they were removed surgically. The dog needed several blood transfusions, due to the severe hemolytic anemia. The dog improved clinically and has gone on to make a complete recovery. One American penny revealed etching of the copper coat and the appearance of a gray metal under this coat.

Metal analysis on the various coins revealed a content of 98% zinc in the American penny. The various Canadian coins contained less than 2% zinc.

True zinc toxicosis is an infrequent occurrence in mammals. Many of the toxic effects attributed to zinc may have been due to other metals, such as, cadmium, lead, arsenic and antimony. Zinc toxicosis is more common in birds, such as water fowl and psittacines that either ingest metal objects or chew on metal-containing objects. Toxic quantities of zinc can be brought into solution when acidic-foods and water are stored in galvanized containers.

1. Hoff B, Boermans HJ, Baird JD. Retrospective study of toxic metal analysis requested at a veterinary diagnostic toxicology laboratory in Ontario (1990-1995). Can Vet J 1998; 39: 39-43. 2. Latimer K, Jain AV, Inglesby HB, Clarkson WD, Johnson GB. Zinc-induced hemolytic anemia caused by ingestion of pennies by a pup. J Am Vet Med Assoc 1989; 195: 77-79.

SHEEP

Cysticercus ovis infection in a slaughter lamb

Dr. Jeff Caswell, AHL; Dr. Andrew Peregrine, OVC Pathobiology; Dr. Ab Rehmtulla, OMAF Food Inspection; Dr. John Martin, OMAF Health Management; Dr. Ramon Carreno, OVC Pathobiology

The carcass of a single lamb was condemned at slaughter in a provincially-inspected abattoir, on the basis of discrete, raised, white, 3-8 mm nodules in the heart and skeletal muscles, including masseter muscle and diaphragm. Most of the nodules contained a caseous friable center surrounded by a fibrous rim. Occasional nodules in the skeletal muscle were fluctuant and filled with clear fluid. Histologic examination of the fluctuant nodules revealed a single cyst containing a single larval cestode, surrounded by a fibrous cyst wall containing macrophages, eosinophils, and few giant cells. Many of the nodules, particularly in the heart, contained caseous cellular debris; definitive diagnosis would not be possible if only these nodules were examined.

A larval cestode was extracted from one of the fluctuant nodules. The scolex had four suckers and a single row of six rostellar hooks. The hooks were irregularly distributed, suggesting that some small hooks and the entire row of large hooks had fallen off during processing. A provisional diagnosis of *Cysticercus ovis* was made, on the basis of hook measurements and anatomic location of the cysts.

***Cysticercus ovis* is the larval form of the tapeworm *Taenia ovis*, which inhabits the small intestine of dogs and other canids.** Infections have been documented rarely in cats. Eggs are passed in the feces, contaminate pastures, and are ingested by sheep. Larval cysts develop in the muscle of the heart, diaphragm, and head, and less frequently in the tongue or muscle of the limbs and body. The life cycle is completed when cyst-laden muscle is ingested by canids. Other larval cestode cysts in sheep include *C. tenuicollis* in the mesentery and liver, Coenurus cerebralis in the brain and spinal cord, and Echinococcus granulosus in the liver and lung.

***Cysticercus ovis* is apparently uncommon in Ontario, as this is only the fourth case reported since**

1991. The concern over this infection is two-fold. First, unlike other cysticerci in sheep, the cysts are present in muscle and may result in condemnation of the entire carcass. Second, there is concern that if the infection becomes established in wild canids, such as foxes and coyotes, in Ontario, the disease may be difficult to control. The source of infection in this closed sheep flock is not known with certainty, but we have anecdotal evidence that introduction may have been from a guard dog puppy acquired from a second Ontario farm, which in turn purchased dogs from an area of western Canada endemically infected with *C. ovis.* Dogs on the farm apparently passed tapeworms following anthelmintic treatment, but these tapeworms were not available for examination.

Measures to prevent *Cysticercus ovis* infection include the following:
1. Treat domestic dogs regularly with anthelmintics such as praziquantel that are effective against cestodes.
2. Do not feed sheep carcasses or offal to domestic dogs.
3. Bury sheep carcasses deeply enough to prevent scavenging by wild canids. Freezing or boiling carcasses will kill larval cestodes in muscle and other tissues, if owners insist on feeding muscle or offal to dogs.

CATTLE

Identification of BVDV persistently infected animals using skin biopsies

Dr. Susy Carman, AHL

Cattle persistently infected with bovine virus diarrhea virus (BVDV) have infection of hair follicle epithelium. The Prairie Diagnostic Services laboratory at the Western College of Veterinary Medicine in Saskatoon has developed a skin test to determine if cattle are persistently infected with BVDV. **This test is useful for dead animals or when a blood sample is not available. This test has good correlation with virus isolation using blood. This test is not appropriate for acutely infected animals.**

The site of biopsy should be a haired area (neck, rump). The site should be clipped. Skin biopsies are collected using a brisket punch ($20-$30) or disposable skin biopsy punches (about $2 ea.). Biopsy punches are best disinfected between animals and used only when sharp. Be sure not to squash the biopsy during cutting.

The samples are fixed in formalin in individually labeled containers (can use 1 mL of formalin in a 2 mL leakproof screw top tube with writing block for animal identification). Skin biopsies should be forwarded without delay for they should be in formalin for no more than one week (24 to 48 hours best), before they are embedded in paraffin and tested using the immunoperoxidase (IP) technique. The approximate cost of transportation of a 1-5 lb. box (under 12" X 12" X 12") to Saskatoon using Purolator is $35.00 by air or $23.00 by ground.

Charges from **Prairie Diagnostic Services** are $30/block plus GST. This fee includes trimming. Where more than one animal is to be tested, the skin from five animals from the same case can be included in one block if special divided cassettes are used for paraffin embedding. Hence, when a minimum of five animals is tested, the average charge to test one animal would be $6 (plus the cost of biopsy punches and transportation).

For more information about this IP test using skin, please call Dr. Keith West at 306-966-7211

Specimens should be forwarded to: Prairie Diagnostic Services 52 Campus Drive Saskatoon, Sask, S7N 5B4 Phone (306) 966-7234

Isolation of BVD virus from bulk milk samples

Dr. Susy Carman, AHL

To enhance our BVD herd screening program, the AHL now offers BVD virus isolation on bulk milk samples using a protocol developed at the New York State Diagnostic Laboratory at Cornell. **This test is used to screen lactating dairy cow populations for persistently infected animals.** If the test is negative, individual animals need not be tested.

Please remember that the majority of persistently infected animals die before 2 years of age. Since they rarely survive to enter the milking herd, they would not be evaluated using this test. Non-lactating animals must to be evaluated using individual blood samples.

- Please submit 200 mL of milk from a WELL-STIRRED BULK TANK in a sterile container. Leakproof sterile urine containers are acceptable. The sample should represent a pool of no more than 400 cows.
- Keep milk chilled.....DO NOT FREEZE.....for we need to harvest the living leukocytes for co-culture with cell cultures.
- Ship to the laboratory on the day of collection using overnight courier delivery.
- Ship with ice packs to decrease the growth of bacteria and fungi which can destroy cell cultures used for virus isolation.
- If possible, please collect your samples at the beginning of a week or give us a call a few days before collection so that we can be certain to have cells ready and waiting for your samples.
- Results will be available in about 3 weeks. This test requires a 3 week turn-around time to accommodate 3 passages in cell culture, each taking one week.
- The charge is $40 per test.

For more information on how to best use this new test in your BVD herd screening programs, please call Dr. Susy Carman at 519-824-4120 ext 4551.

OAHSN - Surveillance needs you

Dr. Grant Maxie, AHL

The AHL is a partner in the **Ontario Animal Health Surveillance Network (OAHSN)**, which includes the Provincial Veterinarian, veterinarians from Health Management, the Education, Research & Laboratories Division, and the Food Inspection Branch in OMAF, and the Chair of Population Medicine, OVC. The AHL aims to provide timely information on new and emerging diseases, disease outbreaks, trends in endemic diseases, and diseases of public health importance to OAHSN and through this, to OMAF, CFIA, the Ministry of Health, and Health Canada, **but this aim is fulfilled only if submitters provide complete demographic information on their submissions - please help us to help you by completing our forms.** Your information helps decision makers to perform risk assessments, evaluate control strategies, identify research needs and facilitate planning, and also helps to maintain national and international confidence in Ontario agriculture and trade.

Animal Health Laboratory Accreditations:
American Association of Veterinary Laboratory Diagnosticians (AAVLD) (lab system)
Thyroid Registry of the Orthopedic Foundation for Animals Inc. (OFA) (thyroid function)
Canadian Food Inspection Agency (CFIA) (EIA)
Canadian Association of Environmental Analytical Laboratories (CAEAL) (metals) ISO 9002 (toxicology)

Mailing list If you would like to be added to, or removed from, the AHL Newsletter mailing list, please fax your request to Ms. Helen Oliver at 519-821-8072 or E-mail to holiver@lsd.uoguelph.ca

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