Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

AHL Newsletter
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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. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

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Specimen Reception update – **more** on getting the most from your diagnostic laboratory

Jim Fairles

Animal Health Laboratory, University of Guelph, Guelph, ON.

AHL Newsletter 2021;25(3):2.

Your relationship with your diagnostic laboratory is vital in the continuum of patient care. I wrote recently about this (1) with some excerpts from an excellent write-up by Dr. Belinda Thompson (now retired) from Cornell (2). As a follow-up from this article, below are some questions that you may want to ask as you assess laboratory testing and how to minimize diagnostic error:

1. Am I trying to rule out the worst-case scenario? Am I trying to rule out all important scenarios?
2. Does the diagnostic test requested reflect the problem list and differential diagnoses?
3. Will I be able to use the results of each of the tests I have requested for decision-making/treatment/management changes?
4. Have I considered which test is the best test ("fit for purpose") if more than one are available? Is there a reason to use more than one test for the same disease/condition?
5. Do I have the correct specimen for each test?
6. Have I collected every specimen that would be required? If not, can they be collected?
7. Have I handled each sample exactly according to the guidelines provided by the laboratory? Do I know what those guidelines recommend?

AHL has many resources to help you with these questions – all are available on our website:

https://www.uoguelph.ca/ahl

User Guide and searchable **Fee Schedule** (access to fees available to veterinarians and researchers by following the instructions on the website):

https://www.uoguelph.ca/ahl/tests-users-guide/ahl-users-guide
https://www.uoguelph.ca/ahl/tests
https://www.uoguelph.ca/ahl/user/login?current=tests

Labnotes – provide more detailed information on specific tests:

https://www.uoguelph.ca/ahl/publications/ahl-labnotes

Previous AHL Newsletters – many updates on testing:

https://www.uoguelph.ca/ahl/publications/newsletters

Diagnostic Plans (currently ruminant and small ruminant are available – more being added):

https://www.uoguelph.ca/ahl/diagnostic-plans/diagnostic-plans-bovine
https://www.uoguelph.ca/ahl/diagnostic-plans-small-ruminant

We are also glad to discuss any testing questions and concerns. Please feel free to reach out! *AHL*

ahlinfo@uoguelph.ca 519-824-4120 extension 54530

References

1. https://www.uoguelph.ca/ahl/getting-most-out-your-diagnostic-laboratory-submissions
2. Thompson BS. On the journey for great diagnostics – Tips and tricks to let the magic happen. AABP, St. Louis, Sept. 2019.

Update from the Director

The view from the Director's office

It has been an eventful summer at the AHL! After the lifting of most COVID-related restrictions in July, many staff have finally been able to take time off to relax and get together with families and friends at the cottage, for a picnic, at an outdoor patio or in small gatherings. As we approach the fall semester and the return of students on the University of Guelph campus, there are many uncertainties related to how we will operate during this 4th wave of COVID-19 infections involving the Delta variant. Beginning September 7, the U of G will implement a proof of vaccination policy for all staff, students and visitors who access buildings on campus. The details of how this compliance will be documented are still being worked out. We do not expect this vaccination mandate to affect veterinarians or clients who are just dropping off samples at AHL Specimen Reception or Postmortem facilities, but we may obtain clarification on this detail soon – stay tuned! We'll update any access requirements on our web-site and email blasts if the situation changes.

We take note of the feedback provided by our clients during meetings, surveys and informal discussions. A common request is "more test panels please!" We recognize the utility of organizing species-specific tests into a disease panel that is easy to order and provides broad diagnostic coverage. In our June newsletter, we rolled out our new foal and adult equine diarrhea panels. The AHL bovine comprehensive respiratory panel has been available for several months, and is described on page 13 of this newsletter. As the cooler weather of fall approaches, respiratory diseases will begin their seasonal rise in occurrence, and bovine practitioners may find this comprehensive panel useful in managing outbreaks. Please also check out several AHL staff changes in our Staff highlights section on page 5.

I hope you have had some time off this summer to relax and recharge. Take care and stay safe!

Maria Spinato, Director

Animal Health Laboratory, University of Guelph, Guelph, ON.

OAHN Update – September 2021

Mike Deane

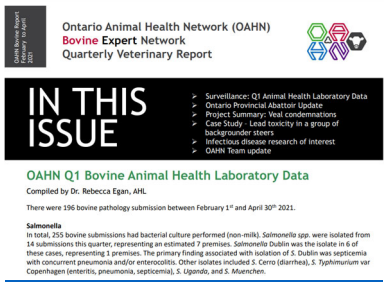
Animal Health Laboratory, University of Guelph, Guelph, ON.

The Ontario Animal Health Network has been busy throughout the summer, with our ongoing work on species-specific producer and veterinary reports, research projects, and podcasts. Read on to find links to all of the exciting animal health and disease research and resources we have been working on.

New Reports

The latest network reports for companion animals, bovine, swine, and poultry have been posted to the OAHN site. Below, find links to the veterinary reports (login needed). To access the producer and industry reports, navigate to your desired network on **OAHN.ca**, and click on the reports tab.

Bovine

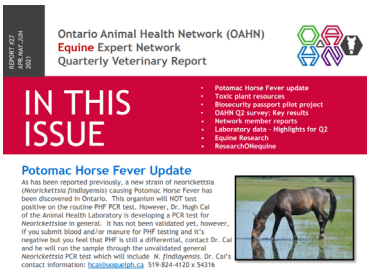


- Ontario Provincial Abattoir Update
- Case Study – Lead toxicity in a group of backgrounder steers
- Infectious disease research of interest

Companion Animals

- New Canadian dog importation rules
- US bans dogs from 113 countries
- Ontario rabies update: Revised control zone, rabid dog from Iran
- Pentobarb shortage

Equine

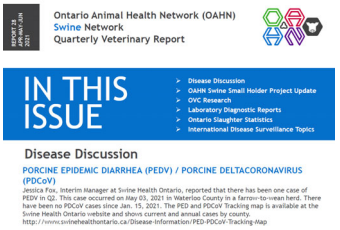


- Potomac Horse Fever update
- Toxic plant resources
- Biosecurity passport pilot project
- Network member reports

Poultry

- Poultry Veterinary Survey Highlights – Q2
- Events and News

Swine



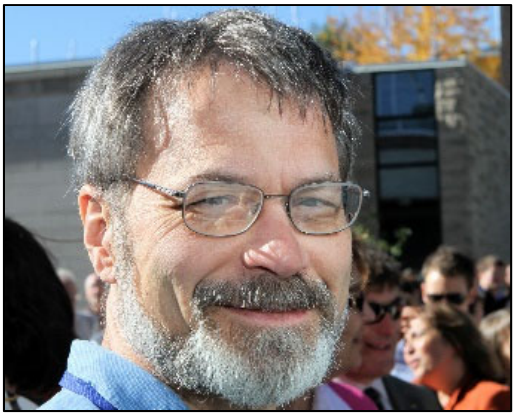
- Disease Discussion
- OAHN Swine Small Holder Project Update
- OVC Research
- Topics of Interest

New Bovine Podcast

The latest OAHN bovine quarterly podcast report gives a quick update on laboratory data from Q1, an overview on risk of lead toxicosis in cattle, and a global surveillance update focusing on Lumpy Skin Disease. Access it here: OAHN Bovine Network Quarterly Update: Lab Data, Lead Toxicosis, Lumpy Skin Disease *AHL*

Staff highlights

Murray Hazlett retires



Well, it is the end of an era! After working at VLS/VLSB and AHL for over 35 years, Dr. Murray Hazlett has retired. His last day of work was June 30.

Murray has made a significant contribution to the animal health sector in Ontario throughout his distinguished career as a pathologist, educator, mentor and colleague.

We wish him many happy, healthy years with his family and friends in his well-deserved retirement.

Murray Hazlett wins Laboratorian of the year at CAHLN 2021

The Canadian Animal Health Laboratorians Network (CAHLN) awards a plaque annually to a laboratorian based on his or her noteworthy contributions to veterinary laboratory medicine in Canada.
A nominee might be an outstanding diagnostician, educator, researcher, mentor of future laboratorians, or other contributor to the field.
This award was presented to Dr. Murray Hazlett at the conclusion of the CAHLN Annual Meeting held June 2021.
Congratulations Murray!

Melanie Barham leaves AHL

Dr. Melanie Barham has accepted a position as Executive Director of the National Farmed Animal and Health Welfare Council (**NFAHWC**) and left the AHL on July 15. While we are sad to see her leave, we are happy that she has this exciting opportunity for career growth. We are appreciative of her tremendous accomplishments during her time here at AHL, including the development of OAHN into a superbly-managed and nationally-recognized animal health surveillance network.

RUMINANTS

Systemic vasculitis in sheep associated with ovine herpesvirus-2

Andrew Brooks, Amanda Mansz, Jan Shapiro

Animal Health Laboratory, University of Guelph, Guelph and Kemptville, ON

AHL Newsletter 2021;25(3):6.

Pathologists at the AHL have observed rare cases of systemic vasculitis in sheep which have lesions similar to malignant catarrhal fever (MCF) in cattle. Between 2007 and 2009, the AHL laboratory in Kemptville observed multiple cases in eastern Ontario (1), and since then, there have been sporadic cases from other regions of the province. Cases of MCF-like disease in sheep have also been reported in Alberta, Europe, and the United States (2-4).

The clinical signs of this syndrome may include anorexia, oculonasal discharge, drooling, depression, recumbency and death. Excessive salivation -"slobbering sheep" - was a characteristic clinical sign noted by the pathologists at Kemptville. Affected sheep may be purebred or crossbred, lambs or adults. Flocks may have single or multiple affected animals. At postmortem, there may be prominent ulceration of the gastrointestinal mucosa including the oral cavity, tongue, esophagus and forestomachs **(Fig. 1)**.

Histologically, vasculitis affecting multiple organs is a common feature of this syndrome **(Fig. 2)**.
Ovine herpesvirus-2 (OvHV-2) is one of the ruminant gammaherpesviruses that causes MCF in cattle and other ungulates. Since sheep are the adapted host for OvHV-2, infection is widespread and mostly asymptomatic in this species. However, OvHV-2 can also cause disease in sheep. There are reports of natural disease resembling MCF in domestic and wild sheep (2, 3), and lambs experimentally infected with OvHV-2 developed lesions similar to MCF (5).

Investigating OvHV-2 as a cause of disease in ovine laboratory submissions is challenging because infection would likely be detectable in most sheep by routine methods such as qualitative PCR or serology, regardless of disease status. However, a novel in situ hybridization (ISH) method has revealed an association between the distribution of OvHV-2 nucleic acid and the vascular lesions in sheep with MCF-like disease, suggesting a causal role for OvHV-2 in this syndrome (4).

Three cases of systemic vasculitis in sheep were selected from the AHL archives for OvHV-2 ISH testing **(Table 1)**. A subset of formalin-fixed paraffin-embedded tissues containing vascular lesions were tested for each case. ISH was performed and interpreted by Dr. Patricia Pesavento (Department of Pathology, Microbiology & Immunology, University of California). With respect to other potential causes of vasculitis, these cases did not have complete diagnostic testing but PCR tests for bluetongue virus (case 1 and 2) and border disease virus/BVDV (case 1) were negative. The cases did not have lesions consistent with ovine lentivirus.

Cases 1 and 2 tested positive for OvHV-2 by ISH, and case 3 tested negative. In the positive tissues, probe hybridization was detected in the nuclei of a subset of lymphocytes surrounding some, but not all, vessels with vasculitis in the kidney or heart **(Fig. 3)**. The ISH signal in the two positive cases was weak compared to the controls which may be due to prolonged tissue fixation in formalin.
These results suggest that OvHV-2 is likely responsible for some cases of systemic vasculitis observed in sheep at the AHL. OvHV-2 should be considered a potential etiology for sheep that exhibit clinical signs

and lesions similar to MCF such as excessive salivation, gastrointestinal ulceration and systemic vasculitis.

The authors would like to thank Dr. Pesavento (University of California) for performing and interpreting the ISH, and the OAHN Small Ruminant Expert Network for financial support. *AHL*

Table 1. Summary of ovine cases of systemic vasculitis tested for OvHV-2 by in situ hybridization.

Signalment	**Submission history summary**	**Main pathology diagnoses**	**OvHV-2 ISH result**
Case 1. East Friesian ewe, 1 year of age	Postpartum agalactia, thin body condition, pale mucous membranes, recumbent	Systemic lymphocytic vasculitis Oral ulceration Pulmonary abscess Bacterial metritis Lymphocytic enteritis and abomasitis	Positive
Case 2. Jacob ram, 9 months of age	Moribund	Systemic arteritis Emaciation Coccidiosis	Positive
Case 3. Crossbred ram, 18 months of age	Off-feed, ulcerative lesions in large and small intestine	Systemic fibrinoid and necrotizing vasculitis Abomasal ulceration Hepatic and renal necrosis Coccidial enteritis	Negative

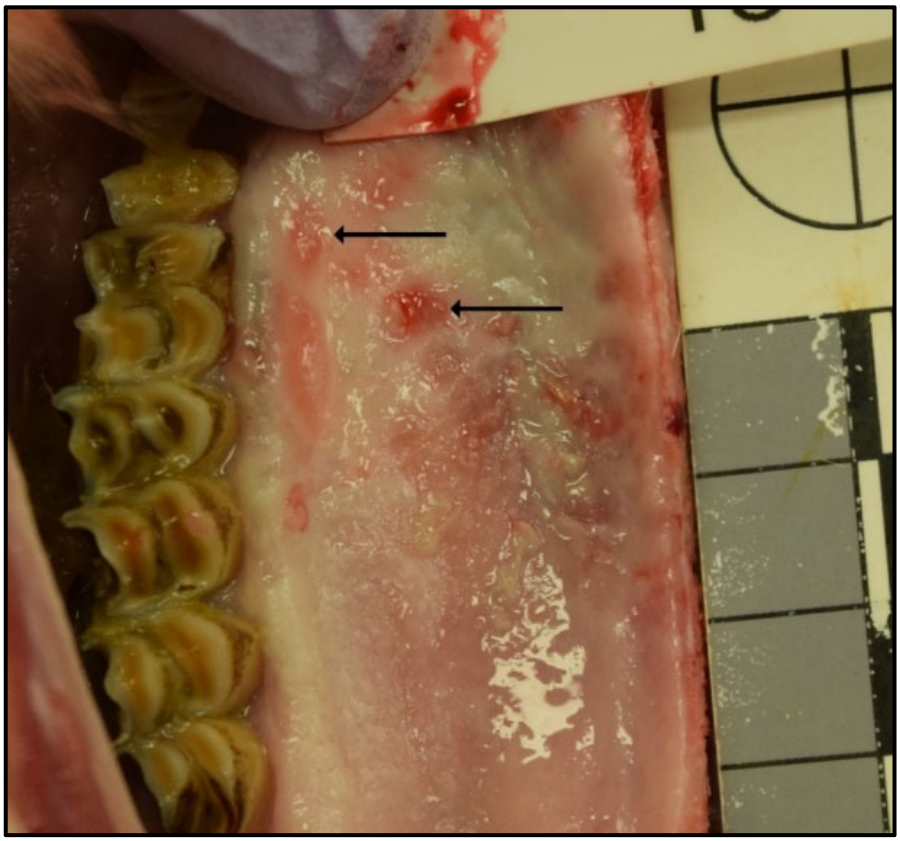


Figure 1. Multifocal ulceration (arrows) of the mucosa of the hard palate, case 1.

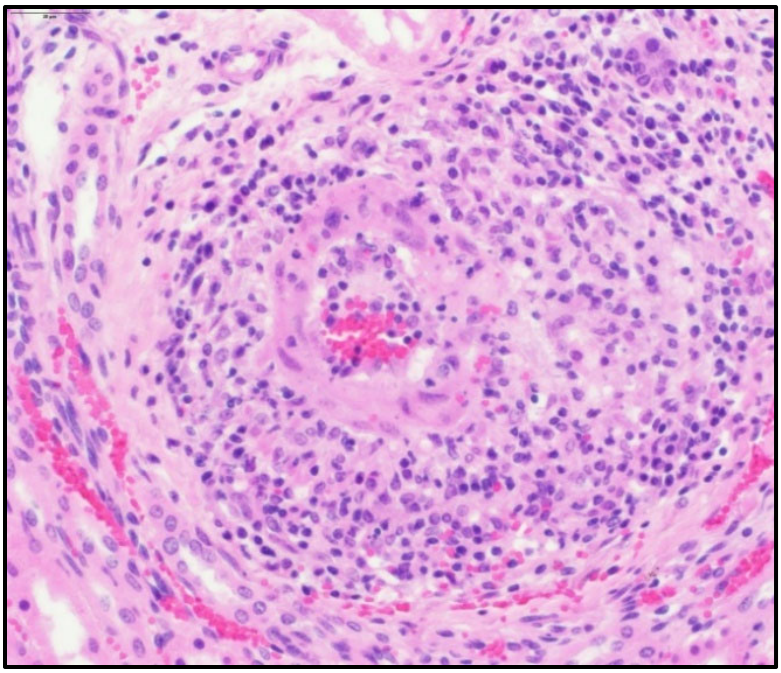


Figure 2. Lymphocytic vasculitis of a renal arteriole, case 2. H&E stain

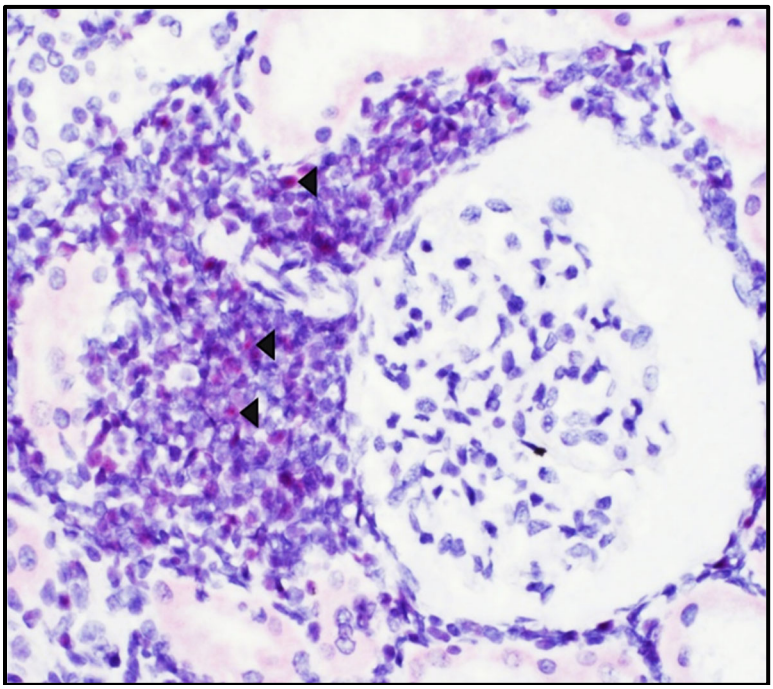


Figure 3. Positive OvHV-2 in situ hybridization (red chromogen, arrowheads) in lymphocytes surrounding an arteriole adjacent to a renal glomerulus, case 2

References

1. Shapiro J, Binnington B. Be on the look-out for an unusual mucosal disease in slobbering sheep. Ceptor 2009;17:4.
2. Slater OM, et al. Sheep-associated malignant catarrhal fever-like skin disease in a free-ranging bighorn sheep (*Ovis canadensis*), Alberta, Canada. J Wildl Dis 2017;53:153-158.
3. Gaudy J, et al. Possible natural MCF-like disease in a domestic lamb in Scotland. Vet Rec 2012;171:563.
4. Pesavento PA, et al. Systemic necrotizing vasculitis in sheep is associated with ovine herpesvirus 2. Vet Pathol 2019;56:87-92.
5. Li H, et al. Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2. J Vet Diagn Invest 2005;17:171-175.

Malignant catarrhal fever in a bison cow

Margaret Stalker, Christiane Buschbeck

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Markdale Veterinary Services, Markdale, ON (Buschbeck).

AHL Newsletter 2021;25(3):9.

A 15-year old bison cow that had calved 3 months previously was found dead with evidence of non-specific trauma including horn marks and subcutaneous hematomas. A postmortem examination revealed abomasal ulcers.

Histologic examination of tissues confirmed acute abomasitis, as well as chronic lymphoplasmacytic portal hepatitis (**Fig. 1**) and mild pulmonary perivascular lymphoplasmacytic cuffing with localized acute alveolitis (**Fig. 2**). Clostridia FA testing was negative. A herd history of previous cases prompted PCR testing for malignant catarrhal fever (MCF). PCR was positive at a low cycle threshold (Ct value 22.08), indicating the presence of ovine herpesvirus-2 (OHV-2) in high concentration in the sample of liver tissue tested.

There have been four Ontario bison herds with individual animals diagnosed with MCF over the last 10 years at the AHL. Bison are considered to be extremely susceptible to infection. The clinical presentation of disease in bison is variable, with an acute clinical disease being most common, as well as rarer subclinical forms (1, 2). The clinical history may be brief, <1-4 days, and mortality rates in bison herds can be high, particularly if animals are stressed or crowded. Affected animals may be found depressed and isolated from their herd mates, or more commonly found dead or dying. Clinical signs may be subtle and include oculonasal discharge, conjunctival hyphema, pyrexia, dysentery and hematuria/stranguria.

On postmortem, lesions include erosive rhinitis, stomatitis and esophagitis, ulcers in the forestomachs and abomasum, and a necrohemorrhagic typhlocolitis. Hemorrhagic cystitis is also a common finding in bison with MCF. Unlike cattle, generalized lymph node hyperplasia, while present, is not pronounced. Terminally ill bison may be attacked and wounded by herd mates, as in this case. On histology, vasculitis lesions are typically less florid than in cattle.

Previously, additional single cases of MCF have been diagnosed in bison on this premises over the past 4 years. Proximity to sheep, the carriers of OHV-2, is required for transmission. However, the virus can spread via aerosol over surprisingly long distances (up to 5 km), based on natural outbreaks (1). In this herd, the source of the virus may be a small ruminant farm located across the road.

The OAHN Small Ruminant Network has recently released a short update MCF in Bison
https://www.oahn.ca/resources/managing-the-risk-of-malignant-catarrhal-fever-mcf-from-sheep-to-bison/

AHL

References

1. O'Toole D and Li H. The pathology of malignant catarrhal fever, with an emphasis on ovine herpesvirus 2. Vet Pathol 2014;51(2):437-52.
2. Schultheiss PC, et al. Malignant catarrhal fever in bison, acute and chronic cases. JVDI 1998;10:255-262.

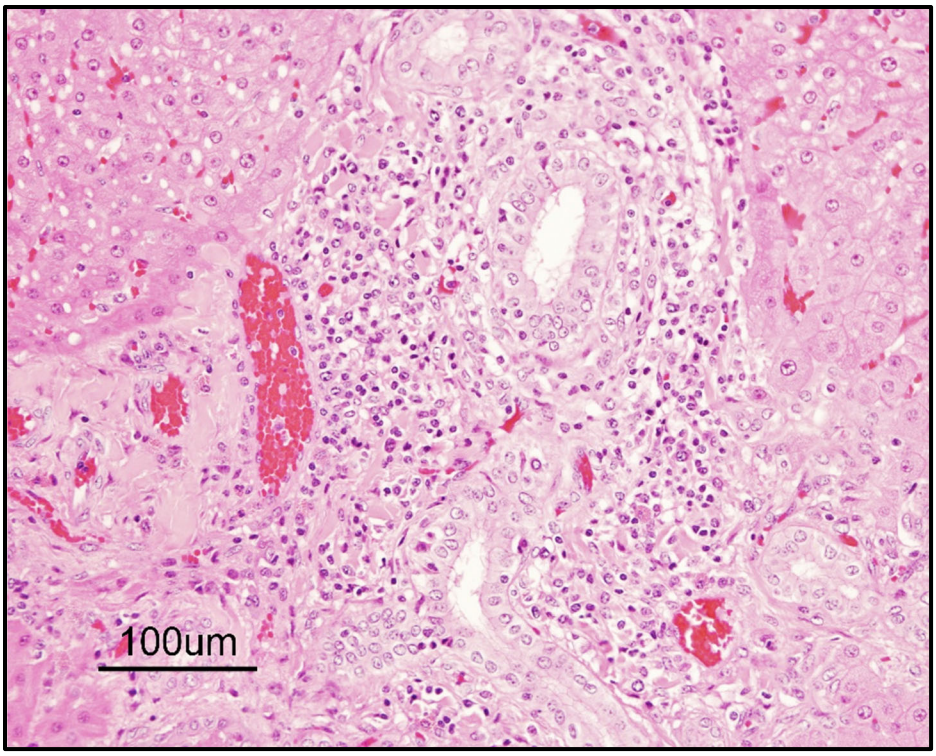

Figure 1. Lymphoplasmacytic portal hepatitis. H&E stain.

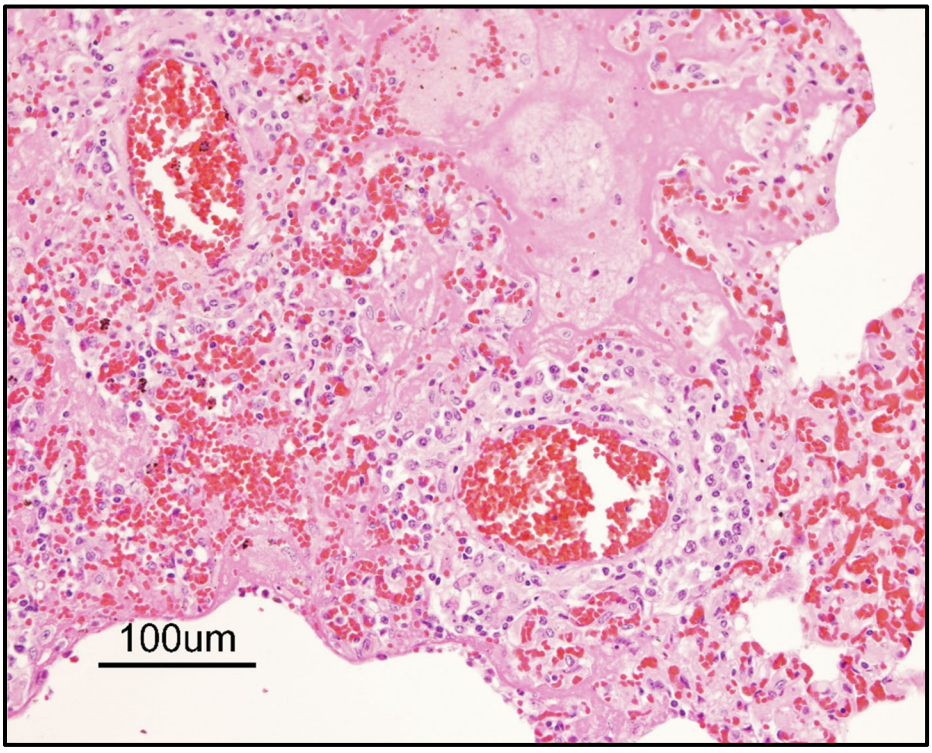

Figure 2. Mild pulmonary perivascular lymphoplasmacytic cuffing with localized acute alveolitis. H&E stain.

Abomasal coccidiosis (*Eimeria gilruthi*) in a goat kid

Emily Brouwer, Sebastian Ruszkowski

Animal Health Laboratory, University of Guelph, Guelph, ON (Brouwer);
Cheltenham Veterinary Services, Caledon, ON (Ruszkowski)

AHL Newsletter 2021;25(3):11.

A 3-month-old male Boer goat kid presented to the veterinarian for a history of coughing. The producer had noted that starting two weeks previously, the kids would start to cough and those affected would die within two days. Of the group of eleven kids, five were sick and two had died. The affected group had been treated with Draxxin, and this untreated kid was sacrificed for postmortem diagnostic workup.

On gross postmortem examination, the kid was in poor body condition, with visible bony protuberances of the scapulae and pelvis, and there was marked reduction in fat stores and mild dehydration. Approximately 50% of the right lung had marked cranioventral consolidation. On cut section, the pulmonary parenchyma was dark plum red, and there were irregular tan, necrotic foci throughout the affected tissue. A small amount of purulent exudate could be expressed from small airways. The forestomachs contained a small amount of finely ground plant fibres, and the small intestines contained brown-green fluid. The colon was empty.

Histologic examination identified a severe necrotizing bronchopneumonia, from which large numbers of bacteria including *Bibersteinia trehalosi*, *Mannheimia haemolytica*, *Pasteurella multocida* and *Trueperella pyogenes* were isolated. Large numbers of encysted apicomplexan parasites were identified within the abomasal mucosa, as well as within the small intestinal mucosa and the subcapsular sinus of a mesenteric lymph node in fewer numbers.

The apicomplexan parasites were contained within large schizonts (megaloschizonts) with thick eosinophilic walls that contained innumerable small round merozoites that were frequently arranged in circular blastophores. No inflammation was evident within the regional abomasal mucosa, nor was there any associated proliferation of the mucosa. In conjunction with anatomic location, the morphology of these megaloschizonts is consistent with *Eimeria gilruthi.*

Eimeria gilruthi is a poorly-characterized species of apicomplexan parasite that sporadically causes gastrointestinal disease in small ruminants. These parasites have been found incidentally on postmortem / microscopic examination, as in this case, but are capable of causing clinical disease. Clinical signs, if present, can include anemia, diarrhea, anorexia, and weakness.

Grossly, there is a range of potential lesions. The megaloschizonts may appear as small white raised foci throughout the abomasal mucosa in less severe cases. Proliferative abomasitis may be present in more severe cases, characterized by a thickened and nodular abomasal mucosa resembling infection by *Teladorsagia* spp.

Although this parasite is infrequently diagnosed, infection with *Eimeria gilruthi* should remain a differential diagnosis for small ruminants experiencing diarrhea and weight loss. *AHL*

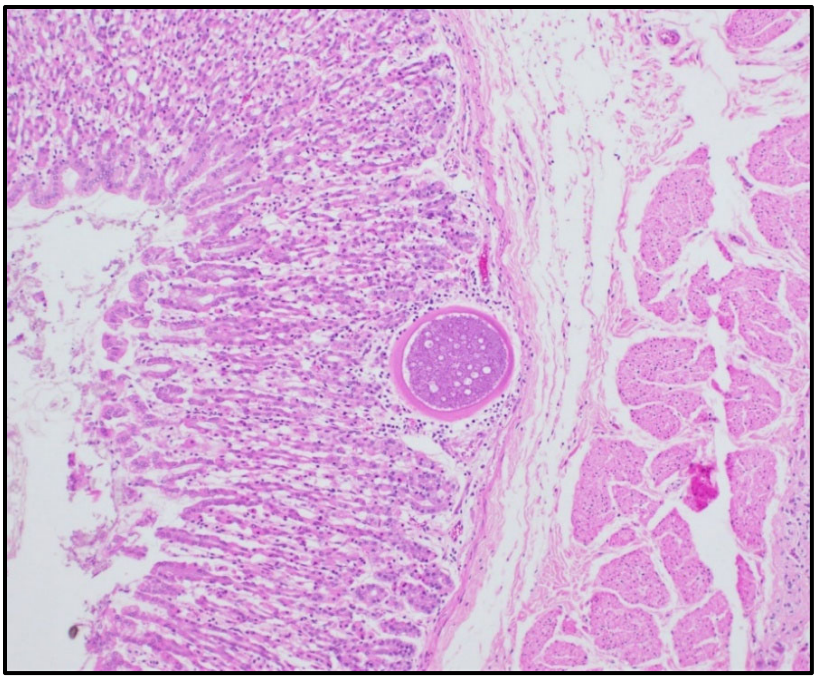

Figure 1. *Eimeria gilruthi* megaloschizont in the abomasal mucosa with thick eosinophilic capsule, 10X magnification. H&E stain

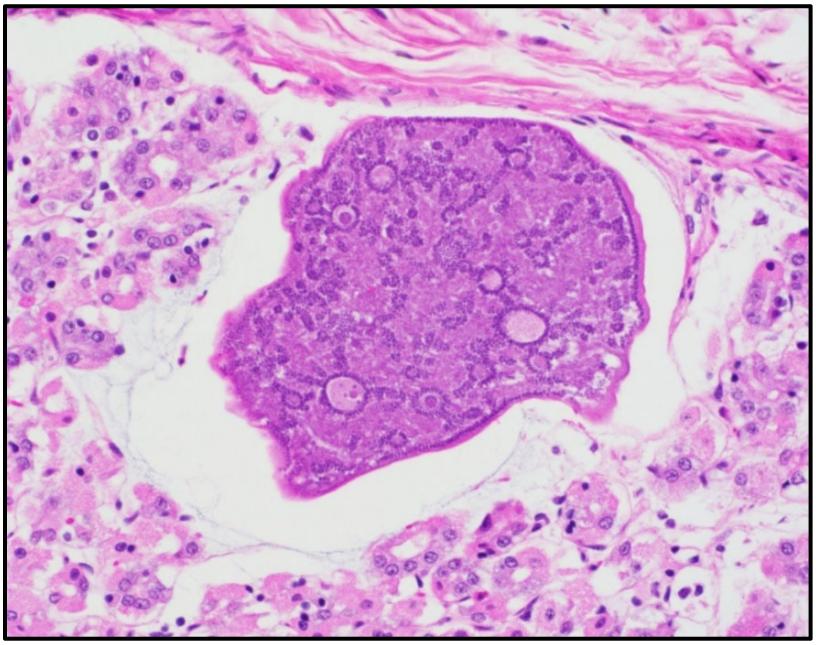

Figure 2. *Eimeria gilruthi* megaloschizont in abomasal mucosa. Note merozoites arranged in circular blastophores, 40X magnification. H&E stain.

References

1. Ammar SI, et al. *Eimeria gilruthi*-associated abomasitis in a group of ewes. J Vet Diagn Invest 2019; 31:128-132.
2. Maratea KA, Miller MA. Abomasal coccidiosis associated with proliferative abomasitis in a sheep. J Vet Diagn Invest 2007; 19:118-121.
3. Bowman DD. Protozoans. In: Georgis' Parasitology for Veterinarians, 9th ed. Elsevier, 2009:93-94.
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Comprehensive bovine respiratory disease panel

Jim Fairles

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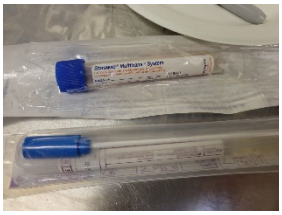

AHL Newsletter 2021;25(3):13.

Many practitioners have asked for complete panels as an aid in diagnostic testing. AHL continues to develop and combine tests to help in providing more comprehensive diagnostic planning. The comprehensive bovine diagnostic panel has been developed for this reason, and combines several existing tests.

The panel includes:

1. rotavirus/coronavirus (bovine/equine) PCR. Coronavirus PCR result is reported only (rotavirus PCR is not relevant as a respiratory pathogen, but the same test is used primarily for enteric disease)
2. bovine respiratory virus PCR panel - BoHV-1/IBR, BPIV-3, BRSV
3. BVDV PCR
4. *Mycoplasma bovis* PCR
5. Bacterial culture, food/fiber producing animals (other than swine).

Samples required:

A) For postmortem samples – **lung** is the required sample

B) For the live animal there are four choices:

1. Nasal swab (BacT swab for culture, Virus Transport Media (VTM) swab or dry swab for PCR)
2. Deep pharyngeal swab (normally guarded uterine swab – for Bacteriology into gel media; for PCR into VTM or dry swab)
3. Bronchoalveolar lavage (BAL)
4. Transtracheal wash (TTW)

Note: separate samples are needed for both bacteriology (gel media) and PCR (VTM or dry swab). The swabs cannot be transferred into alternate media upon arrival at the lab.

A reference article (1) outlines a comparison of sample types. There is good agreement when samples types are compared to TTW except for bovine coronavirus (BCV) and BRSV. Deep pharyngeal swab appears to be the best non-invasive method for sampling. It is also important that a representative number of affected animals are sampled to provide a good cross-section of the problem. The actual number needed is always centered around a representative sample and cost of testing.

This panel is intended for individual animal testing only. If pooled PCR testing is desired (up to 5 samples), each pooled test must be ordered individually. Pooled testing is not available for bacterial culture, as these samples are subject to contamination and pooling will amplify this problem. *AHL*

We are also glad to discuss any testing questions and concerns. Please feel free to reach out! ahlinfo@uoguelph.ca or jfairles@uoguelph.ca; 519-824-4120 extension 54530.

Reference

1. Doyle, D, et al. Agreement among 4 sampling methods to identify respiratory pathogens in dairy calves with acute bovine respiratory disease. J Vet Intern Med 2017;31(3):954–959.

Glasser's disease: An uncommon presentation of a common disease

Amanda Mansz, Suzanne Burlatschenko

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Goshen Ridge Veterinary Services, Tillsonburg, ON (Burlatschenko)

AHL Newsletter 2021;25(3):14.

Three mature boars were submitted to the Animal Health Laboratory for postmortem with a history of severe scrotal swelling, decreased appetite and increased respiratory effort. Postmortem examination of all three boars revealed a severe fibrinosuppurative polyserositis characterized by thick mats of fibrin coating all peritoneal and pleural organs with multiple fibrous adhesions between abdominal organs and within the pleural cavity. Two of the three boars had severe scrotal swelling with cutaneous hemorrhage and ulceration (**Fig. 1**). Incision through the scrotum and testes revealed massive peritesticular edema with an outer layer of fibrotic thickening (**Fig. 2**). Bacterial culture confirmed isolation of *Glaesserella* (formerly *Haemophilis*) *parasuis* as the causative agent of the polyserositis. The massive scrotal swelling was concluded to be an extension of the severe peritoneal inflammation along the tunica vaginalis surrounding the testes.

Glaesserella (formerly *Haemophilis*) *parasuis*, is the causative agent of "Glasser's disease". *G. parasuis* is a commensal organism of the upper respiratory that has the potential to cause disease, most commonly affecting young pigs. Clinical symptoms range from fever, nasal discharge, coughing, lameness, central nervous dysfunction, often progressing to death. As a septicemic agent, *G. parasuis* has a predilection for growth on serosal surfaces (peritoneum, pleura, pericardium, joints, meninges) with infection typically resulting in polyserositis, polyarthritis and/or pneumonia.

This case is an uncommon presentation of a fairly common disease, and an important reminder that Glasser's disease can present sporadically in naive adult porcine populations with the unusual lesion of intense scrotal swelling. *AHL*

Reference

1. Uzal FA, Plattner BL, Hostetter J M. Alimentary System. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, 6th ed. Maxie MG, ed. Elsevier, 2016; vol 2:252.

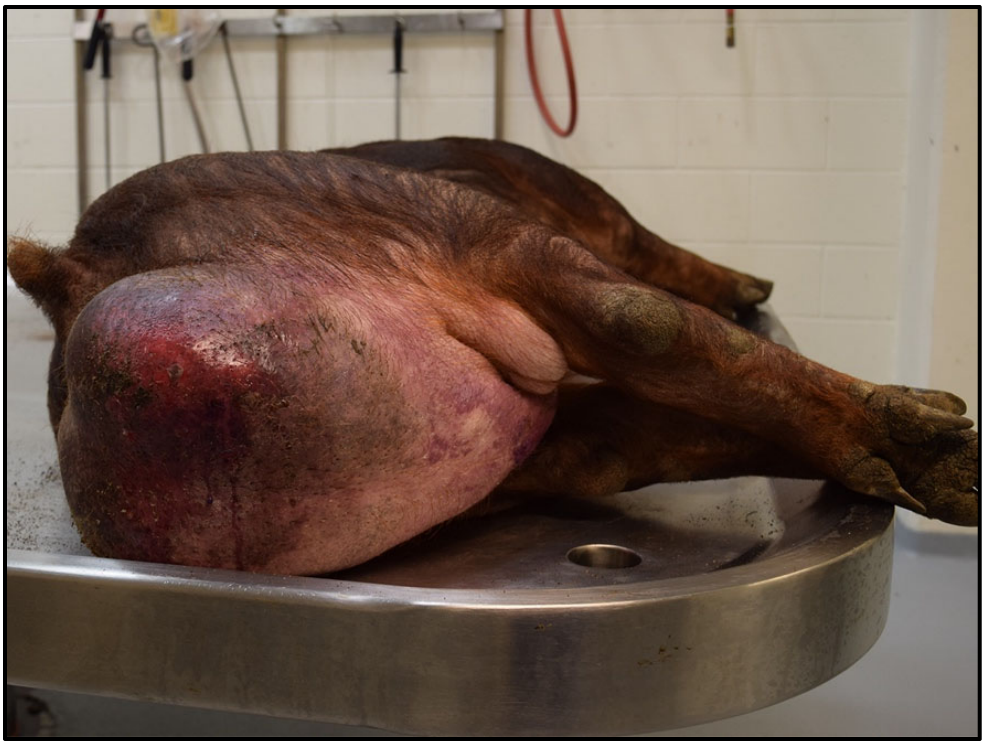

Figure 1. Severe scrotal swelling with cutaneous hemorrhage and ulceration.

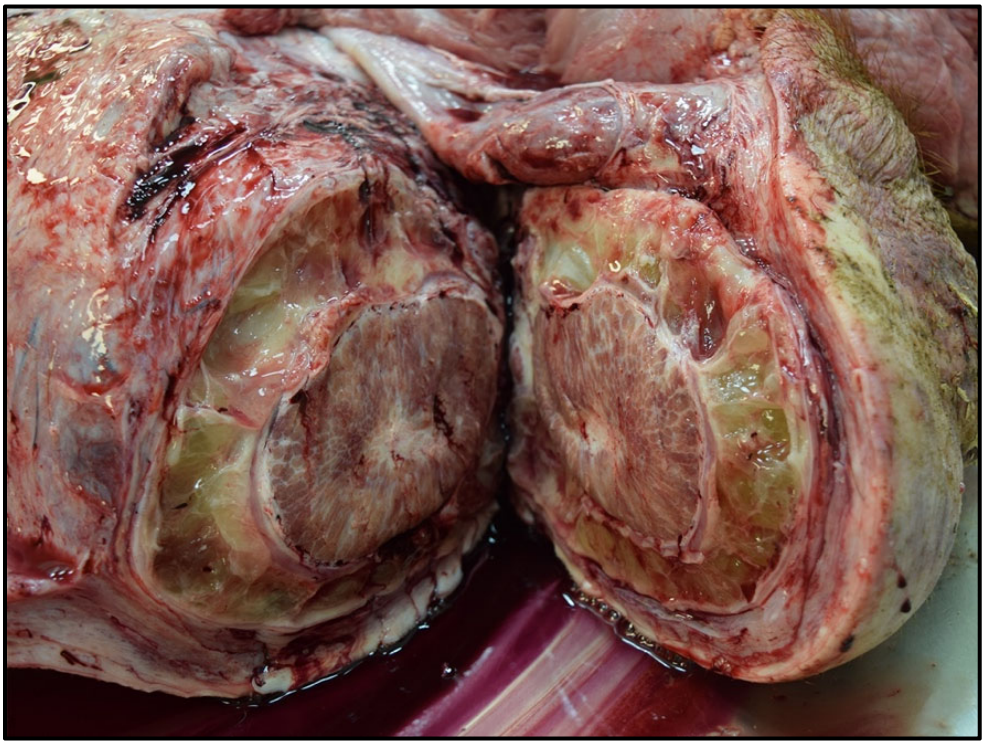

Figure 2. Massive peritesticular edema with fibrinous inflammation and fibrosis.

Update: OAHN swine small herd postmortem project

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The Ontario Animal Health Network (OAHN) swine small herd project has been in place since May 2020 due to the minimal documented information available about small swine herds in this province. The goals of this project are threefold:

1. identify disease problems in small-scale swine herds in Ontario;
2. establish communications between small scale producers, vets, and OAHN;
3. increase awareness of zoonotic diseases and FADs among small scale producers.

The postmortem project provides free laboratory testing and free or subsized postmortems for small swine herds in Ontario which meet the following criteria:

- herd size is ≤ 50 sows, or ≤1000 hogs marketed / year
- herd has a Premises Identification Number (PID)
- vet enrolls the herd and case in the project and follows appropriate sampling protocols
- producer submits a completed herd management survey

Postmortems may be done at the Animal Health Laboratory (Guelph or Kemptville). Alternatively, the herd vet may conduct field postmortems for which a stipend is provided by the project. All test results are released directly to the herd vet.

To date, 21 cases have been submitted to the project, originating from 16 herds and 9 veterinary clinics. The majority of submissions have been Kunekune pigs (10 cases), followed by commercial breeds (9 cases), minipig (1 case), and wild boar (1 case).

A range of disease conditions have been diagnosed in submitted pigs (**Table 1**). Many of these diseases are common in larger swine operations; however, other conditions are less common in commercial herds. Testing for PRRSV and influenza A virus (IAV) is carried out on all submissions: PRRSV has been detected in only 1/21 herds, whereas IAV has not been identified in any of the pigs. Collated information from producer surveys will be released at the conclusion of the project.

The AHL continues to accept submissions to the small herd postmortem project. To enroll a case in the project, please contact Dr. Josepha DeLay, AHL at 519-824-4120 ext. 54576 or jdelay@uoguelph.ca.

More information about the project is located on the AHL website: https://www.uoguelph.ca/ahl/oahn-swine-small-scale-herd-postmortem-project-may-2020. *AHL*

Table 1. OAHN swine small scale herd postmortem diagnoses, May 2020-August 2021.

Septicemia (*S. suis*) - 1
Atrophic enteritis (rotavirus) - 2
Pneumonia (*M. hyopneumoniae*, *S. suis*, and / or PRRSV) - 3
GI parasitism (whipworms, roundworms) - 2
Parasitic pneumonia (*Ascaris suum*) - 2
Non-suppurative encephalomyelitis (idiopathic) - 3
Mulberry heart disease, hepatosis dietetica – selenium deficiency - 1
Endometritis (*S. suis*) - 1
Acute idiopathic myonecrosis - 1
Salt toxicity /water deprivation - 1
PCVAD, with cerebral vasculitis - 3
Congenital cardiac anomalies - 2
Idiopathic abortion - 1
Polyserositis - 2

Excellent resources for small scale producers and veterinarians:

https://www.ontariopork.on.ca/producers/small
https://www.ontariopork.on.ca/Portals/0/OP%20ASF%20Poster_Backyard_web.pdf
https://www.casv-acvp.com/uploads/1/2/7/4/127429484/canadian_small-scale_pig_farming_manual_-_march_2021.pdf

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PRRSV sequencing surveillance for the pathogenic 1-4-4 lineage 1C variant at the AHL

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AHL Newsletter 2021;25(3):17.

Restriction fragment length polymorphism (RFLP) analysis is been one of the tools used to study the molecular epidemiology of porcine reproductive and respiratory syndrome virus (PRRSV). Currently, PRRSV ORF5 sequencing is the major method of PRRSV sequencing whereby homology tables and phylogenetic trees are generated and used to compare viruses.

The current sequencing software also automatically provides the traditional RFLP "number". This number is used to help name and categorize similar PRRSV viruses. There are some concerns regarding this methodology however, as RFLP only analyzes very specific areas of the PRRSV ORF5 sequence and can produce results that are open to misconception. For example, two 144 RFLP viruses can be quite different.

However, this nomenclature is still commonly used in the industry, therefore, AHL continues to report RFLP results to clients when sequence comparisons are performed. Recently, a highly pathogenic PRRSV 1-4-4 lineage 1C variant in the USA has been associated with high rates of sow and piglet mortality, abortions and slow growth in finishers: https://farmscape.ca/f2ShowScript.aspx?i=27596. AHL has obtained a copy of this sequence and added it to the standard list of sequences that are used to compare new sequences. Historical data have been reviewed and 1-4-4 sequences have been compared with this new 1-4-4 lineage 1C variant, as seen in Table 1.

Recent Ontario sequences (2019-2021) have a maximum of only 87.9% homology to the US 1-4-4 variant. To be considered "similar", **greater than 98% homology** is required. It is hoped that current biosecurity standards in the swine industry prevent this virulent variant from surfacing in Ontario. AHL will continue to provide surveillance around this threat by monitoring sequence homology closely. An incursion of this US PRRSV strain into Ontario would be considered a new and unusual event under the OMAFRA Animal Health Act, and as such, would be immediately notifiable. *AHL*

Table 1. Percentage homology of PRRSV 1-4-4 sequences identified at the AHL since 2019 compared to the pathogenic US 1-4-4 lineage 1C variant.

AHL	Minnesota_2020 1-4-4_severe
21-056811-1-4-4	86.7
21-030489-1-4-4	86.4
21-004666-1-4-4	87.1
20-080910-1-4-4	87.6
20-035315-1-4-4	87.1
19-089285-1-4-4	87.2
19-068991-1-4-4	86.9
19-053819-1-4-4	87.9
19-006718-1-4-4	87.4

Fungal osteomyelitis in meat turkeys

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AHL Newsletter 2021;25(3):19.

In early July 2021, 6-week-old meat turkeys presented with increased mortality and lameness. The birds had been moved a week prior to presentation. The birds were raised on shavings in the brooder barn and were moved onto shavings in a clean and disinfected grower barn at 5 weeks of age. Three barns were affected. Birds were submitted for postmortem examination from the most severely affected barn.

On postmortem examination, the birds were noted to have air sac lesions suspicious for fungal organisms (**Fig. 1**), as well as osteomyelitis and tibial dyschondroplasia. Tissues were fixed in formalin and submitted to the AHL for histopathology.

On histopathology, the air sacs had mats of fungal organisms (**Fig. 2**), and the lungs had multiple tiny fungal granulomas (**Fig. 3**). The bone section had a large abscess spanning the growth plate and articular cartilage, extending across 1/3 of the width of the growth plate (**Fig. 4**). Fungal organisms were identified within the abscess, growth plate and infiltrating the articular cartilage (**Fig. 5 and 6**). Air sac swabs were positive for E. coli (1+ and 4+), and the fungus was isolated and identified as *Aspergillus fumigatus* complex. The reovirus PCR on tendons was negative.

Fungal osteomyelitis is an uncommon lesion and could be related to extension of infection from thoracic air sacs into bone air sac diverticula; however, the affected tibiotarsal bone in this case is not one of the aerated bones in birds. Fungemia (fungal septicemia) may therefore be a possible route of infection. Considering the air sac and lung lesions were mild, it is unclear why bone was affected in this case. *AHL*

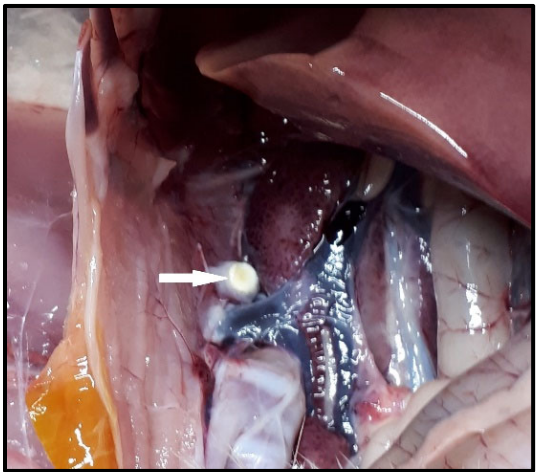

Figure 1. White nodule in air sac (arrow).

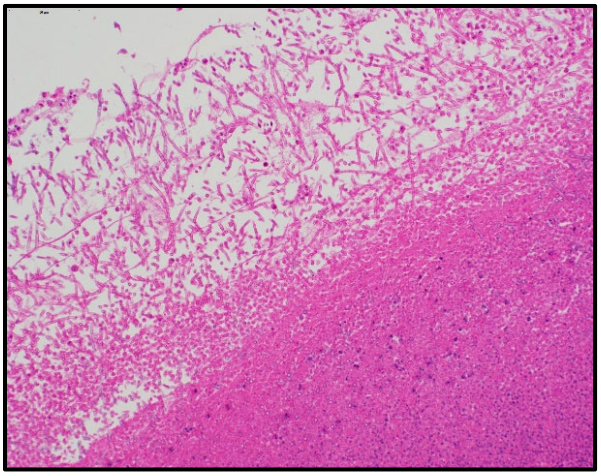

Figure 2. Mat of fungi over air sac nodule. H&E.

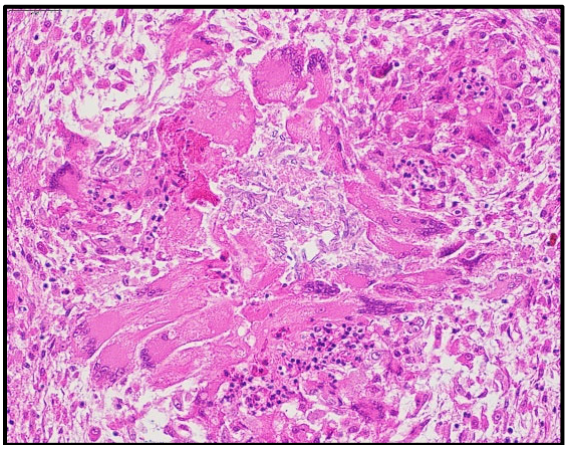

Figure 3. Pulmonary fungal granuloma. H&E.

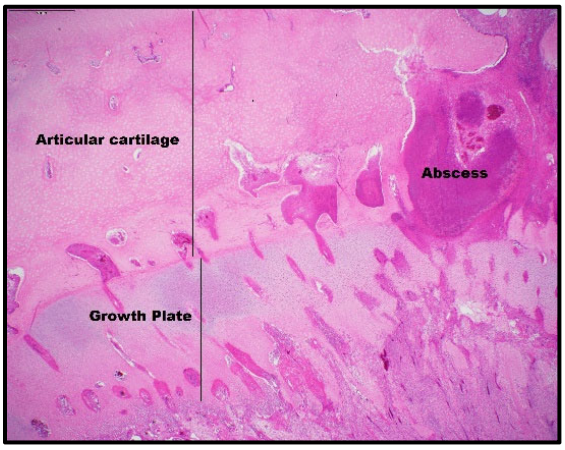

Figure 4. Abscess spanning growth plate and articular cartilage. H&E.

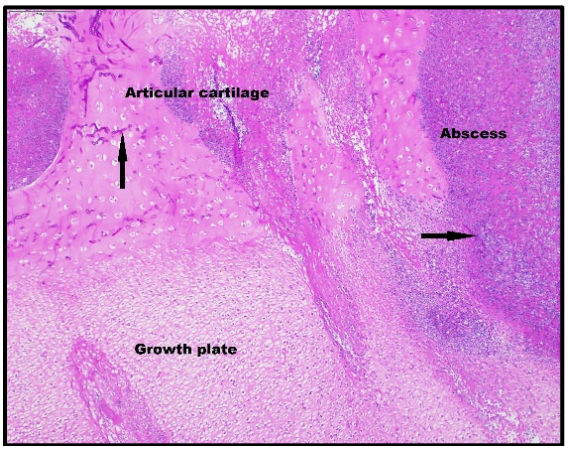

Figure 5. Fungal organisms (arrows). PAS.

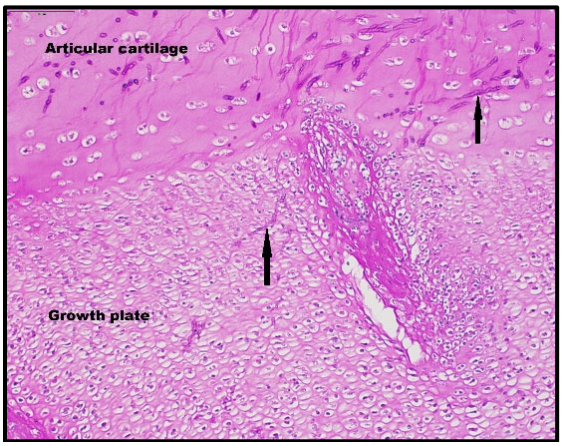

Figure 6. Fungal hyphae (arrows). PAS.

The threat of histomoniasis: turkeys and chickens cannot be housed together!

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AHL Newsletter 2021;25(3):21.

Histomonas meleagridis, the causative agent of Blackhead disease (histomoniasis), is a protozoan parasite with a complex life cycle. Avian species that are susceptible to natural infection include, in order of susceptibility: turkeys, pea fowl, guinea fowl, chicken, chukar, pheasant, and bobwhite quail. *Histomonas* is a fragile organism and can only last minutes to hours in the environment, therefore, an intermediate host is needed for it to survive. Direct transmission is thought to be possible only in turkeys, by direct contact or 'cloacal drinking'. Ducks can also be asymptomatic carriers of *Histomona*s.

Intermediate hosts for *Histomonas* include the cecal roundworm, *Heterakis gallinarum*, as well as earthworms. *Heterakis* eggs are resistant to commonly-used disinfectants and can live in the soil for months to years, harbouring *Histomonas*. Mechanical transmission of *Heterakis* eggs is also possible. The bird hosts for *Heterakis gallinarum* that are most likely to contaminate soil are pheasants, guinea fowl and chickens.

Lesions of histomoniasis are located primarily in the ceca. Liver lesions are common in turkeys but not in other birds. Protozoa may also migrate through the bloodstream to other organs such as spleen, kidney, and pancreas.

Prevention of histomoniasis is based on three principles:

1) controlling *Heterakis gallinarum*;
2) separation of susceptible avian species from birds that are reservoirs of *Histomonas meleagridis* and/or *Heterakis gallinarum*;
3) stringent biosecurity measures.

Please refer to '**AHL LabNote 54 – Blackhead (histomoniasis) in small turkey flocks**' for more information and a list of preventative measures to take for this disease:

https://www.uoguelph.ca/ahl/ahl-labnote-54-blackhead-histomoniasis-small-turkey-flocks *AHL*

Reference

Abdul-Aziz T, McDougald LR, Barnes HJ. Histomoniasis slide study set #33, AAAP Conference; 2012 Aug 4-7; San Diego, California.

Pneumocystis pneumonia in a Thoroughbred colt

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AHL Newsletter 2021;25(3):22.

A 60-day-old Thoroughbred colt presented with a history of pneumonia, pyrexia, and swollen legs. A complete blood count (CBC) revealed evidence of a mild left shift with mild neutrophil toxicity characterized by cytoplasmic basophilia and occasional Doehle bodies. Inflammation was further supported by a markedly increased serum amyloid A concentration of 4721 mg/L (reference interval 0-20 mg/L).

Concentrated slide preparations of bronchoalveolar lavage fluid (BAL) from this colt were evaluated. The slides contained scant mucus in which the majority of sample material was entrapped. This material included numerous clusters of clear to slightly basophilic, round, variably intact cystic structures which were occasionally found to contain internal evidence of four to eight, round, deeply basophilic, peripherally located intra-cystic bodies. These structures were consistent with *Pneumocystis jirovecci* (*P.cariini*) cysts. Concurrent inflammation consisting of a predominance of macrophages was also identified (**Fig. 1**).

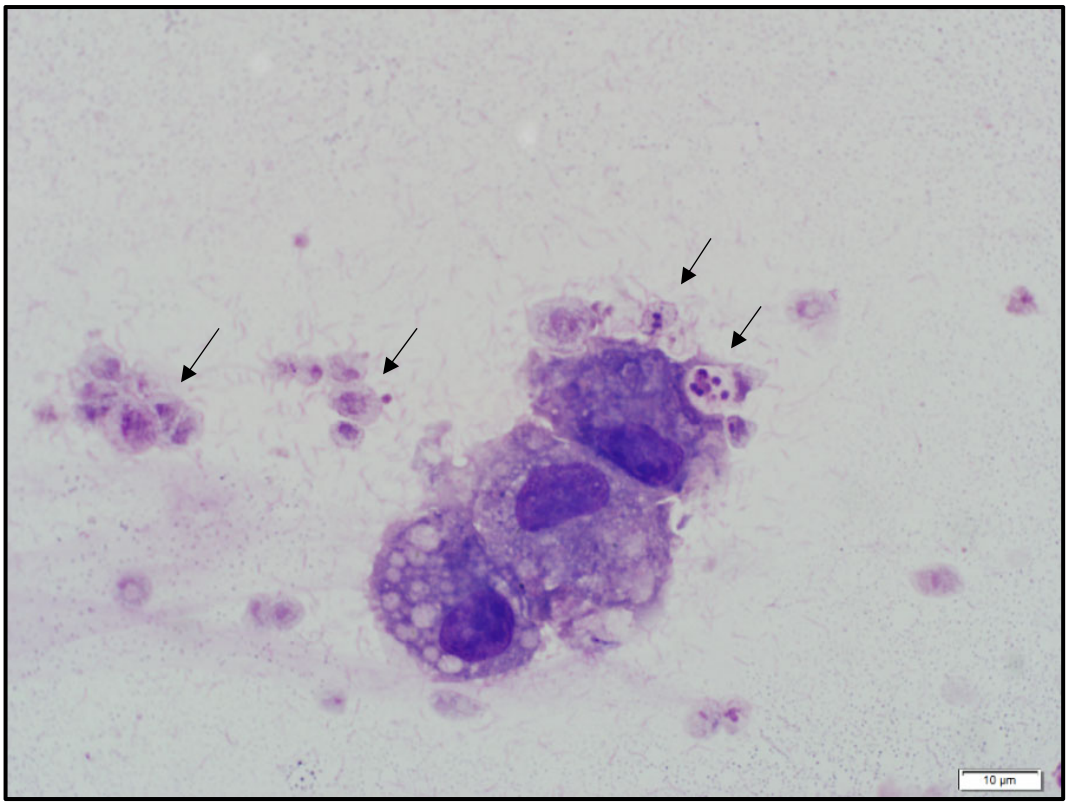

Figure 1. Three macrophages and associated, variably intact *P.jirovecci* cysts containing intra-cystic bodies (arrows). Wright's stain. Image credit Dr. Janet Beeler-Marfisi.

Pneumocystis is an opportunistic extracellular fungus that can be seen as a thin-walled trophozoite-like form with a single nucleus, as well as a thick-walled, cyst-like organism with multiple inner bodies. The procyst is considered either a product or a subtype of the trophozoite stage.

Pneumocystis pneumonia has been documented in several species, including: humans, horses, foals, goats, pigs and dogs. Historically, disease has been thought to be a problem primarily in immunocompromised hosts. However, in addition to SCID (severe combined immunodeficiency) Arabian foals and foals concurrently infected with other bacterial and viral pulmonary pathogens, immunocompetent foals from 4 months to one year of age and adult horses have also been diagnosed with pneumonia due to *Pneumocystis*. Recent evidence supports that *Pneumocystis* may be a component of the normal upper respiratory flora of immunocompetent animals, and humans are typically exposed to *P.jirovecci* early in childhood with primary infections ranging from asymptomatic to mild upper respiratory signs in immunocompetent individuals.

Pneumocystis infection in horses is associated with two disease conditions: fulminant acute infections, and secondary infections with pulmonary fibrosis (pneumocystosis). Localization of *Pneumocystis* to the alveoli results in accumulation of proteinaceous fluid which impairs oxygenation. In equine infections, BAL is essential for detection of fungal elements as this organism cannot be grown in culture. PCR testing is also possible, but not widely available.

The identification of *Pneumocystis* infection should prompt investigation of the immune status of the horse. *Pneumocystis* infections in immunocompetent humans and horses with normal numbers of CD4+ lymphocytes have been documented, but the requirement for CD4+ cells for protection against disease has also been demonstrated in murine models and in humans with acquired immunodeficiency syndrome.

Flow cytometry of this colt's peripheral leukocytes revealed a decreased proportion of CD8+ lymphocytes relative to adult values, but CD4+ lymphocyte proportions were unremarkable. Evaluation of serum IgG revealed a decreased value of 637 mg/dl (reference interval for adult horses 984-1685 mg/dl, and the expected value for foals having received adequate colostrum is 800 mg/dl or greater).

The colt was treated with a systemic antibiotics and an anti-inflammatory drug, and was provided supportive care. It is clinically well three months following initial diagnosis and treatment. It is unknown if the immunodeficiency suggested by the decreased IgG concentration was transient or persistent, as follow-up testing has not been possible to date. *AHL*

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Zakrzewska M, et al. *Pneumocystis* pneumonia: Still a serious disease in children. Dev Period Med 2019;3:159-162.
Sellon D, Long M, Kohn C. Miscellaneous fungal diseases. In: Equine Infectious Diseases, 2nd ed. Sellon D and Long M, eds. Elsevier, 2014:446-448.

Canine parvovirus 2 antigen ELISA: Interpretation of negative test results

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AHL Newsletter 2021;25(3):24.

Fecal testing for Canine parvovirus 2 (CPV-2) using an antigen detection enzyme-linked immunosorbent assay (ELISA) is a useful rapid test modality for investigating the possibility of parvovirus infection in the clinical setting. However, confusion may arise when CPV-2 antigen is not detected by the ELISA in feces from animals exhibiting classic clinical signs, or typical lesions at postmortem.

The most likely cause for a false-negative fecal antigen ELISA is the presence of endogenous plasma antibodies which may develop as rapidly as day 3 following infection. Significant viral-mediated damage to proliferating intestinal crypt epithelium disrupts the gut mucosal barrier by 6-9 days post-infection, allowing leakage of plasma antibodies. If present in sufficient concentration to bind and neutralize remaining virus in the intestinal lumen, these antibodies may result in false-negative test results in the antigen ELISA. Parvovirus can however persist inside the surviving intact mucosal epithelial cells, and may remain detectable by immunohistochemistry staining (**Fig. 1**) or by PCR testing, and may continue to be shed in feces.

This reinforces the importance of considering history, signalment and clinical signs in conjunction with results of laboratory testing when diagnosing infectious diseases. A positive fecal antigen ELISA result is helpful to confirm CPV-2 infection; however, a negative result does not exclude CPV-2, particularly if clinical signs are consistent with parvoviral enteritis.

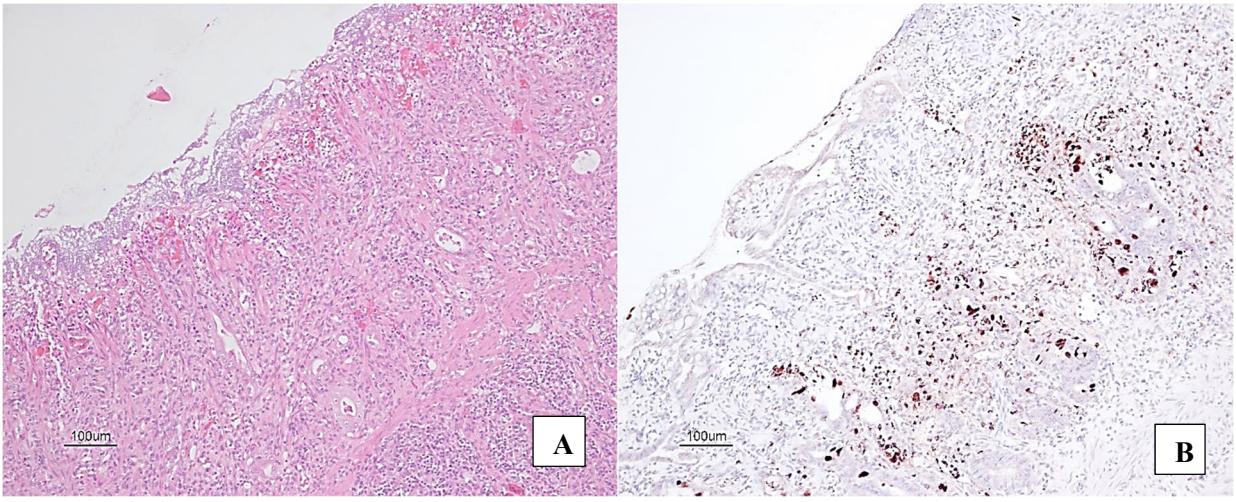

Figure 1: Histology (A; H&E) and parvovirus immunohistochemistry (B; Nova Red) of the small intestine of a 13-week-old puppy that **tested negative on two sequential CPV-2 antigen ELISA tests**, demonstrating late-stage infection with widespread crypt epithelial necrosis and complete collapse of intestinal villi (A). Note positive immunostaining for canine parvovirus within surviving crypt epithelium (B).