Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

AHL Newsletter, Volume 24, Number 3 **September 2020**

In this issue:

AHL Newsletter
September 2020 - Volume 24, Number 3 ISSN 1481-7179
Editor: **Maria Spinato,** DVM, DVSc, MBA, Diplomate ACVP
Editorial Assistants: **Helen Oliver, Kate Artuso**

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.*

Mailing address & contact information:
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

To receive an **electronic copy of this Newsletter**, please send your email address to: holiver@uoguelph.ca

Update from the Director

The view from the Director's office

As fall and the back-to-school period approaches, life is shifting into an altered homeostasis dictated by COVID-19. People and organizations are still learning how to modify plans and processes, based upon our most up-to-date understanding of how to prevent the spread of SARS-CoV-2. At the AHL, we are fortunate in being able to work in a well-equipped facility with sufficient PPE supplies available to protect staff. Masks are worn by all staff members in the laboratory, as mandated by the Wellington-Dufferin-Guelph Public Health unit. Since the current level of COVID-19 infection in Ontario is low, AHL laboratory sections have resumed normal work hours rather than the split teams approach employed during the early weeks of the pandemic. In addition, the AHL Virology section has restarted virus neutralization (VN) testing.

Similar to many of you, we are cautiously optimistic about the current stage of recovery and re-opening in Ontario. Laboratory submissions that were reduced during the early stage of the pandemic because of restricted veterinary activities have recovered to normal levels over the summer months. These include diagnostic and surveillance submissions from veterinary clinics, in addition to research samples from OVC, other University of Guelph departments and agricultural industries. We thank you for your patience over the past 6 months as AHL modified business operations to adjust to fluctuating requirements for maintaining a safe working environment for laboratory staff, supply chain disruptions and inconsistent courier deliveries.

Check out the improved mobile functionality of our LIMS (laboratory information management system) at https://sapphire.lsd.uoguelph.ca:8443/labservices/logon.jsp. The pages will adapt to the screen size on whichever tablet or mobile device you choose to use. When your web browser contacts our secured website, it is now an encrypted connection. You will see a padlock beside the link to indicate all communication sent from your browser to our server is secure. You will be able to access test results in addition to information about every test AHL performs in a user-friendly format while on the road. Thanks to our hard-working IT staff for completing this important initiative!

We wish you, your staff, and families continued health and safety.

Maria Spinato, Director

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

AHL Guelph Specimen Reception update

Jim Fairles

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

AHL Newsletter 2020;24(3):3.

1. Since the spring, AHL Guelph Specimen Reception has been closed on Sundays and holidays. A new automatic door was installed in June and since then, submission drop offs are available from 7AM to 8PM every day. Beginning September 6th (and including the holiday Monday September 7th), AHL Guelph Specimen Reception will be staffed and open for receiving from 9AM to 5PM on Sundays and statutory holidays (excluding Christmas Day). Regular hours remain the same: 8AM to 6PM Monday to Friday and 9AM to 5PM Saturday.

2. As we move into the new "normal", we are still occasionally experiencing Purolator courier delays. We have no control over these delays, and express courier service is also not guaranteed. When you can, please make sure you are shipping early in the week with appropriate cold chain provisions so that an extra day in transit will not impact the quality of submissions.

Please visit our website for any updates in service: https://www.uoguelph.ca/ahl/

Please direct any questions to ahlinfo@uoguelph.ca or 519-824-4120 ext. 54530.

Stay safe!

AHL postmortem room floor renovation successfully completed!

Andrew Brooks

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

AHL Newsletter 2020;24(3):3.

We wish to thank all AHL clients for their patience and understanding during our recent renovation of the postmortem room. From June 19 to July 1 2020, the entire postmortem room flooring (approximately 7500 sq. ft.) was replaced with a new epoxy-based surface. This important renovation was necessary to address deterioration of the initial floor that was installed during building construction. We also sincerely thank Mary Fowler (Campus Animal Facilities, University of Guelph) and Tom Smith (Atwood Resources Inc.) for kindly allowing us to perform emergency postmortems at their facilities.

Ontario rolls out CanSpot ASF enhanced surveillance pilot

Christa Arsenault

Ontario Ministry of Agriculture and Rural Affairs, Guelph, ON.

AHL Newsletter 2020;24(3):4.

Canada has a comprehensive surveillance program in place for the early detection of foreign animal disease such as African Swine Fever (ASF) virus. The newly launched CanSpot ASF surveillance pilot will enhance this surveillance with several tools in a phased approach. The goal of ASF surveillance is to protect the Canadian commercial swine sector from the adverse effects of ASF on production and trade. The primary objective of surveillance in the domestic swine population is to enhance our ability to detect ASF rapidly should it enter Canada. A secondary objective is to support the claim that the domestic pig population is **NOT** infected with ASF.

The first tool being launched as part of the pilot project is the "risk-based early detection testing" with the goal to improve pathogen detection. This pilot is similar to surveillance recently launched in the USA. The pilot project is aimed at enhancing the diagnostic testing of ASF in Canada. The Canadian Animal Health Surveillance Network (CAHSN) laboratories will run routine diagnostic rule-out tests where ASF is **NOT** suspected. Certain diseases/conditions may mask the clinical signs of ASF and delay detection. Herds with a history of these diseases/conditions, or cases with a compatible clinical or pathological presentation are eligible for testing.

There is no change in the protocol for cases where ASF is suspected and these must be immediately reported to CFIA.

The CanSpot ASF will add other surveillance tools such as risk-based surveillance from abattoirs, increased veterinary presence and sampling on small-scale swine farms, and samples from wild pigs.

Both herd practitioners and pathologists can initiate this new ASF rule-out testing. Any case submitted for pathology to the Animal Health Lab in Guelph may be tested for ASF if the following criteria are met:

- sufficient information to trace the animal (Premises ID or animal location); and
- appropriate tissues are submitted and include some or all of the following: whole blood, body fluids and sections of tonsil, spleen, kidney, lymph node and terminal ileum.

What happens if the rule-out testing (real-time PCR) is **NEGATIVE**?

- If the sample/animal is ASF-negative, the laboratory will report this result as per usual protocols. The diagnostic test result will be included in the CanSpot ASF reporting (pilot period or annual reporting).
- No movement restrictions will be placed on the farm.

What happens if the rule-out testing (real-time PCR) is **POSITIVE OR SUSPECT**?

- The sample/animal yielded a positive or suspicious test result, but without further confirmatory testing and epidemiologic investigation, the true status of this case is **UNKNOWN**. This result could be a false positive result or a true case of ASF. The ASF PCR test is an excellent test so these results will be taken seriously.
- The approved laboratory will immediately inform the herd veterinarian and the local CFIA district office where the herd is located. If the approved laboratory is conducting the test for another laboratory, they will immediately notify that laboratory and the original laboratory will be responsible for notifications.

• The local CFIA district office will:

✓check the health of the animals on the premises together with the herd veterinarian;
✓may collect additional samples from pigs on the premises and coordinate collection and shipment of samples to the NCFAD in Winnipeg; and
✓complete a risk assessment. If the risk assessment finds no evidence of ASF, the CFIA will place a quarantine to stop movement of swine off the premises until the NCFAD confirmatory testing is completed (estimated 48 to 96 hours). If the risk assessment finds a suspicion of ASF, the CFIA will place a quarantine to stop movement of swine, and may make a declaration of infected place to stop other traffic on and off the premises until the NCFAD confirmatory testing is completed (estimated 48 to 96 hours).

Clinicopathological presentations eligible for additional ASF testing at approved laboratories include:

1. Septicemia and/or multiorgan hemorrhage such as caused by *E. rhusiopathiae*; *S. suis*; *S. zooepidemicus; A. suis*; *S. cholerasuis*; other bacteria
2. Porcine Reproductive and Respiratory Syndrome virus (PRRS), especially when it causes cyanotic skin
3. Porcine Dermatitis and Nephropathy Syndrome (PDNS) and vasculitis that can be caused by PCV 2, PCV 3 or other pathogens
4. Hemorrhagic diarrhea / necrotizing enterocolitis such as caused by *Salmonella* spp; *L. intracellularis; B. hyodysenteriae*; *B. hampsonii*
5. Fibrinous pleuritis / pericarditis / hydropericardium such as caused by *H. parasuis*; *S. suis*
6. Mulberry heart disease
7. Splenic torsion
8. Abortion above historical trend for herd
9. Mortality above historical trend for herd

This pilot on risk-based early detection at approved laboratories is expected to run from July 2020 to June 2021. Reporting and evaluation for the pilot will be completed at the 6 and 12-month time points. Both the 6-and 12-month reports will be provided as part of an annual report for the CanSpot ASF program.

Initial reporting for stakeholders is anticipated to include the number of participating laboratories; the number of cases tested; summarized test results; and where possible, the number of eligible cases and the number of premises included in testing by province or region and time.

Reference

Canadian Food Inspection Agency. CanSpotASF technical description: Enhanced surveillance activities that aim to protect the commercial swine sector from the impacts of African Swine Fever. Ottawa, ON, 2020.

OAHN update – September 2020

Sabrina Di Ilio and Melanie Barham

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

AHL Newsletter 2020;24(3):6.

The Ontario Animal Health Network has been busy throughout the summer releasing new info sheets, reports, and a new website. Read on to find links and descriptions of what we have been working on.

Value of a Postmortem for your Goat Herd

The OAHN Small Ruminant Network created a new video: The Value of a Postmortem for Your Goat Herd. This whiteboard video is directed at producers and outlines the value of performing a postmortem on goats that die unexpectedly in order to keep their herd healthy.

Bacterial Kidney Disease infosheet

The OAHN Fish Network created an infosheet on Bacterial Kidney Disease in Ontario for producers in response to the influx in BKD detections.

Small Ruminant infosheets

The Small Ruminant Network has been creating new infosheets based off of topics reported on in previous quarterly updates. The infosheets released so far are: Guide to Safe Handling and Sheep and Goat Placentas, Toxoplasmosis, Maedi Visna, Fecal Egg Counts: Tips and Tricks, and Campylobacter Abortions.

OAHN Website Update

Take a look at the newly updated OAHN website at **OAHN.ca**! It features more intuitive navigation and improved organization of OAHN network materials. All new reports are also searchable and mobile-friendly so you can find and send them easily.

New Reports and Resources

The latest network reports for companion animals, bovine, swine, poultry, and equine have been posted to the OAHN site under "Network Reports". OAHN released an Ontario Animal Health Annual Update in both English and French versions that outline research and highlights of 2019.

We have lots of other **new reports, lab data, and resources**. Be sure to check out **OAHN.ca**

Campylobacter spp. abortion in ruminants

Murray Hazlett, Đurđa Slavić

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

AHL Newsletter 2020;24(3):7.

Campylobacter spp. abortions in ruminants are relatively common in sheep, and sporadic in cattle and goats. Since 2007, a total of 105 pathology abortion submissions were diagnosed as caused by *Campylobacter* spp. (**Table 1**), 91 of these being in sheep, 7 in goats and 7 in cattle (also 1 canine). In sheep, 20 (22%) of *Campylobacter* spp. abortions were identified in conjunction with other pathogens, most commonly *Coxiella burnetii* (n=17), but also *Chlamydia abortus* and other miscellaneous pathogens including *Histophilus somni, Trueperella pyogenes* and BVDV. Concurrent infections were not identified in goats or cattle; however, case numbers were relatively small.

Campylobacter jejuni and *Campylobacter coli* are leading causes of enteritis in humans and are associated with late-term abortions in sheep. Both are commonly found in the gastrointestinal tract of sheep, and there is some evidence that certain tetracycline-resistant clones are often associated with sheep abortion in the USA. A tetracycline-resistant clone has been identified in Ontario. The use of tetracyclines in the feed or to mass medicate the ewe flock for the control of infectious abortion (e.g., if abortion due to *Chlamydia abortus* is suspected) may actually increase the incidence of tetracycline-resistant *C. jejuni* abortions. For this reason, diagnostic investigation of ovine abortions should always be pursued to confirm the etiology and to implement appropriate control measures.

Similarly, *Campylobacter fetus* subsp. *fetus* is also a zoonotic disease, and is commensal in a wide range of animals, including sheep. *AHL*

Table 1. AHL Pathology submissions with a diagnosis of *Campylobacter* spp. Abortion, 2007-2020.

Species	*C. coli*	*C. jejuni*	*C. fetus fetus*	Total Campylo-bacter	Total abortions	% Campylo-bacter
Ovine	3	46	42	91	**480**	**18.96%**
Caprine	0	7	0	7	**329**	**2.13%**
Bovine	0	4	3	7	**1275**	**0.55%**

References

1. Ertas HB et al. Isolation of *Campylobacter jejuni* and *Campylobacter coli* from the gall bladder samples of sheep and identification by polymerase chain reaction. J Vet Med B, 2003:50; 294–297 (2003)
2. Slavić D et al. Tetracycline susceptibility of *Campylobacter* isolates causing ovine abortions in Ontario. AHL Newsletter, Volume 16, Number 2, June 2012.

Investigation of the increase of swine erysipelas in Ontario (OAHN 009115)

Đurđa Slavić, Daniel Bogema, Tim Pasma, Narelle Sales, Ian Marsh, Sue Burlatschenko and Josepha DeLay

Animal Health Laboratory, University of Guelph, Guelph, ON (Slavić, DeLay); Elizabeth Macarthur Agricultural Institute, New South Wales, Australia (Bogema, Marsh, Sales); Goshen Ridge Veterinary Services, Tillsonburg, ON (Burlatschenko), Ontario Ministry of Agriculture and Rural Affairs (Pasma)

AHL Newsletter 2020;24(3):8.

Since the fall of 2015, the OAHN Swine Network has noted an increase in activity of swine erysipelas, based on data from practitioners completing the quarterly survey and from provincial and federal slaughter plants. The purpose of this study was to isolate and, using whole genome sequencing (WGS), to characterize isolates of swine erysipelas from abattoirs and swine farms in Ontario. During 2019, tissue samples (e.g., lung and spleen) collected from hogs in slaughterhouses and clinical cases in Ontario with lesions suspicious for swine erysipelas were submitted to Animal Health Laboratory (AHL) for culture. In total, 8 cases were received comprised of 25 samples. Eleven samples were collected from clinical cases whereas 14 samples were from slaughterhouses.

Only 6 isolates of *Erysipelothrix rhusiopathiae* were recovered from the samples submitted (3 from the clinical cases), even though enrichment method was used for culturing. This low recovery of isolates can potentially be explained by previous antimicrobial treatment, chronic course of the disease or possibility of other septic causes with cutaneous involvement resembling *E. rhusiopathiae*. To compensate for low number of isolates recovered, *E. rhusiopathiae* isolates conveniently archived by the AHL (5) and Gallant Custom Laboratories (3) were also included in the study, bringing the total number of isolates for sequencing to 14. Based on limited data available for each isolate, it is likely that isolates originated from 11 different premises; however, this cannot be confirmed. Most of the sequenced isolates were archived isolates recovered in 2015 (1), 2016 (2) and 2018 (5).

Isolates were sequenced using Illumina MiSeq and whole genome sequence data were used to detect the resistance genes, virulence genes and to establish multi locus sequence types (MLST) of Ontario isolates. The MLST was done by analyzing 7 housekeeping genes. As no *E. rhusiopathiae* MLST scheme is available in a public MLST database, sequence types (ST) were randomly assigned to each isolate, indicating that there are 8 different MLSTs present among 14 isolates. When comparing MLST of Ontario isolates to a MLST scheme developed by an Australian research group, it was determined that only 2 of Ontario isolates belong to the existing ST4. All other STs were new and were assigned ST from 110 to 116 (**Fig. 1**).

The presence of antimicrobial resistance genes was not detected in all Ontario isolates, but when detected, resistance to tetracyclines and lincosamides was consistently present. In addition, most of the isolates sequenced in this study had all putative virulence genes as defined previously.

Because of the limited number of isolates, all data presented here are considered preliminary and more work is needed before any solid conclusions can be drawn. Our data indicate that isolates from the same premises are likely uniform as they have the same MLST, AMR and virulence genes patterns. Similar to other studies, a significant diversity in MLST was observed for Ontario isolates with the newly detected MLSTs. However, there are some indications that, in general, isolates from the same geographical

locations tend to cluster together (**Fig. 1**). To confirm this hypothesis, more isolates from Ontario need to be sequenced and included in the MLST database in order to monitor their epidemiological relatedness. From the clinical perspective, there is currently no correlation between a specific MLST and virulence potential of the isolate. *AHL*

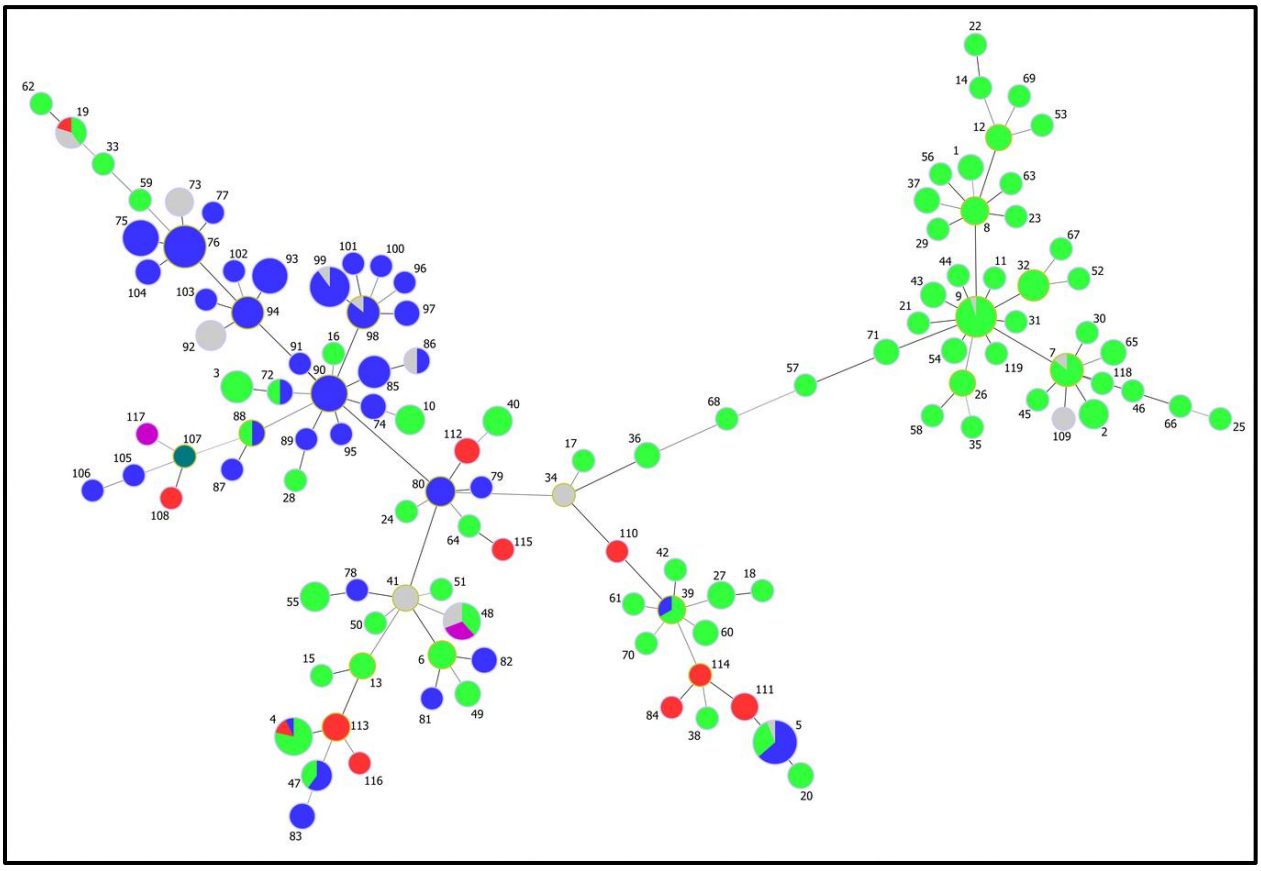

Figure 1. The full MLST location image is a complete minimum spanning tree of all the isolates, with node colours representing continents (Europe - Green; Australia - Blue; North America - Red; Asia - Purple; South America - Teal; NA - Grey). Ontario isolates belongs to ST4, ST110, ST111, ST112, ST113, ST114, ST115, and ST116. Courtesy of D. Bogema, Elizabeth Macarthur Agricultural Institute, NSW.

References

1. Janßen T et al. A combinational approach of multilocus sequence typing and other molecular typing methods in unravelling the epidemiology of *Erysipelothrix rhushiopathiae* strains from poultry and mammals. Vet Res 2015; DOI 10.1186/s13567-015-0216-x.
2. Ogava Y et al. The genome of *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas, reveals new insights into the evolution of Firmicutes, and the organism's intracellular adaptations. J Bact 2011;193**;**2959-2971.
3. Sales N et al. Innovation Grant: 2A-117 – *Erysipelothrix rhusiopathiae* Epi-interface, a new approach to the management of porcine erysipelas. 2018.

OAHN swine small-scale herd postmortem project is underway

Josepha DeLay, Tim Pasma

Animal Health Laboratory, University of Guelph, Guelph, ON (DeLay); Ontario Ministry of Agriculture and Rural Affairs (Pasma)

AHL Newsletter 2020;24(3):10.

The Ontario Animal Health Network (OAHN) is funding a new study at the Animal Health Laboratory (AHL) to identify disease issues in small-scale swine herds in Ontario. The project will also facilitate connection and communication between veterinarians and small-scale producers.

The project funds postmortems (PM) on pigs that die or are euthanized due to disease, and for which the herd meets the qualifying criteria for the project (see below). PM samples will be tested for a variety of diseases depending on the presenting complaint and the age of the animals. All animals will be tested for PRRSV and influenza A virus. Test results will be reported to the herd veterinarian who will communicate these findings to the producer.

The costs of PM at the AHL and all diagnostic tests will be covered by the project, at no charge to the client. For cases with on-farm PM, veterinarians will receive a subsidy for conducting the PM, and all diagnostic test costs will similarly be covered by the project. Importantly, on-farm PMs must follow the project sampling protocol specific for each age group and disease syndrome (see links to submission forms and sampling guides at https://www.uoguelph.ca/ahl/oahn-swine-small-scale-herd-postmortem-project-may-2020).

Summary of herd requirements for participation in the project:

- The swine herd is located in Ontario and has ≤50 sows, or markets ≤1000 hogs per year.
- The herd has a Premises Identification Number (PID).
- The producer completes and submits a herd management survey (included with submission form).
- The herd veterinarian has enrolled the herd / case in the project.

For more information, to enroll a small herd in the project, and to receive a sampling kit for field PMs, veterinarians may contact Dr. Josepha DeLay at the AHL (jdelay@uoguelph.ca or 519-824-4120 ext. 54576). *AHL*

Necrohemorrhagic tracheitis in swine – New project supported by SHIC

Josepha DeLay, Tim Blackwell

Animal Health Laboratory, University of Guelph, Guelph, ON (DeLay); Ontario Ministry of Agriculture, Food and Rural Affairs (Blackwell).

AHL Newsletter 2020:24(3):10.

Severe tracheitis is seen sporadically in Ontario swine and can cause significant morbidity with typically low mortality. No etiologic agent has been definitively or consistently associated with the syndrome. It is possible that either a novel infectious agent or a novel strain of a known infectious agent is responsible for severe tracheitis. Thorough diagnostic workups of tracheitis cases are hampered by sampling deficits (trachea not included for various diagnostic tests) and the sporadic nature of the condition.

The Swine Health Information Center (SHIC) is working together with the Animal Health Laboratory, University of Guelph, the Iowa State University Veterinary Diagnostic Laboratory and the Laboratoire de Santé Animale, MAPAQ to support consistent testing of severe tracheitis cases. SHIC funding will supplement routine respiratory disease diagnostic workup on applicable cases. A tissue bank will also be established from these cases for further investigation of novel pathogens.

The case definition for participation in the project is as follows: growing pigs (including breeding stock), within an age range of 14-30 weeks and a weight range of 70-160 kg that develop an acute onset of a 'honking' cough, progressing to dyspnea (affecting a low % of animals). Necropsied animals feature edema and hemorrhage within tracheal submucosa, resulting in marked luminal impingement (**Fig. 1**).

The minimum sample required for testing is an intact pluck, including trachea from larynx to bifurcation, and both lungs. Additional samples include bilateral caudal deep cervical and costoaxillary lymph nodes, spleen, stomach, ileum and heart.

Link to SHIC project description, with a full list of desired samples:
https://www.swinehealth.org/hemorrhagic-tracheitis-standardized-submissions-to-help-find-etiology-supported-by-shic/

Link to hemorrhagic tracheitis webinar (April 2020):
https://iastate.app.box.com/s/lvwkqcenddb29pusgy3sa7f6xo75zn4f

For more information or to participate in the project, contact Dr. Josepha DeLay, AHL: 519-824-4120 ext. 54576 or jdelay@uoguelph.ca. For assistance with on-farm sampling, please contact Dr. Tim Blackwell, OMAFRA: 519-820-2680 or tim.blackwell@ontario.ca. *AHL*

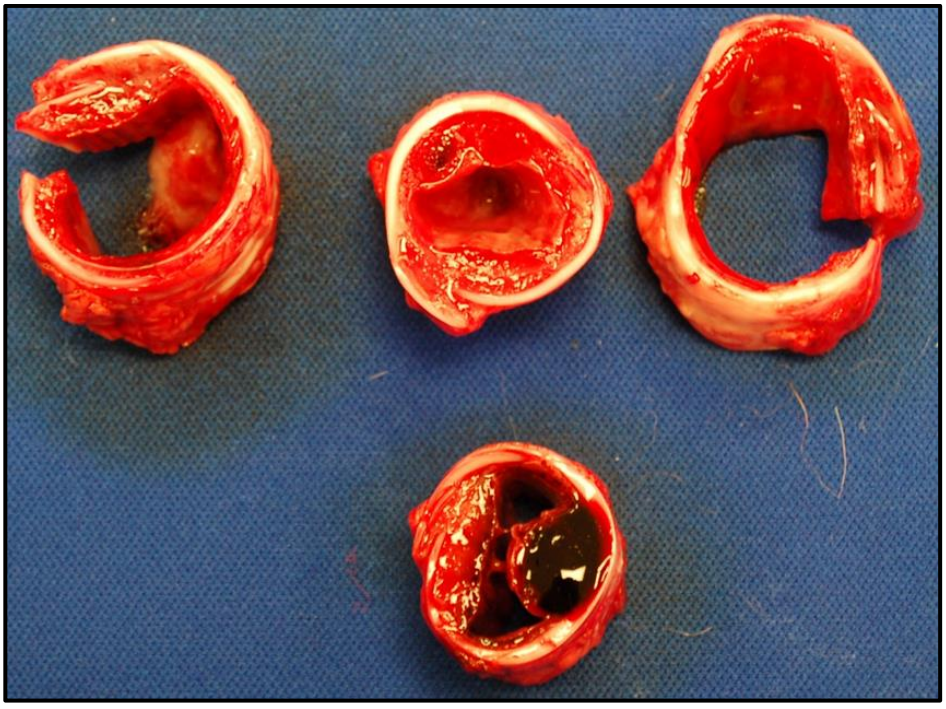

Figure 1. Tracheal cross-sections from multiple finisher pigs with necrohemorrhagic tracheitis. Note severe submucosal hemorrhage and edema, with resulting reduction in tracheal luminal area.

Eimeria necatrix in chickens

Emily Martin

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

AHL Newsletter 2020;24(3):12.

Over the past few months, we have identified a number of cases of *E. necatrix* by histopathologic examination. The tissue damage caused by this organism is impressive and warrants a review of this infection. Chickens infected by *Eimeria necatrix* can have bloody droppings that contain fluid and mucus. The birds will appear depressed, lose weight, the flock can become uneven, and mortality can be greater than 25 percent. Egg layers can have decreased egg laying potential and can lose pigmentation.

On postmortem examination, the small intestines are markedly dilated, thickened, congested and white to yellow foci or plaques and petechial hemorrhages may be present over the serosal surface. In dead birds, these serosal lesions can appear white and black, leading to the description of a 'salt and pepper' appearance. When the intestines are opened, the lumen is often filled with fluid, blood and necrotic debris. The mucosa may be markedly thickened, irregular and dark brown due to severe confluent necrosis. *Eimeria necatrix* is found primarily in the jejunum, but can extend into the duodenum and ileum in severe cases.

Eimeria necatrix does not easily reproduce so it does not compete well with other coccidia. This low ability to reproduce results in this organism taking a long time to propagate. Consequently, older birds such as breeder or layer pullets 9-14 weeks of age are most commonly affected. In the life cycle of *Eimeria necatrix*, the asexual cycles occur in the small intestines and the sexual cycles occur in the ceca. As a result, the oocysts can only be found in the ceca. Since oocyst production is also poor, birds often die before oocysts are present in the feces.

The reason for *Eimeria necatrix* causing extensive tissue destruction can be observed histologically as the schizonts penetrate deep into the mucosa and submucosa and destroy blood vessels and smooth muscle (**Fig. 1**). This makes epithelial regeneration difficult and if birds survive, areas of scar tissue can develop in the intestine.

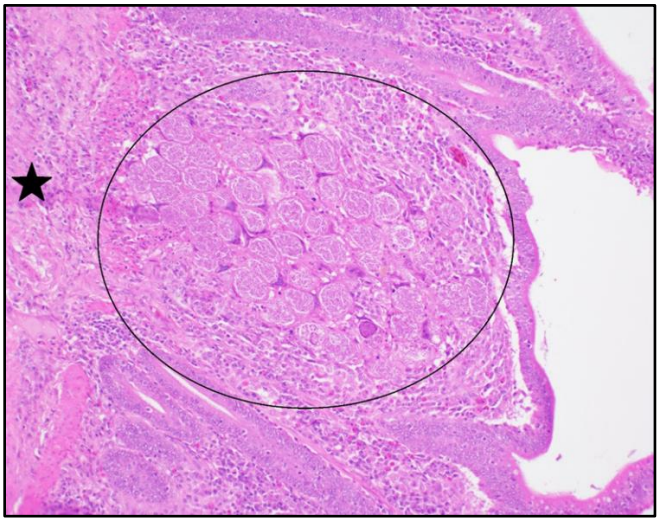

Figure 1. Large schizonts of *Eimeria necatrix* (circle) in deep lamina propria of jejunum. Tunica muscularis (star). (20x) (H&E)

Eimeria necatrix is considered one of the most pathogenic *Eimeria* species as it only requires 10^4 - 10^5 sporulated oocysts for infection. In older birds with intestinal signs and lesions, this infection should be on the differential diagnosis list. *AHL*

References
1. Cervantes HM, et al. Coccidiosis. In: Diseases of Poultry, 14th ed. Swayne DE, ed. Wiley Blackwell, 2020; vol 2:1193-1217.
2. Fitz-Coy SH. Parasitic Diseases. In: Avian Disease Manual, 7th ed. AAAP, 2013:153-178.

Bacterial gill disease in a research colony of lake whitefish

Heindrich Snyman, Marcia Chiasson, Calvin Kellendonk, Patricia Bell-Rogers, Qiumei You, Lisa Ledger, Jason Eidt, Nathan Bennoit, Hugh Cai

Animal Health Laboratory, University of Guelph, Guelph, ON (Snyman, Kellendonk, Bell-Rogers, You, Ledger, Eidt, Bennoit, Cai); Alma Aquaculture Research Station, University of Guelph, Elora, ON (Chiasson).

AHL Newsletter 2020;24(3):13.

A research colony of lake whitefish (*Coregonus clupeaformis*) held in flow-through tanks at 8.5°C were experiencing a sudden increase in daily mortality, losing ~ 93 fish out of a population of 574 over a 5 day period (~ 16% cumulative mortality rate). Fish were exhibiting flared gills with foci of hemorrhage along the gill base and occasional fin erosions. Dissolved oxygen was ~ 7.0 mg/L, which is within an acceptable range for salmonid fishes. Feed was withheld and aeration was increased in an effort to alleviate the respiratory distress. Although this did slow the mortality rate, fish were still exhibiting respiratory distress and a subset of four fish were submitted fresh dead to the Animal Health Laboratory for analysis.

Fish ranged from 28- 35 cm in length and all fish contained flared opercula and diffusely congested gill arches. There were scattered hemorrhages throughout the gill filaments, along the base of the gill arches and the pectoral fins, and two of the fish contained intra-ocular hemorrhage. Gill wet mounts of all four fish revealed widespread lamellar hyperplasia with interspersed dense meshed mats of filamentous bacteria (**Fig. 1**). Tissues were collected for histopathology and bacterial culture, and acute viral hemorrhagic septicemia (VHS) was ruled out by PCR testing.

Histologically, there was widespread lamellar epithelial hyperplasia that extended along the entire length of the filaments, often filling the interlamellar spaces and resulting in lamellar fusion (**Fig. 2**). Filling the remaining interlamellar spaces and lining the hyperplastic epithelium were scattered dense mats of long slender filamentous bacteria (**Fig. 3**). Aerobic culture of gill surface swabs yielded *Flavobacterium branchiophilum*, which together with the histological changes were consistent with a diagnosis of bacterial gill disease (BGD).

BGD is caused by the gram negative, non-motile, slender filamentous bacterium *Flavobacterium branchiophilum*. The disease is typically seen in young intensively reared salmonids and is considered one of the most significant infectious diseases affecting freshwater salmonid aquaculture worldwide. Rainbow trout aquaculture represents the majority of these cases; however, all Salmonidae are theoretically susceptible. Despite rearing lake whitefish for four years and having typical historic outbreaks of BGD in rainbow trout, this is the first time this disease has been identified in lake whitefish in this facility and at the AHL. The affected tank of lake whitefish was effectively treated with a 1-hour static bath of 10 ppm Chloramine-T on three occasions with one day in between treatments. *AHL*

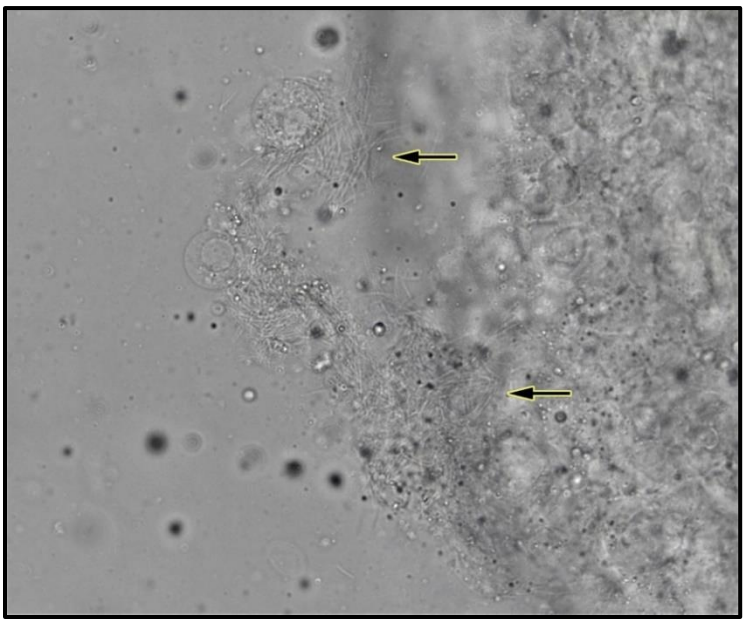

Figure 1. Gill wet mount with meshed mats of filamentous bacteria (arrows).

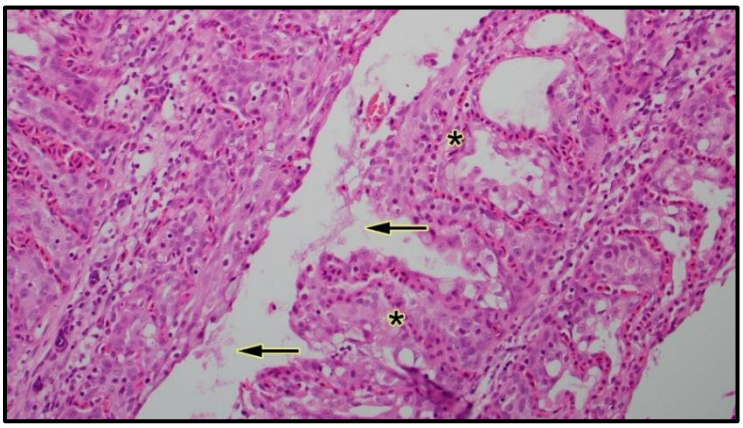

Figure 2. Histopathology of gill with fused gill lamellae (asterisks) and surface colonies of filamentous bacteria (arrow). (H&E)

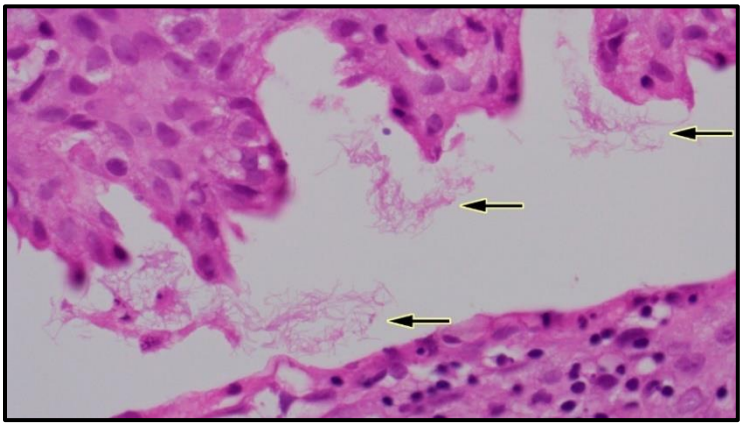

Figure 3. Higher magnification of surface colonies of meshed *Flavobacterium branchiophilum bacteria* (arrows). (H&E)

References

1. Ferguson H, et al. Systemic Pathology of Fish, 2nd ed. Scotian Press, 2006. ISBN-10: 0955303702.
2. Ferguson H, et al. Gill diseases of fish in Ontario farms. OMAFRA fact sheet. 1994. ISBN 0-7778-2306-3.
3. Noga E. Fish Disease: Diagnosis and Treatment, 2nd ed. Wiley-Blackwell; 2010. ISBN-10: 0813806976.

Disseminated toxoplasmosis in a captive slender-tailed meerkat

Heindrich Snyman, Sherry Davidson, Ron Mergl

Animal Health Laboratory, University of Guelph, Guelph, ON (Snyman); Niagara Falls Animal Medical Centre, Niagara Falls, ON (Davidson, Mergl)

AHL Newsletter 2020;24(3):15.

A ~ 2 year old, intact male, slender-tailed meerkat (*Suricata suricatta*) from a captive zoo colony presented with sudden acute bilateral hind limb ataxia. The hind limbs did not reveal any observable pain response and no obvious central proprioceptive deficits were apparent although a full detailed neurological examination was difficult in this animal. Immediate differentials included trauma (spinal fracture/luxation) with spinal cord compression or some other cause of central nervous system inflammation. The ataxia progressively worsened to paralysis and due to a lack of clinical response and a poor prognosis, the animal was euthanized.

An in-clinic necropsy was performed, revealing a regional area of sublumbar intramuscular hemorrhage at the level of L4 that was consistent with localized trauma. Other findings included mottled congestion of the ventral apical surfaces of the heart, bilateral pallor of the kidneys, and pale mottling of the splenic parenchyma. A broad array of representative tissues were collected in formalin and submitted to the Animal Health Laboratory for histopathology.

Histological sections of the brain and spinal cord contained multiple randomly scattered ~ 50 to 200 µm foci of malacia and gliosis (**Fig. 1**) with associated lymphoplasmacytic perivascular cuffing and few similar loose meningeal infiltrates of lymphocytes, plasma cells and macrophages. The heart contained multiple dense ~ 50 to 350 µm diameter coalescing interstitial aggregates of macrophages, lymphocytes, plasma cells, and rare scattered neutrophils with regional associated myofiber degeneration and necrosis (**Fig. 2**). Similar inflammatory aggregates were also scattered throughout the liver with accompanying acute hepatocellular necrosis, as well as the kidneys. Visceral lymph nodes contained small numbers of draining neutrophils and macrophages. The spleen appeared unremarkable and splenic mottling was attributed to red pulp congestion and barbiturate euthanasia.

The progressive neurological decompensation in this meerkat was attributed to this pleocellular and necro-inflammatory disease that was highly suggestive of infection with *Toxoplasma gondii*. Disseminated toxoplasmosis was confirmed by IHC staining with localization of *T. gondii* antigens to inflammatory foci within the brain (**Fig. 3**).

Meerkats are members of the mongoose family and are considered highly susceptible to infection with *T. gondii*. A number of high mortality outbreaks in captive zoo settings have been described. Similar to other domestic animal species, transmission is through the fecal-oral route via ingestion of contaminated feed. This occurs commonly in captive settings where feral cats can be attracted to feed in enclosures, which allows for the subsequent shedding of infectious oocysts into the environment.

Although the clinical presentation did not reveal any central nervous system deficits in this case and a traumatic incident was initially suspected, the multifocal nature of this disease often results in widely varied clinical presentations which can complicate antemortem diagnosis. Therefore, given the species predilection, toxoplasmosis should always be considered in any cases of central or peripheral neurological disease or acute unexplainable death in meerkats. *AHL*

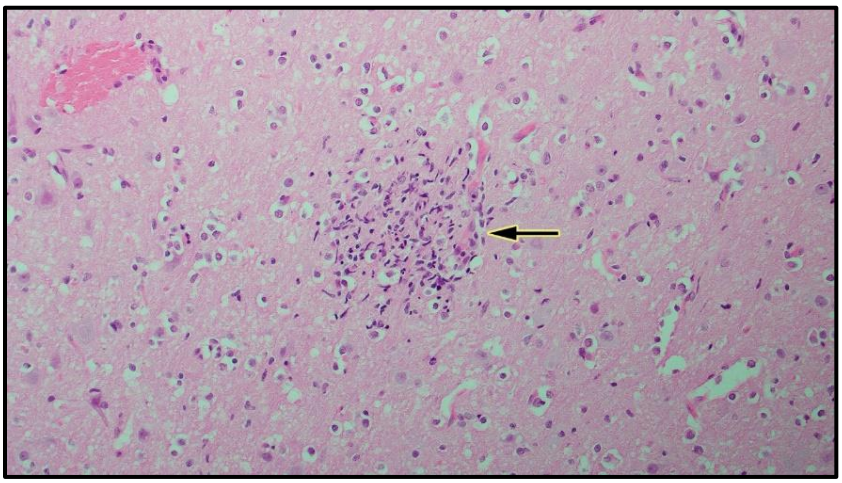

Figure 1. Focus of malacia and gliosis in the brain (arrow). (H&E)

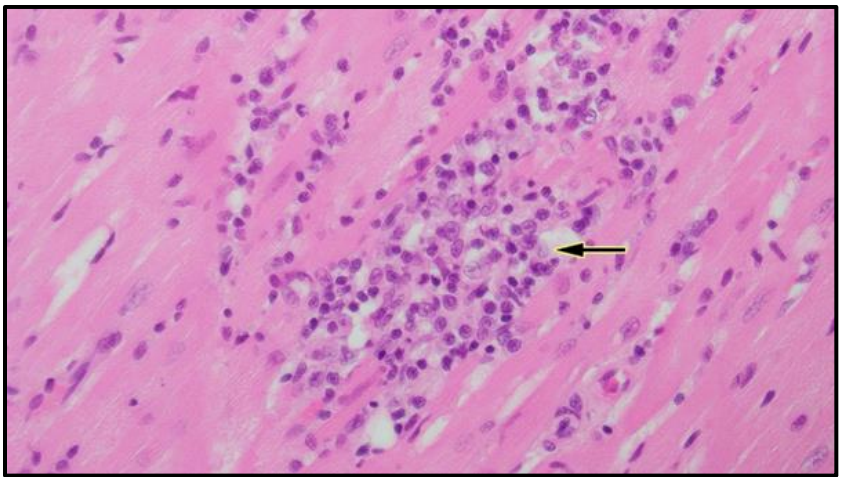

Figure 2. Interstitial myocarditis comprised of aggregates of macrophages, lymphocytes and plasma cells. (H&E)

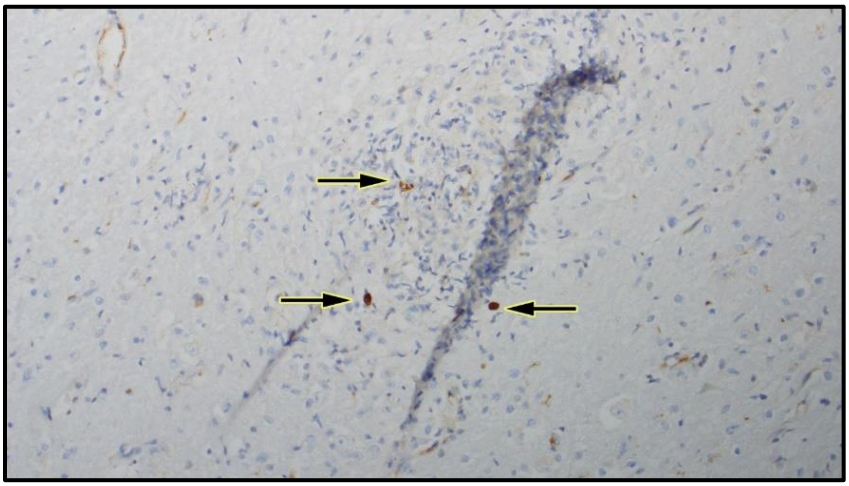

Figure 3. Positive IHC staining for *Toxoplasma gondii* antigen in a malacic and gliotic focus in the brain (arrows).

References

1. Molly E, *et al*. Chapter 14 - Procyonidae, Vivverridae, Hyenidae, Herpestidae, Eupleridae, and Prionodontidae. In: Pathology of Wildlife and Zoo Animals, 1st ed. Terio K, McAloose D and St. Leger, J, eds. Elsevier, 2018: 315.
2. Juan-Sallés C, *et al.* Epizootic disseminated toxoplasmosis in captive slender-tailed meerkats (*Suricata suricatta*). Vet Pathol 1997;34(1):1-7.
3. Basso W, *et al.* Isolation and molecular characterization of *Toxoplasma gondii* from captive slender-tailed meerkats (*Suricata suricatta*) with fatal toxoplasmosis in Argentina. Vet Parasitol 2009;161(3-4):201-6.
4. Burger M, *et al.* Fatal disseminated toxoplasmosis in a zoological collection of meerkats (*Suricata suricatta*). J S Afr Vet Assoc 2017; Mar 31;88(0):e1-e5.

We wish to express our sincere condolences to the Niagara Falls Animal Medical Centre on the passing of our colleague and friend, Dr. Ron Mergl. *AHL*

Actinobacillus equuli peritonitis and septicemia in an adult horse

Josepha DeLay, Đurđa Slavić

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

AHL Newsletter 2020;24(3):17

A 5-year-old thoroughbred gelding was found dead following recent transient (1 day) pyrexia (40^{o}C). Gross lesions at postmortem examination included fibrinous peritonitis localized to colonic serosa and severe bilateral nephritis. Kidneys were swollen and renal capsule was adherent to the cortical surface. On capsular and cut surfaces of kidney, numerous 1-3 mm tan-white nodules were present in cortex, with few similar lesions in renal medulla **(Fig. 1)**. Synovial fluid in single carpal and fetlock joints was opaque, with orange-pink discoloration.

Histologically, renal cortical and medullary parenchyma was replaced multifocally by discrete 0.5-3mm aggregates of neutrophils mixed with clusters of coccobacilli and variable fibrin and hemorrhage **(Fig. 2)**. Aggregates of similar bacteria filled lumens of adjacent glomerular capillaries and cortical tubules. Similar clusters of inflammatory cells and bacteria or a few intravascular bacterial aggregates were present in myocardium, brain, adrenal gland, colon, and pancreas. *Actinobacillus equuli* ssp. *equuli* was isolated from kidney and synovial fluid, and *A.equuli* ssp. *haemolyticus* was isolated from peritoneum.

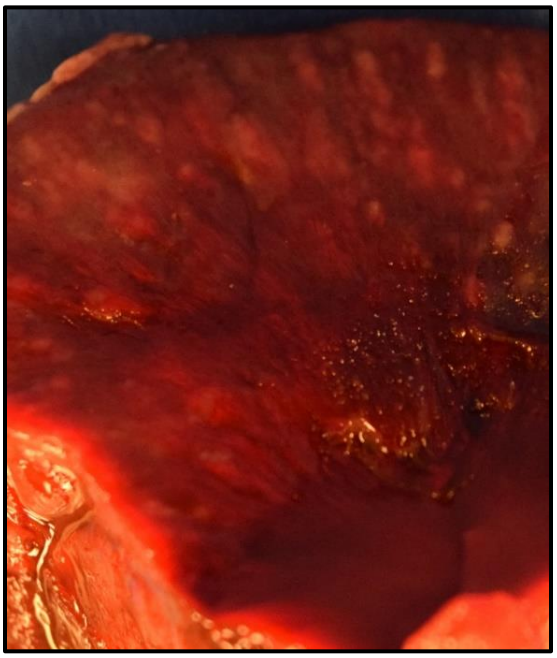

Figure 1. Kidney, gross appearance, cut surface. Numerous tan-white nodules in cortex.

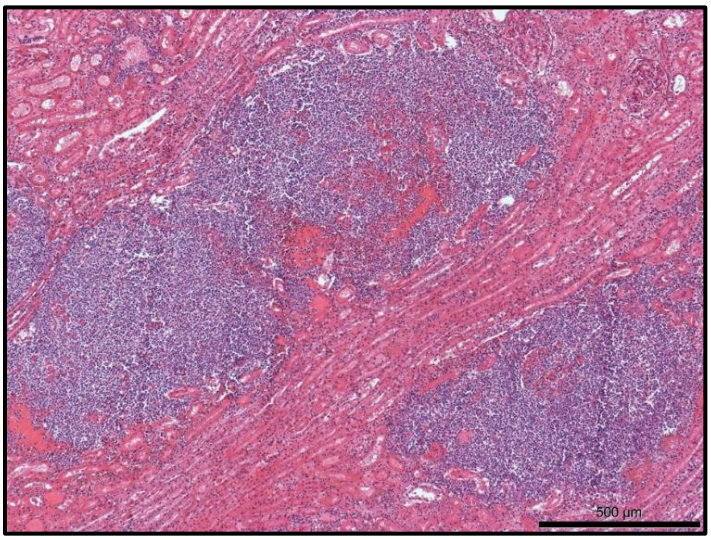

Figure 2. Kidney, histologic section. Nodules identified o gross exam correspond to multiple aggregates of neutrophils mixed with hemorrhage, fibrin, and bacteria. Hematoxylin and eosin stain.

Septicemia and peritonitis are unusual in adult horses, but both conditions have been occasionally described in association with *A.equuli*. The organism is a normal inhabitant of the equine oral cavity and intestine. Opportunistic infection from intestinal mucosal injury or larval nematode migration has been suggested as the pathogenesis of *A.equuli* peritonitis and septicemia in adult horses. *A.equuli* may cause abortion in mares, as well as septicemia in neonatal foals, and has been associated with hemorrhagic diarrhea in foals. Prominent renal lesions are often present in foals and adult horses with *A.equuli* septicemia, similar to the lesions described in this horse.

Among AHL pathology cases from 2010-2020, *A.equuli* septicemia was diagnosed in 12 adult horses, with concurrent peritonitis in 2 horses, and in 6 neonatal foals (1-3 days of age). Three equine abortion cases during this time period were attritubuted to *A.equuli*. *AHL*

References

1. Patterson-Kane JC, et al. Septicemia and peritonitis due to *Actinobacillus equuli* infection in an adult horse. Vet Pathol 2001;38:230-232.
2. Matthews S, et al. Peritonitis associated with *Actinobacillus equuli* in horses: 51 cases. Aust Vet J 2001;79:536-539.

Equine neurologic disease – getting a diagnosis

Murray Hazlett, Maureen Anderson, Jim Fairles

Animal Health Laboratory, University of Guelph, Guelph, ON (Hazlett, Fairles); Veterinary Science Unit, Animal Health and Welfare Branch, OMAFRA (Anderson).

AHL Newsletter 2020;24(3):18.

Getting a diagnosis on fatal neurologic disease in horses can be difficult. Even if rabies is considerably low down on the list of considerations, it is often a concern; however, submission of the brain directly to CFIA for a rabies test can mean samples for more likely causes of encephalitis cannot be collected. Often the owner does not want to pay for transportation for a complete autopsy at the lab.

As per the OMAFRA web page regarding rabies: if there is any human exposure risk, contact the local public health unit http://www.health.gov.on.ca/en/common/system/services/phu/locations.aspx. If exposure of other domestic animals (e.g. herd-mates) is a concern, **veterinarians** can contact an OMAFRA veterinarian for assistance with the risk assessment by calling the Agriculture Information Contact Centre (AICC) at 1-877-424-1300. If testing of the suspected rabid animal is warranted, veterinarians must contact OMAFRA for shipping and laboratory submission information. Please see http://www.omafra.gov.on.ca/english/food/inspection/ahw/rabies.htm#17 for additional information.

Some veterinarians opt to perform brain removal on the farm. Brain removal should always be done with proper PPE, including mouth/nose, and especially, eye protection. Details of two different brain removal techniques for large animals can be located on the AHL website: https://www.uoguelph.ca/ahl/rabies-sample-collection-veterinarians.

If submitting brain, it is often a good idea to call ahead to advise the lab it is coming so that it can be unpackaged promptly and flagged for the duty pathologist's attention. A removed brain should be submitted chilled if it can reach the lab within 24 hours of death; otherwise freezing prior to transport is likely the best option. Please pack with the submission form on top within a separate plastic bag. For cases within a reasonable driving distance of either the Guelph or Kemptville laboratory, the horse's entire head can be submitted directly instead, although fees for brain removal and disposal of the rest of the head may apply.

Once the AHL pathologist receives the brain (or removes the brain from the head), the pathologist prepares brain slices that meet the requirements for the rabies fluorescent antibody test (FAT) at the CFIA

rabies lab, in the event that rabies testing is required (**Fig. 1 A-C**). This allows the remainder of the brain to undergo sample collection for other neurologic diseases. Samples are collected – usually from cerebral cortex and medulla – for virology (PCR for EEE, WNV and EHV-1) as well as for histology. If the sample is anatomically disrupted or autolyzed and rabies testing is required as per the public health or OMAFRA risk assessment, then only small samples are collected for virology and the remaining (almost entire) brain is submitted to the CFIA rabies lab. All ancillary tests of brain samples – other than histology – are held until rabies has been ruled out.

It is a good idea to also submit fixed liver, kidney and colon in cases of "encephalitis" in horses, as hyperammonemia (hepatic encephalopathy – **Fig. 1 D**) can be mistaken for encephalitis and can have a fairly rapid clinical onset. Serum samples and whole blood collected prior to euthanasia, when possible, can also be submitted for IgM testing for WNV and EEE (depending on the season), and PCR for EHV-1, respectively. A compilation of equine CNS disease diagnosed at the AHL can be found on page 20 of the June 2016 AHL newsletter https://www.uoguelph.ca/ahl/sites/uoguelph.ca.ahl/files/ANwsl20-2-Jun2016-pub2010.pdf . *AHL*

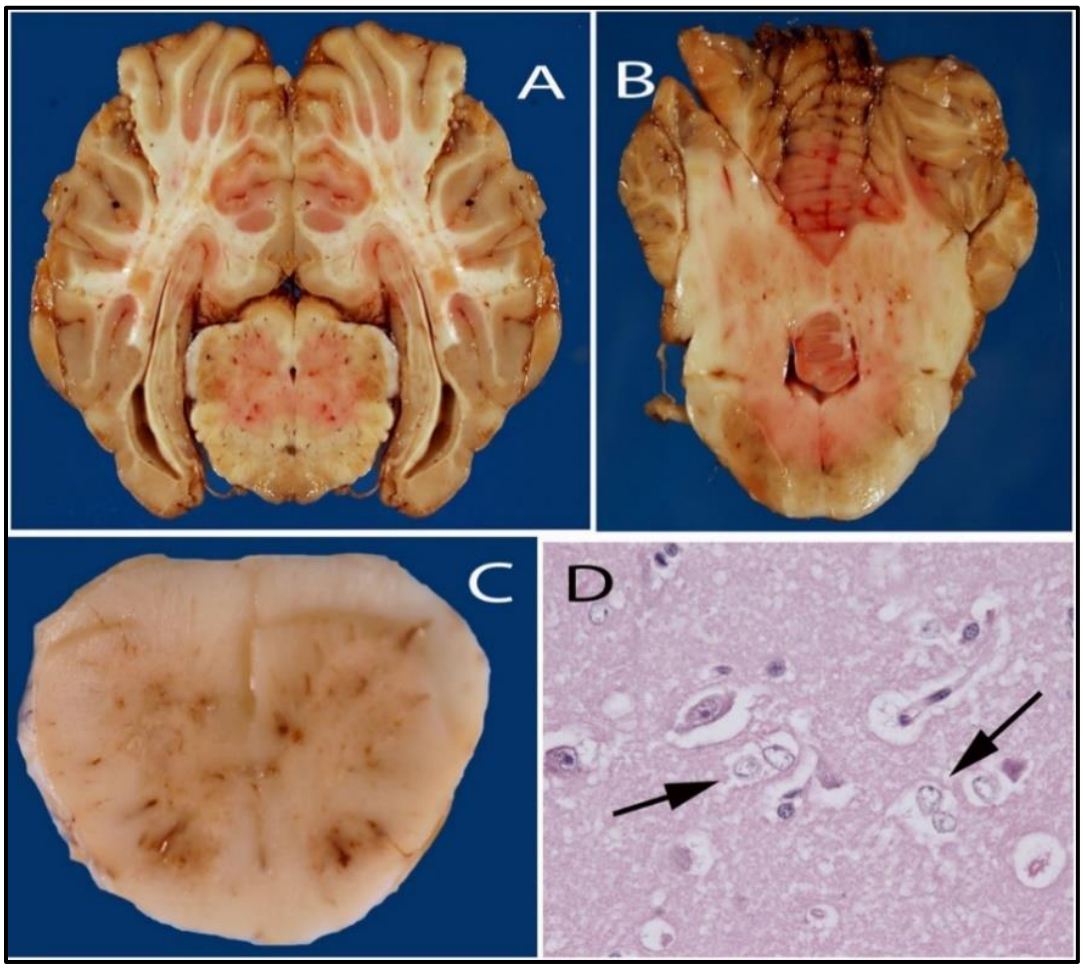

Figure 1. A,B – Sections of brain containing required bilateral areas of brain required for rabies testing, including hippocampus (**A**) cerebellum (**B**) and brain stem (**A** and **B**). **C**. Spinal cord should be submitted in cases of ascending myelitis. **D**. Alzheimer type II astrocytes seen in cases of hepatic encephalopathy (arrows).

Enterotoxigenic colibacillosis in two puppies

Andrew Brooks, Đurđa Slavić

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

AHL Newsletter 2020;24(3):20.

Two golden retriever puppies from the same litter were submitted to the Animal Health Laboratory (AHL) for postmortem with a history of fading, weakness and sudden death. The puppies were 12 days of age and were the offspring of a naïve, unvaccinated dam. In both puppies, the major gross lesion was enteritis. The jejunum and ileum contained bloody fluid in the lumen and the intestinal mucosa was diffusely red. There were no other significant gross lesions.

Histologically, the main lesion in both puppies was prominent bacterial enteritis characterized by a thick layer of gram-negative bacilli covering the villi of the small intestine (**Fig. 1.**). *E. coli* were isolated in large numbers from the small intestine of both puppies, along with *Clostridium perfringens*. Neither *Yersinia* spp., *Salmonella* spp., nor *Campylobacter* spp. were isolated. *C. perfringens* enterotoxin ELISA tests performed on the intestinal contents were negative. PCR tests for canine herpesvirus and adenovirus were also negative. There were no histological lesions to suggest infection with canine parvovirus or canine distemper virus.

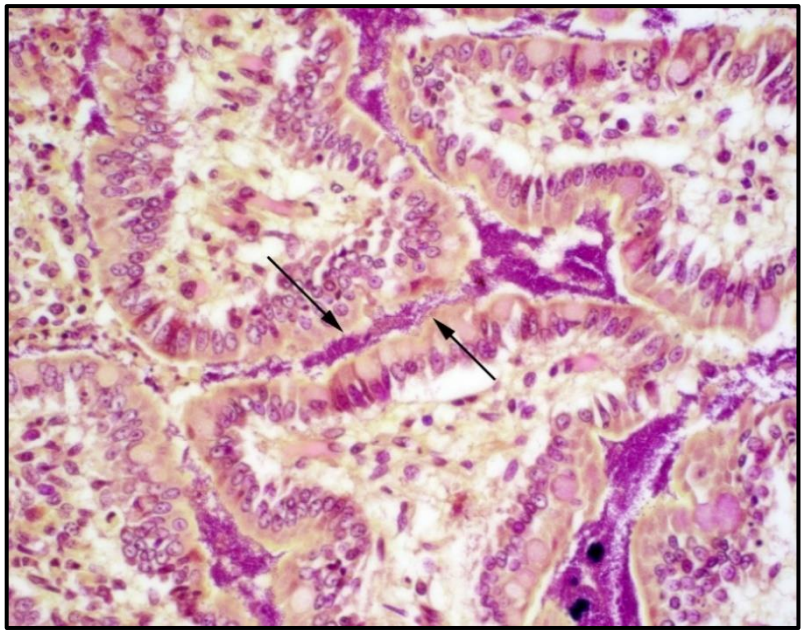

Figure 1. The small intestine villi are coated by a thick layer of gram-negative bacilli (arrows) (Gram stain).

The *E. coli* isolates from both puppies were positive for the genes encoding heat-stable toxins STa and STb, consistent with enterotoxigenic strains (ETEC). As the fimbriae of canine ETEC strains are not fully characterized (1), AHL does not provide testing for them. As expected, no genes for bovine- and swine-specific fimbriae F18, F4/K88, F41, F5/K99 or F6/987P were present.

A search of the AHL database over the past 10 years identified 19 other pathology submissions where enteric colibacillosis was confirmed or suspected in dogs. The dogs ranged from 4 days to 9 months of age, and the most frequent clinical problems were diarrhea and mortality. In 7 submissions, genotyping revealed enteropathogenic *E. coli* (EPEC). Genotyping was not performed or was inconclusive in the other 12 submissions. In contrast to ETEC, EPEC strains are characterized by the presence of the *eaeA* gene and produce the typical attaching-and-effacing lesion on the enterocyte brush border.

Although enteric colibacillosis a more common problem in ruminants and swine, it should also be considered in young dogs with diarrhea (2). *AHL*

References

1. Dubreuil JD, Isaacson RE, Schifferli DM. Animal Enterotoxigenic *Escherichia coli*. EcoSal Plus 2016; doi:10.1128/ecosalplus.ESP-0006-2016.
2. Drolet R, Fairbrother JM, Harel J, Hélie P. Attaching and effacing and enterotoxigenic *Escherichia coli* associated with enteric colibacillosis in the dog. *Can J Vet Res* 1994;58(2):87-92.

Fatal chronic copper-associated hepatitis in a Labrador retriever

Felipe Reggeti, Glenna McGregor, Nick Schrier

Animal Health Laboratory, University of Guelph, Guelph, ON (Reggeti, Schrier); Animal Health Centre, Abbotsford BC (McGregor)

AHL Newsletter 2020;24(3):21.

A 10-year-old female spayed Labrador retriever died 3 days after showing clinical signs consisting of tremors, drooling, mydriasis and profound depression/coma. The owner noted the dog was chewing on grass and roots in the yard. The area had endemic *Amanita phalloides* and *Amanita pantherinoides* and this dog had been hospitalized months prior with elevated liver enzymes suspected to be caused by mushroom toxicity.

The body was submitted to the Animal Health Center, Ministry of Agriculture, Abbotsford, BC for postmortem examination. The animal was in good body condition (BCS 3/5) with moderate autolysis and diffuse yellow discoloration (icterus). The abdominal cavity contained approximately 500 mL of clear yellow to blood-tinged fluid. The liver was shrunken, firm, with numerous 1-2 cm diameter nodules, consistent with macronodular cirrhosis (**Fig. 1**). No intact pieces of mushroom were noted in the gastrointestinal tract.

Histopathology of the liver revealed thick bands of fibrous tissue with mixed inflammatory cells, surrounding and separating variably-sized regenerative nodules and more normal hepatic parenchyma. Centrilobular hepatocellular necrosis was prominent. In a rhodamine red-stained section, large amounts of copper were present in virtually all hepatocytes and Kupffer cells, with larger quantities in centrilobular hepatocytes (**Fig. 2**).

Liver samples were sent to the Animal Health Laboratory (AHL), University of Guelph for heavy metal screen by inductively coupled plasma mass spectrometry (ICP-MS). The analysis identified: copper (Cu) 1560 ppm (adequate 90 - 300 ppm) and iron (Fe) 1620 ppm (adequate 300-900 ppm) on a dry weight (dw) basis. Based on histopathological evidence of chronic hepatitis with accumulation of Cu in centrilobular areas, identification of toxic levels of Cu (in excess of 1000 ppm dw) and exclusion of infectious diseases, a diagnosis of Cu hepatitis was made. These findings are compatible with the current recommendations for the diagnosis of Cu-associated chronic hepatitis (1).

Copper accumulates in the liver as a result of genetic defects limiting Cu excretion (primary); in association with underlying inflammation, fibrosis or cholestasis (secondary), or due to increased consumption. It has been shown that the pattern of Cu accumulation in primary Cu storage disease is always centrilobular, as noted in this case. Cu levels in these animals are commonly higher than 2000 ppm dw, but may be lower with advanced cirrhosis, as Cu does not accumulate in hyperplastic nodules and fibrous tissue, which can be quite abundant (2). Concomitant high Fe is consistently reported in cases of Cu toxicity. Fe commonly accumulates in the liver in the context of underlying diseases (e.g. inflammation), and it is speculated that hepatic Fe sequestration might contribute to oxidative injury underlying the cell damage in Cu toxicity.

The owner indicated this dog was fed a high Cu diet, which could have been a contributing factor, but the distribution of the lesions and Cu levels are more consistent with a primary storage disorder. Furthermore, the brother of this dog died 8 months prior with elevated liver enzymes of undetermined origin. This could point to a familial genetic mutation resulting in copper accumulation and copper-associated hepatitis in both dogs. Recent evidence suggests that mutations in the gene coding for the transporter ATP7B is associated with high levels of Cu in the liver of Labrador retrievers, as seen in humans with Wilson's disease (3). *AHL*

Figure 1. Gross image of the liver showing macronodular appearance (cirrhosis).

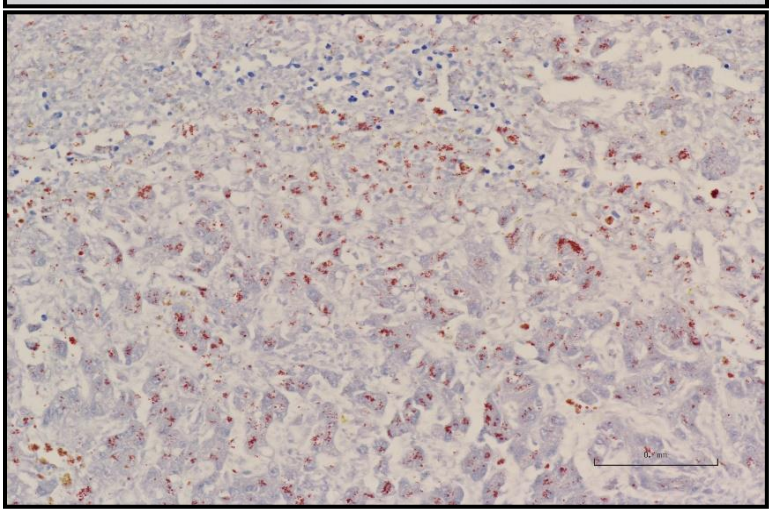

Figure 2. Abundant coper accumulation (red granules) in hepatocytes and Kupffer cells. Qualitative copper score 4/5. Rhodamine red. 200X

References

1. Webster CR et al. ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs. J Vet Intern Med 2019;33:1173–1200.
2. Smedley R et al. Copper-associated hepatitis in Labrador retrievers. Vet Pathol 2009;46:484–490.
3. Pindar S, Ramirez C. Predicting copper toxicosis: relationship between the ATP7A and ATP7B gene mutations and hepatic copper quantification in dogs. Hum Genet 2019;138(5):541-546.

Intra-ductal mammary tumor in a male neutered dog

Rebecca Egan, Kristiina Ruotsalo

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

AHL Newsletter 2020;24(3):23.

Direct smears prepared from a fine needle aspirate of a 2 cm mass associated with the teat of a 7.5-year-old male neutered mixed-breed dog were submitted for cytological evaluation. The dog was otherwise clinically well. The slides contained a hemorrhagic background with moderate numbers of variably preserved neutrophils and a predominance of foamy macrophages that often contained evidence of blue-green granular intracytoplasmic material. This material was somewhat similar in colour to the clusters of amorphous material (likely secretory) which were also present in the background of slides. Scattered clusters of epithelial cells were noted (**Fig. 1 A-B**). These epithelial cells contained scant to moderate amounts of lightly to moderately basophilic cytoplasm with occasional punctate vacuolation, and one oval to round nucleus with fine chromatin and a variably prominent nucleolus. Occasional evidence of two to three-fold anisokaryosis and anisocytosis was also noted. The cytological features of this sample were highly suggestive of an epithelial mammary tumor, which is very uncommon in male dogs (1), and as with all mammary masses, excision and histologic evaluation were recommended.

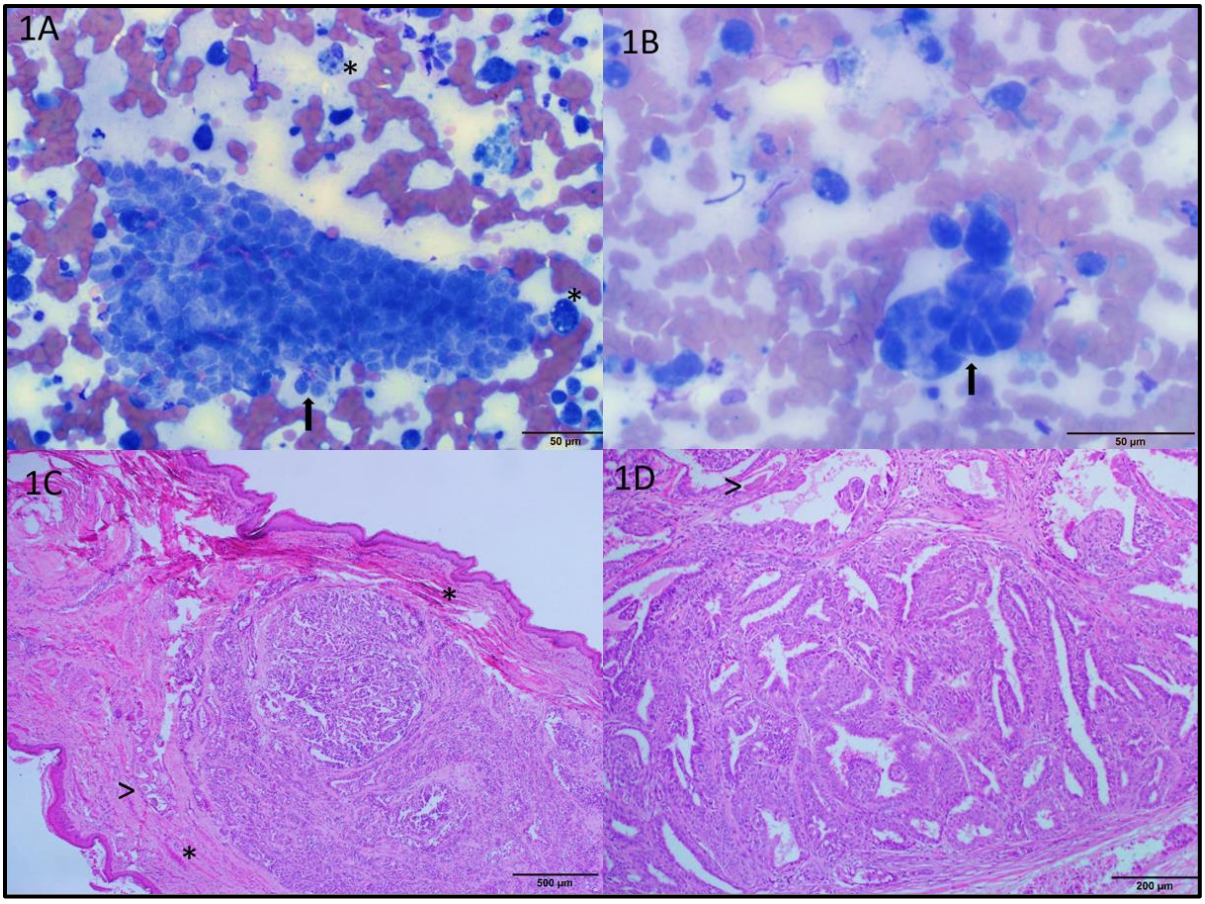

Figure 1. Mammary tumor in the teat of a male neutered dog. **A**,**B** - Direct smears from a fine needle aspirate (Wright's). Clusters of epithelial cells (arrows) and macrophages (*) within a background of hemorrhage. **C**,**D** - Excisional biopsy (H&E). An epithelial mammary tumor within the mammary duct of a teat. **C**. There is smooth muscle surrounding the expanded mammary duct (*). **D**. Secretion is evident within tubules (>).

The teat mass was excised and sent for histologic examination which confirmed the presence of an intraductal mammary carcinoma with low-grade features of malignancy. The mass expanded the mammary duct and was multilobular, locally infiltrative and comprised of neoplastic epithelial cells arranged in papillary projections and tubules, in addition to occasional micropapillae and small solid clusters of cells (**Fig. 1 C**). In some areas, neoplastic cells were relatively well-differentiated and orderly, whereas in others, neoplastic cells displayed jumbling, piling and clustering, accompanied by moderate anisocytosis and anisokaryosis and patchy squamous differentiation. In well-differentiated areas, mitoses were sparse, and a total of 10 mitotic figures were observed in ten consecutive 400x high powered fields in areas that displayed greater cellular variation. There were also occasional areas of necrosis within the mass, and as noted on cytologic examination, the lumens of some neoplastic tubules contained flocculent fluid, secretion and/or neutrophils and macrophages with finely granular yellow-brown cytoplasmic pigment (**Fig. 1 D**).

The current case demonstrates a common presentation of a relatively uncommon finding in veterinary practice, as mammary gland tumors are rarely reported in male dogs. The majority of documented cases report a benign clinical course following surgical excision (1). *AHL*

References

Saba, Corey F., et al. Mammary gland tumors in male dogs. *J Vet Diagn Invest* 2007;21(5):1056-1059.