Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

AHL Newsletter, Volume 24, Number 2 **June 2020**

In this issue:

AHL Newsletter
June 2020 - Volume 24, Number 2 ISSN 1481-7179

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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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AHL operations during the COVID-19 pandemic

The view from the Director's office

To say that life has changed since our last AHL Newsletter issue in March would be an understatement! Similar to your operations, we have had to rethink all of our processes in order to maintain some degree of functionality, while also ensuring a safe work environment for our staff and clients. AHL is considered an essential service as per Government of Ontario guidelines related to the agricultural sector, and therefore, we had to find a way to continue receiving samples, testing and reporting results. In the early days of the lock-down, submissions dropped in number, and most laboratory sections were able to complete their workload with reduced staff, thereby meeting appropriate physical distancing guidelines. We had to restrict laboratory hours, hold non-critical samples and batch others. Specimen Reception is now closed on Sundays, and the vestibule had to be locked after hours due to security concerns. Only 1 person at a time is permitted in the Specimen Reception vestibule space, and a plexiglass barrier was installed to protect both clients and staff during receiving interactions. Access to restrooms, lunch room and break areas had to be reconfigured to maintain physical distancing requirements.

As veterinary services began to adapt to the "new normal" modus operandi, submissions to AHL increased. Since a reduced staffing level was insufficient to manage this increased caseload, other adjustments had to be devised. These are very similar to processes currently employed in veterinary diagnostic laboratories throughout Canada and the United States. They include splitting a laboratory section into two teams that each works a separate shift during an expanded workday. Split teams accomplish two important goals: they allow enhanced physical distancing, and reduce the risk that the entire laboratory section would be required to self-isolate in the event of a COVID-19 positive staff member, since the two teams do not interact. And of course, we are so fortunate to have highly competent and motivated staff that are already well-trained in scientific procedures and disinfection protocols. Similar to other businesses, AHL is struggling with delivery disruptions, back-ordered supplies and sourcing sufficient PPE to protect staff.

We thank our clients for their patience and understanding as AHL manages our operations during this pandemic. If there is anything we can do to support your business as we work through this next stage of reopening and recovery, please contact us. We wish you, your staff and families continued health and safety during this challenging time.

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

Purolator update

Jim Fairles

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter, Volume 24;(2):3.

As you are likely aware, Purolator has been affected by COVID-19 operational requirements like all other businesses, including ours. Due to increased volume of shipments and operational adjustments required to respect physical distancing in order to keep employees, customers and communities safe, we are experiencing occasional delays of 1-3 days for our prepaid Purolator Ground® shipments.

We are asking clients to help by ensuring that samples are packaged so they can tolerate an extra day or two in transit. This will become even more critical as the weather gets warmer and we transition into the summer months.

Shipping Monday to Wednesday will help prevent samples from sitting over the weekend in warehouses.

From our **LabNote 27**: https://www.uoguelph.ca/ahl/submissions/ahl-labnote-27-submission-instructions

- Separate serum from clot after complete blood clotting has taken place.
- Include a blood smear for all complete blood count (CBC) samples.
- Use styrofoam insulating containers and ice packs **(Fig. 1)**.

Figure 1. Styrofoam container, icepacks and packaging material for optimal transport of lab submissions.

Thanks very much for your patience and understanding during these trying times.

Please contact ahlinfo@uoguelph.ca if questions or concerns.

SARS-CoV-2 (COVID-19): Animal testing at the AHL

Jim Fairles and Davor Ojkic

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter, Volume 24;(2):4.

AHL is now offering a PCR test for SARS-CoV-2 (COVID-19). Testing must be pre-approved by the Office of the CVO of Ontario, as AHL is supportive of the CCVO and CFIA position statements on testing of animals for SARS-CoV-2.

The Council of Chief Veterinary Officers' Position Statement on testing of animals for SARS-CoV-2 states:

- Public health testing must be the priority to protect human health.
- The SARS-CoV-2 pandemic is driven by person-to-person transmission with current data suggesting that the risk for human infection through animal contact is low.
- Testing animals for SARS-CoV-2 may consume the personal protective equipment and supplies needed for safe sampling and testing in people.
- In general, test results will not change the clinical management of the animal or change the recommended measures to manage the potential risks if an animal tests positive.
- There is the risk of stigmatizing animals that test positive for SARS-CoV-2 which could result in damaging the human-animal bond or threatening animal welfare.

Under the following circumstances, testing may be considered:

- Testing is deemed to contribute to advancing the understanding of the epidemiology of SARS-CoV-2 in animals.
- Testing will inform the need for public health and animal health disease control activities that may alter the current recommended measures to manage potential risks.
- There is an animal health concern based on clinical signs not explained by other etiologies as assessed by diagnostic procedures performed by a veterinarian, AND there is a need which will inform public health action.
- The animal has had contact with a person diagnosed with COVID-19.
- In specific circumstances, an asymptomatic animal that could have been exposed to the SARS-CoV-2 virus may be tested if it is:
    - part of a cluster of animals showing compatible clinical signs
    - part of an endangered species
    - in a special situation such as therapy animals in close contact with vulnerable people.

If your requirement for SARS-CoV-2 testing meets one of the appropriate circumstances, please contact the Office of the Chief Veterinary Officer of Ontario to discuss your request: **OMAFRA: 1-877-424-1300** ag.info.omafra@ontario.ca

Only after permission is granted will AHL proceed with testing for SARS-CoV-2. Please contact AHL (ahlinfo@uoguelph.ca 1-519-824-4120 ext. 54530) to discuss the submission and transportation of the sample. Please note there are specific requirements for sending these samples to the laboratory that must be discussed ahead of time. For the biosafety of all, AHL MUST know when these samples are to arrive (courier tracking number required). Please refer to AHL LabNote 63 for additional details.

References

1. Council of Chief Veterinary Officers Position Statement: SARS-COV-2 testing in animals: **https://www.canadianveterinarians.net/documents/council-of-chief-vet-officers-p-s-testing-animals-sars-cov-2**
2. CFIA - Interim Guidance For Laboratories Testing Animals for SARS-COV-2 **https://www.canadianveterinarians.net/documents/update-interim-guidance-laboratories-testing-animals-sars-cov-2**

OAHN update – June 2020

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AHL Newsletter, Volume 24;(2):5.

The Ontario Animal Health Network has been busy throughout the spring, releasing new infographics, reports, videos, and COVID-19 resources. Read on to find links and descriptions of what we've been working on.

Anti-Parasitics Tables for Dogs and Value of a Postmortem in Your Sheep Flock Video

The OAHN Companion Animal Network created 2020 anti-parasitics tables for dogs and cats outlining which species different anti-parasitics are used for, and what type of parasite they treat. A simplified table was created for pet owners in addition to the veterinary version.

The OAHN Small Ruminant Network created a new video: The Value of a Postmortem in Your Sheep Flock. This video outlines the value of performing a postmortem on sheep that die unexpectedly as a tool to improve flock management.

OAHN Small Flock Poultry Video Series

OAHN has been working with Dr. Victoria Bowes, a diagnostic avian pathologist with a special interest in small flock medicine, to produce a series of videos to assist veterinarians treating small flock poultry. The completed series has a total of 8 lectures, and can be accessed here: https://oahn.ca/small-flock/

Completed Research Projects

The most recently-completed research project was released by the OAHN Swine Network: Ongoing investigation of an outbreak of Senecavirus A: https://oahn.ca/resources/oahn-swine-network-project-ongoing-investigation-of-an-outbreak-of-senecavirus-a/

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 outlining research and highlights of 2019.

- Companion Animal Special Bulletin: https://oahn.ca/reports/companion-animal-special-bulletin/

The OAHN Bovine Network and Equine Network put together resources for veterinarians during the COVID-19 pandemic:

- COVID-19 Information for Cattle Veterinarians and Farmers: https://oahn.ca/resources/covid-19-bovine/
- COVID-19: Caring for Your Horse During a Pandemic: https://oahn.ca/resources/covid-19-caring-for-your-horse-during-a-pandemic/
- Farriers: Working in a COVID-19 Pandemic: https://oahn.ca/resources/farriers-working-in-a-covid-19-pandemic/

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

Neonatal diarrhea in small ruminants: AHL pathology diagnoses, 2018-2020

Josepha DeLay, Andrew Brooks, Emily Brouwer, Rebecca Egan, Murray Hazlett, Amanda Mansz, Jan Shapiro, Heindrich Snyman, Maria Spinato, Margaret Stalker

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

AHL Newsletter 2020;24(2):6.

Between January 1, 2018 and May 1, 2020, diarrhea was identified in 35 postmortem and/or histopathology submissions of neonatal goat kids or lambs (age 1-14 days, average 8 days) submitted to the AHL. These included 29 (83%) caprine and 6 (17%) ovine cases. A consistent set of ancillary diagnostic tests was carried out on 22/35 (63%) cases, all of which had postmortem examination done at the AHL. The test panel included: histopathology, fecal sucrose wet mount, bacterial culture of small and large intestine, and rotavirus and coronavirus PCR tests on intestinal mucosa or content. For the remainder of the cases, histopathology plus various combinations of these same tests were requested by the submitting veterinarian.

Etiologic agents of neonatal diarrhea identified in this group are listed in **Table 1**. A single enteric pathogen was confirmed in the majority of cases [22/29 (76%) goat kids and 4/6 (67%) lambs]. *Cryptosporidium* spp. was the most frequently identified cause of diarrhea in neonates of both species and was detected in 12/29 (41%) goat kid cases and 4/6 (67%) lamb cases. Concurrent rotavirus enteritis was present in 3 of the goat cases. Of the 4 lambs with cryptosporidiosis, concurrent coccidiosis was identified in one 14-day-old animal, and concurrent colibacillosis was present in one 7-day-old lamb.

Rotavirus A or B was the sole pathogen associated with neonatal diarrhea in 2 young (1 and 2-day-old) kids. Coronavirus was not detected in any of the cases. Of the 10 *E.coli* cases in both species, 6 (67%) bacterial isolates were confirmed by genotyping as verotoxigenic (VTEC) or enteropathogenic (EPEC) serotypes. Other bacteria identified in 2 neonates with diarrhea included *Clostridium perfringens* and *C. difficile*, although the clinical significance of either isolate was not confirmed. Conditions affecting other body systems were present concurrently with neonatal diarrhea in 6/29 (21%) goat kids and 1/6 (17%) lambs, and included bacterial pneumonia and septicemia.

Table 1. Etiologic agents identified (number of cases) and age range of affected animals (in days, D) in 35 AHL small ruminant neonatal diarrhea pathology submissions, 2018-2020.

Species	*Cryptosporidium*	*E.coli*	Rotavirus	Coccidia	Other bacteria (confirmed or suspected)	Idiopathic (autolyzed samples)
Kids (n=29)	12 (3-14 D)	7 (6-14 D)	5 (1-14 D)	2(7-14 D)	5 (1-10 D)	2
Lambs (n=6)	4 (4-14 D)	3 (3-7 D)	0	1 (14 D)	0	0

This pathology case review provides a reminder of the common causes of neonatal diarrhea in kids and lambs, and of the tests available for diagnostic evaluation in both clinically-affected live animals and postmortem cases. *AHL*

Clostridium ramosum abomasitis in a dairy goat

Murray Hazlett, Đurđa Slavić, Lisa Sharko

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AHL Newsletter 2020;24(2):7.

An investigation of a dairy goat herd with issues of pregnancy toxemia, ketosis, hypoglycemia and hypocalcemia resulted in submission of two poor doing 2-year-old does for autopsy at the AHL.

One of the does had a very large polycystic abdominal mass, about 10 litres in volume which was believed to account for the poor performance. The second doe had severe neutrophilic abomasitis with **marked edema, emphysematous enteritis and neutrophilic ulcerative typhlocolitis. A large number of *Clostridium ramosum* was isolated from the abomasum** with a moderate number of *Escherichia coli*. A small number of *Clostridium perfringens* was isolated from the cecum.

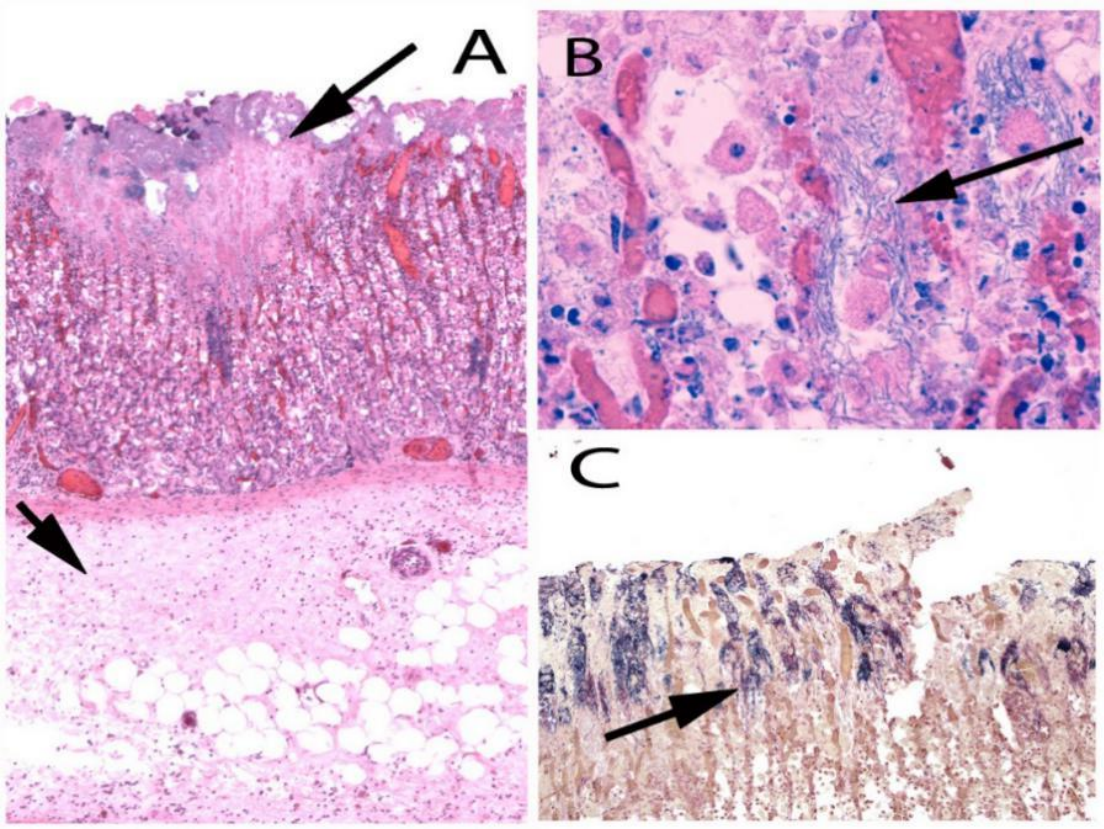

Figure 1. A. Marked edema of the abomasal submucosa (lower arrow) and an area of necrosis and ulceration in the mucosa (upper arrow) (H&E stain). **B.** Clusters of bacterial bacilli within the abomasal mucosa (arrow) (H&E stain). **C.** Gram-positive *Clostridium ramosum* organisms at the interface of dead and living tissue. (Gram stain).

Since 2007, 46 isolates of *C. ramosum* have been reported at the AHL, most being regarded as secondary or opportunistic infections isolated with other bacteria and in various species (10 cattle submissions, 5 caprine, 2 ovine and 7 equine). Most cases of caprine abomasitis diagnosed at the AHL are mild and of unknown cause; many are assumed to be post-parasitic lesions. Parasitic abomasitis is the most commonly-diagnosed cause, followed by *Sarcinia* and unknown bacterial species. Abomasum is not typically submitted for culture in a majority of cases.

Clostridium ramosum is a common enteric bacterial species in humans and is a spore-forming anaerobe. Within the *Clostridium* genus is a subgroup known as the RIC group, named for the initial letters of its constituents' species (*C.* ***r****amosum*, *C.* ***i****nnocuum*, and *C.* ***c****lostridioforme*). Identification can be difficult because of Gram stain variability, spores that are often difficult to see, and atypical colony morphology.[1] It is considered to be rarely pathogenic but has been identified in some opportunistic infections in humans including abscesses, otitis and septicemia.[2] *C. ramosum* has been demonstrated to be obesogenic in mice and possibly in humans. *AHL*

References

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2. Forrester JD, Spain DA. *Clostridium ramosum* bacteremia: Case report and literature review. Surg Infect 2014;15(3):343-346.

OAHN swine small-scale herd postmortem project is underway

Josepha DeLay, Tim Pasma DVM, MSc, MBA

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AHL Newsletter 2020;24(2):9.

The Ontario Animal Health Network (OAHN) has funded a new study at the Animal Health Laboratory (AHL) to identify disease issues in small-scale swine herds in Ontario. Through this work, we hope to also establish and expand connections with small-scale swine producers in the province. The study will build on previous OAHN projects for disease surveillance in poultry small flocks and small ruminant herds, both of which provided a wealth of practical information for producers and veterinarians.

Half of the world's pig population is raised in small-scale herd settings (Dietze et al, 2011), and similar herds also exist in Canada and Ontario. Small-scale swine production has been researched in other countries, but to date, there are no known studies of this subset of swine herds in Ontario.

The goals of the project are to:

- identify disease problems in Ontario small-scale swine herds;
- establish and maintain communications among small-scale swine producers, veterinarians and OAHN;
- increase awareness of zoonotic and foreign animal diseases among small-scale swine producers.

For this project, a small-scale herd is defined as having ≤50 sows OR marketing ≤1000 hogs per year. Enrollment in the project and sample collection for testing must be done through the herd veterinarian who subsequently submits samples to the AHL. Disease surveillance will be accomplished through subsidized postmortem examinations on pigs from participating herds. For individual cases, postmortems may be conducted either at the AHL or on-farm by the herd veterinarian. Laboratory tests on samples from postmortem exams will be done at the AHL and will also be subsidized. Pigs will be tested for a variety of diseases depending on the presenting complaint and the age of the animal. 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.

Completion by the producer of a short, user-friendly survey on herd management will be required in order to qualify for subsidized testing. A premises identification number (PID) is also required to participate. Findings from the project will be made available to industry stakeholders in the winter of 2020-2021.

Summary of requirements for postmortem (PM) case enrollment in the project and qualification for PM and testing subsidies:

- swine herd is located in Ontario and has ≤50 sows or ≤1000 hogs marketed per year;
- herd has a Premises Identification Number (PID);
- producer completes and submits the herd management survey;
- veterinarian has enrolled the case in the program (contact jdelay@uoguelph.ca or 519-824-4120 ext. 54576);

- animals are submitted to AHL for postmortem OR on-farm postmortem is conducted by the veterinarian, with full completion of the appropriate submission form, strict adherence to sampling requirements, and carcass / sample submission to the AHL.

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

Reference
Dietze K, Beltran-Alcrudo D, Pinto J et al. Factors affecting emergence of diseases in swine. Proc 22nd IPVS Cong 2012:11-15.

Pet pigs: what we are seeing

Murray Hazlett, Josepha DeLay

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

AHL Newsletter 2020;24(2):10.

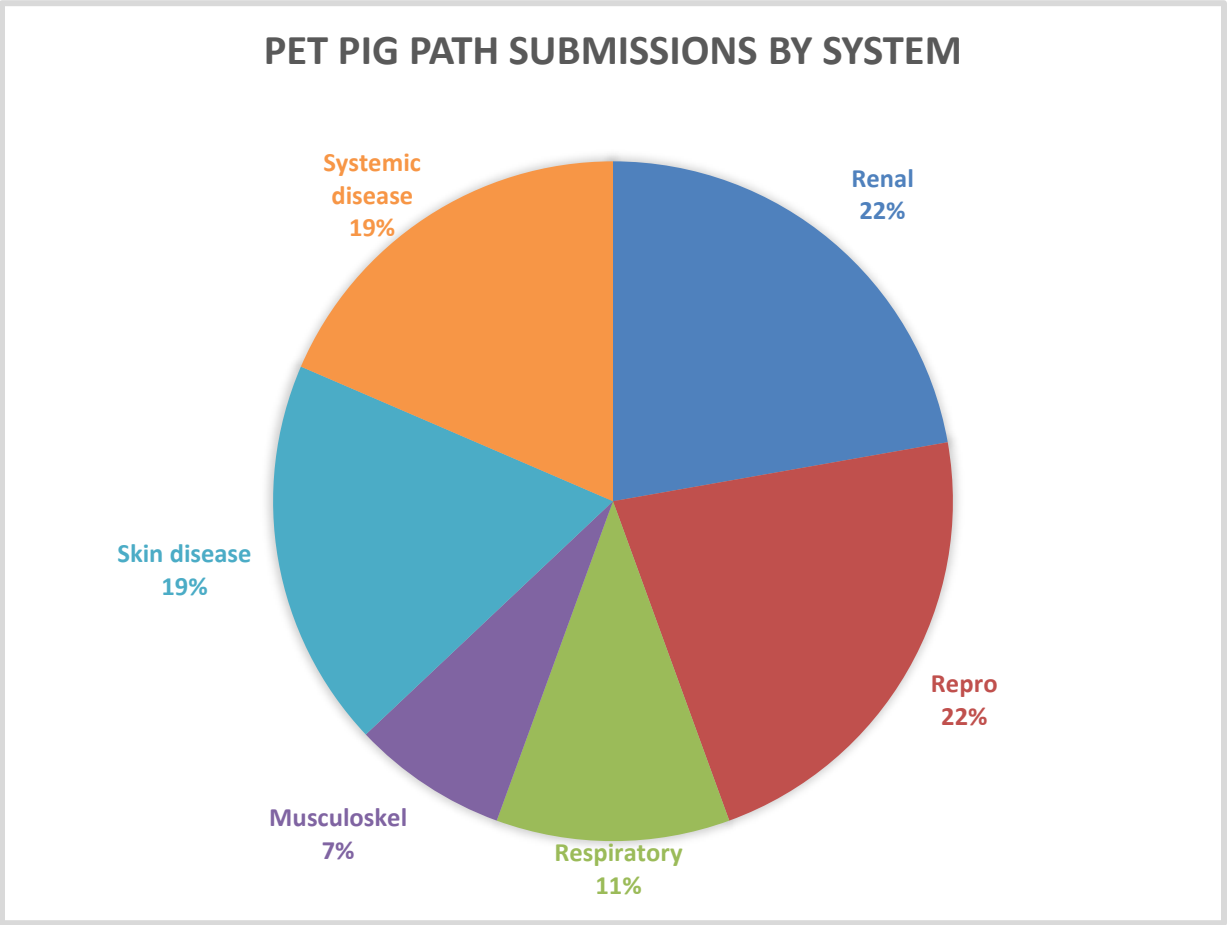
Disease monitoring in pet pigs is important for improving health and welfare in these and other swine species. Pet pigs are susceptible to diseases that also affect commercial swine, including foreign animal diseases such as African swine fever and classical swine fever. They are also susceptible to zoonotic pathogens including rabies virus and influenza virus. Premises with pet pigs may have minimal biosecurity, making these animals a potential portal of entry for these devastating diseases. Increasing awareness of swine diseases and biosecurity issues among pet pig owners and veterinarians can help to ensure the health and welfare of all Canadian swine.

Although not a common submission to the AHL, we have received 206 submissions of pet breed pigs between May 2007 and April 2020. These included 176 Pot-bellied pigs (85%), 27 Miniature pigs (13%), and 3 Micro-mini pigs (2%). Of these 206 submissions, 48 (23%) were pathology submissions: 27 (13%) were postmortems carried out at the AHL and 21 (10%) were biopsies or samples collected for histopathology from field postmortems (**Graph 1.**).

Among these 48 pet pig pathology cases, urinary and reproductive system diseases were the most common categories (6 cases each) with urolithiasis (3) being the most common disease entity. There was 1 case each of ethylene glycol toxicosis and pyelonephritis, and 2 cases of non-specific nephritis. Reproductive system diagnoses were more heterogeneous and included 2 uterine smooth muscle tumors, and miscellaneous inflammatory conditions. Three other diagnosed tumors included a hepatocellular carcinoma, an interstitial cell tumor and a mandibular osteoma. These results are in contrast to those observed at the AHL for commercial pigs, in which respiratory and gastrointestinal diseases are most frequently diagnosed.

Zoonotic pathogens identified from the pet pig submissions included *Streptococcus suis* (5 cases) and *Ascaris suum* (2 cases).

Some animals were tested for common swine pathogens including porcine respiratory and reproductive syndrome virus / PRRSV (2 animals), porcine circovirus 2 and PCV3 (2), *Brachyspira* spp. (4), gastrointestinal coronaviruses (6), and rotavirus (1). All PCR results for these pathogens were negative.



Graph 1. AHL pet pig pathology submissions: May 2007 – April 2020.

Links to excellent resources for pet pig owners and veterinarians can be found at this site: http://www.swinehealthontario.ca/Communications/pet-pig-guide. *AHL*

Turkey Arthritis Reovirus (TARV) and aortic rupture in Ontario turkeys

Emily Martin, Davor Ojkic, Marina Brash, Emily Brouwer and Helen Wojcinski

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Wojcinski Poultry Health Consulting, Michigan, USA. (Wojcinski).

AHL Newsletter 2020:24(2):12.

Reovirus was isolated from joints and ruptured tendons of turkeys with tenosynovitis/arthritis as early as 1980; however, while reovirus was shown to cause the disease, it could not be reproduced experimentally. In 2011, reovirus-associated lameness in turkeys re-emerged with experimental infection causing tendon lesions by 10 days of age and rupture of tendons by 8 to 10 weeks of age. This reovirus, identified as turkey arthritis reovirus (TARV), has been reported from all turkey-producing states of the USA and other countries. The lameness affects male turkeys 12-17 weeks of age (occasionally hens) and is characterized by recumbency with wing tip bruises ("wing walkers"), and uni- or bilateral swelling of the hock (tibiotarsal) joints (**Fig. 1**). Gross lesions include hock swelling, periarticular edema and fibrosis, increased fluid in the tendon sheaths and hock joints, tenosynovitis, and occasional rupture of the gastrocnemius or digital flexor tendon (**Fig. 2**). Lameness affects 15%-70% of a flock, resulting in economic losses due to excessive culling, diminished carcass quality and reduced market weights. The severity of clinical signs is believed to depend on the virus pathotype, route of exposure (vertical or horizontal), age of infection (<2 weeks), maternal antibody (breeder vaccine or field exposure), plus environmental management and nutrition.

Experimentally, the lesions start as lymphocytic infiltration at 4 weeks, progressing to fibrosis by 16 weeks post infection. Tendon fibrosis results in decreased tendon tensile strength, and tendon adhesions may contribute to tendon rupture in older, heavier birds. In addition, there is evidence that aortic ruptures are associated with TARV infection with increased mortality for 2 or more weeks in older birds (>13 weeks). One theory for this association is that there may be damage to collagen/elastin of tendons and aorta; however, no histologic evidence of vascular inflammation has been reported in diagnostic cases or experimental challenge studies. Another theory is when barns housing heavy birds have lights turn on, large birds may have pain on rising, causing increased blood pressure and subsequent aortic rupture.

Based on the USA experience, the National Turkey Federation (USA) has a TARV economic summary and case definition:
https://www.eatturkey.org/wp-content/uploads/2020/01/Economic-Impact-of-TARV_NTF_122019.pdf

Ontario's TARV situation is currently under investigation. Lameness in Ontario turkey flocks can be multifactorial. TARV infection is an additional differential diagnosis that is based on gross lesions, compatible histologic lesions and positive reovirus PCR. Other causes of lameness including osteomyelitis, synovitis/tenosynovitis, tibial dyschondroplasia, muscle/tendon rupture from other causes, footpad dermatitis, *Mycoplasma synoviae*, *Mycoplasma iowae* and dietary deficiencies need to be ruled out. Subsequent reovirus genotyping and phylogenetic analysis will provide further information on strains in Ontario.

The most likely source of TARV is the turkey enteric reovirus (TERV), as they share a high degree of homology. Chickens are not believed to be the reservoir for TARV infection in turkeys. Although turkey arthritis reoviruses are extremely hardy, lasting 2 weeks in unsanitized water and 6 – 8 days in litter, they are susceptible to most commonly-used disinfectants.

There is no specific serological test for TARV. Current commercially-available reovirus ELISA tests are chicken-based and detect both TERV and TARV. The degree of the ELISA response or seroconversion may indicate exposure to TARV and can be used for surveillance purposes. Turkey-validated PCRs are available; however, in many cases, older turkeys showing typical clinical and postmortem signs may test negative by PCR or virus isolation. TARV is best detected prior to or as soon as lameness develops. Follow up testing of subsequent flocks at an earlier age is recommended.

Collecting your own samples for submission to AHL:
Swabs: Aseptically swab the hock joint and/or digital flexor tendon for reovirus PCR, mycoplasma PCR and bacterial culture (individual swabs or in pools of no more than 5).
Histology: Collect gastrocnemius tendon and/or digital flexor tendon (3 to 5 maximum). If applicable, collect the area of aortic rupture (3 to 4 maximum).
Submitting intact legs to AHL for sample collection (submit 5 intact legs maximum):
a) Swabbing and histology only: reovirus PCR, mycoplasma PCR, bacterial culture, and tissue collection (histology).
Fees: Pathologist tissue collection (per 15 minutes), testing fees, and decalcification charge.
b) Full leg dissection for evaluation of bacterial infections, tibial dyschondroplasia (TD), and swabbing: reovirus and mycoplasma PCR, bacterial culture, and tissue collection (histology).
Fees: Full PM charge, testing fees, and bone/tendon decalcification charge.
Virus isolation is available as a send-out test and can be arranged if requested.

If you have any questions, please contact AHL to discuss your sample submission. *AHL*

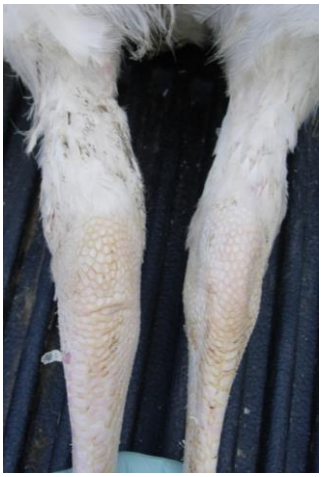

Fig 1. Unilateral (left) hock swelling. (Photo: D. Pyle)

Fig 2. Tendon rupture. (Photo: D. Pyle)

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Acute imidacloprid toxicosis in broiler chickens

Felipe Reggeti, Nick Schrier, Marina Brash, Daniel Venne, Isabelle St-Pierre

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AHL Newsletter, 2020;24(2):14.

High mortality was observed in a barn housing 4-day-old broiler chickens over the first 48 hours of placement. Approximately 27,000 birds were at risk, but deaths were localized to the first 20 feet of the barn. Affected chicks were in ventral recumbency, appeared somnolent with closed eyes and had neurological signs consistent with leg paralysis **(Fig. 1)**.

The barn had been sprayed with the pesticide Credo ® SC (Bayer) to help control darkling beetles; however, due to a presumptive mixing error, excessive amounts of concentrated product were applied to the walls near the barn's entrance. This spray had dried out, leaving a white precipitate on the walls close to the floor **(Fig. 2)**, and it was thought that the chicks had eaten this precipitate. The rest of the barn walls were sprayed with the remaining diluted solution, which did not leave the coating of white precipitate. When the flock veterinarian limited access to the walls using pieces of cardboard, the affected birds showed rapid clinical improvement.

Live chicks without contact to the white precipitate for 12 hours were submitted to the MAPAQ diagnostic laboratory in Québec city the next morning, but the birds appeared to be normal upon arrival, and there were no significant macroscopic or microscopic lesions on postmortem examination.

Liver samples were sent to the Animal Health Laboratory (AHL) for pesticide screening (pestscr). The samples were analyzed by liquid chromatography/electrospray ionization-tandem mass spectrometry, revealing a small peak consistent with trace amounts of imidacloprid (the active ingredient of the pesticide), confirming exposure. This finding, along with clinical presentation and exclusion of infectious diseases, supported a diagnosis of imidacloprid toxicosis. Rapid improvement of clinical signs after restricting contact to the white precipitate suggests fast metabolism of the toxic compound; therefore, submitting birds with clinical signs may increase the probability of identifying the toxin. Liver is the best sample, however, crop/gizzard contents may also be informative.

Imidacloprid is a group 4 neonicotinoid insecticide registered in Canada for applications including control of lesser mealworm (the darkling beetle *Alphitobius diaperinus*) larvae and adults in poultry facilities (1). Neonicotinoid pesticides were derived from modifications of nicotine, a plant alkaloid with insecticidal properties extracted from tobacco leaves. Similar to nicotine, the pharmacological and toxic effects of these new compounds involve activation of nicotinic acetylcholine receptors; however, while these effects are exerted in the central nervous system of insects, they are mostly restricted to the peripheral nervous system in vertebrates due to their low lipophilicity and poor penetration of the blood-brain barrier (2). Because of this relative selectivity, neonicotinoids are generally considered to be less harmful to vertebrates, explaining the popularity of these pesticides. However, some neonicotinoids, including imidacloprid, are particularly toxic to birds. Multiple cases of toxicity in game and wild birds have been recorded from ingestion of insecticide-coated seeds (3). In the poultry industry however, exposure is more likely to occur from ingestion of poisoned beetles or direct exposure to the substance, as reported in this case. Clinicopathological findings in experimental imidacloprid toxicity studies in broiler chickens have been reported elsewhere (4). *AHL*

Figure 1. Affected bird in ventral recumbency, with splayed right leg and closed eyes (4).

Figure 2. White "chalky" precipitate at the bottom of walls near barn entrance (courtesy of Dr. Venne)

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Submitting pet aquarium fish to the AHL for diagnostic evaluation

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AHL Newsletter, 2020;24(2):15.

Pet fish are particularly popular pets in North America and similar to our other domestic animal species, they are also afflicted by various disease conditions that could affect their health and wellness or that of their cohorts. As such, it is not surprising that the AHL often receives inquiries from concerned owners and veterinarians about the submission of pet fish for postmortem examination and diagnostic testing. There are well over 33,000 known species of fish and in addition, countless variations in shape, size, and specialized anatomic structures that easily generate uncertainty on how to best submit these cases to the lab. To address these uncertainties, here are a few general recommendations for submission of pet aquarium fish for diagnostic evaluation at the AHL.

Chilled, recently dead fish (<12-24hours) or live sick fish can be submitted directly for **fish processing and sampling** which includes gross dissection and evaluation, and tissue subsampling for ancillary testing and histopathology. This procedure also includes fish specific testing such as gill clips, fin clips and skin scrapes for cytological evaluation for the presence of parasites, filamentous bacteria, or diatoms. Although this appears to be the easiest option for submission, there are a few important factors to

consider. Fish undergo particularly **rapid postmortem degradation and autolysis**, and even with overnight courier shipping, this can often significantly affect the quality of downstream testing and may even preclude valuable histopathological evaluation. **External parasites also rapidly disappear** following death, leading to negative cytology results while postmortem overgrowth stemming from the particularly **rich aqueous commensal bacterial environment** often complicates interpretation of bacteriology results. Although targeted specific disease agent testing assays (e.g. viral PCRs) are common fodder for the typical farmed finfish aquaculture species, such **specific disease agent testing assays in pet fish are limited** to a few very select agents; e.g. koi herpes virus (KHV), viral hemorrhagic septicemia virus (VHSV). Given these limitations, **histopathology represents the single most valuable test for pet aquarium fish and therefore, submission of in-clinic sampled formalin-fixed tissue samples or whole formalin-fixed fish for histopathology is recommended as the first step for diagnostic evaluation.** Archiving of freshly-frozen tissues (kidney, spleen, and liver) and/or concurrently submitting sterile swabs or tissues (coelomic, kidney, spleen, or skin ulcers) for bacteriology will allow for follow up or ancillary testing if indicated.

Whole formalin-fixed fish are best submitted in sealed screw top containers with a preferred volume of fixative as close to 10 times the volume of tissue as possible. To ensure adequate formalin infiltration and rapid fixation of internal organs and tissues, the coelomic cavity should be opened by cutting a large wedge section out of the coelomic wall **(arrows; Fig 1).** In larger fish (> 6 cm long), the intestine can be detached at the anus and the internal organ mass loosened and pulled out slightly. The gill surfaces should also be exposed by removing the overlying opercular plate from at least one side of the fish **(asterisk; Fig. 1)**.

If in doubt, we encourage you to contact us and discuss your disease investigation so that we may assist you in getting the most out of your diagnostic laboratory submissions. *AHL*

Figure 1. Formalin-fixed pet fish for histopathology submission with the coelomic wall and opercular plate removed for better penetration of formalin.

Bacterial kidney disease (BKD) in rainbow trout (*Oncorhynchus mykiss*)

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AHL Newsletter, 2020;24(2):17.

During early spring, a commercial aquaculture operation was experiencing a slow cumulative increase in the daily mortality rate of a group of rainbow trout (*Oncorhynchus mykiss*). On-farm postmortems were performed on the daily mortalities and it was noted that some of these fish contained pale gray and granular-appearing swollen kidneys. Formalin-fixed tissue samples and plated bacterial cultures of gill, spleen, and kidney from the affected fish were submitted to the Animal Health Laboratory for further analysis and testing.

Histological evaluation of the head- and trunk-kidneys revealed numerous scattered, dense, coalescing interstitial aggregates of macrophages, fewer neutrophils, and occasional central cavitary regions of necrotic cellular debris and fibrin **(Fig. 1A & 1B)**. Diffusely, macrophages contained abundant numbers of **~ 0.5 µm diameter intracellular gram-positive bacilli (Fig. 1C & 1D)**. Within regions not effaced by the histiocytic inflammation, there was widespread expansion of interstitial hematopoietic precursor cells with separation of individual tubules, consistent with chronic systemic inflammation. Similar aggregates with intra-histiocytic coccobacilli were also present in the liver, spleen, and occasional gill filaments, while the same type of infiltrate also expanded the meninges within the brains **(Fig. 1E)**. Within the heart there was widespread endocardial cell hypertrophy with acute myofiber degeneration and necrosis, and similar dense aggregates of macrophages expanded the epicardial surface **(Fig. 1F)**. Both the epicardial macrophages and hypertrophic endocardial cells contained abundant numbers of the same intracytoplasmic coccobacilli. Bacterial cultures did not yield any pathogens; however, the **histological changes were highly consistent with a diagnosis of disseminated Bacterial Kidney Disease (BKD).** BKD is an important infectious disease of salmonid aquaculture worldwide. **It can affect all species of wild and reared fresh- and salt-water salmonids, but is particularly important in pacific salmonid aquaculture (e.g. Rainbow trout, Chinook and Coho salmon).** The causative agent is ***Renibacterium salmoninarum***, a gram-positive, non-motile, intracellular bacterium of the Micrococcaceae family. Inflammation can vary from granulomatous to pyogranulomatous with varying amounts of accompanying necrosis. The bacteria typically infect primarily macrophages and although the head and trunk kidney are a commonly-affected site (as indicated by the name), infections are often disseminated throughout multiple visceral organs and tissues. Vertical transmission through the egg occurs and although all ages are susceptible to infection, disease outbreaks usually tend to occur in older age classes.

On standard bacteriological media, *R. salmoninarum* is very slow-growing and even with the use of specialized media and the addition of antibiotics and antifungals designed to preferentially enhance its growth, cultures often become overgrown by other faster-growing microorganisms. Routine bacteriology is therefore not recommend for detection of infection. There are very few gram-positive bacterial fish pathogens and therefore the presence of characteristic bacteria and consistent histological changes are considered pathognomonic for BKD. Further confirmation can however be sought through PCR testing, ELISA serology, or 16S rRNA sequencing. *AHL*

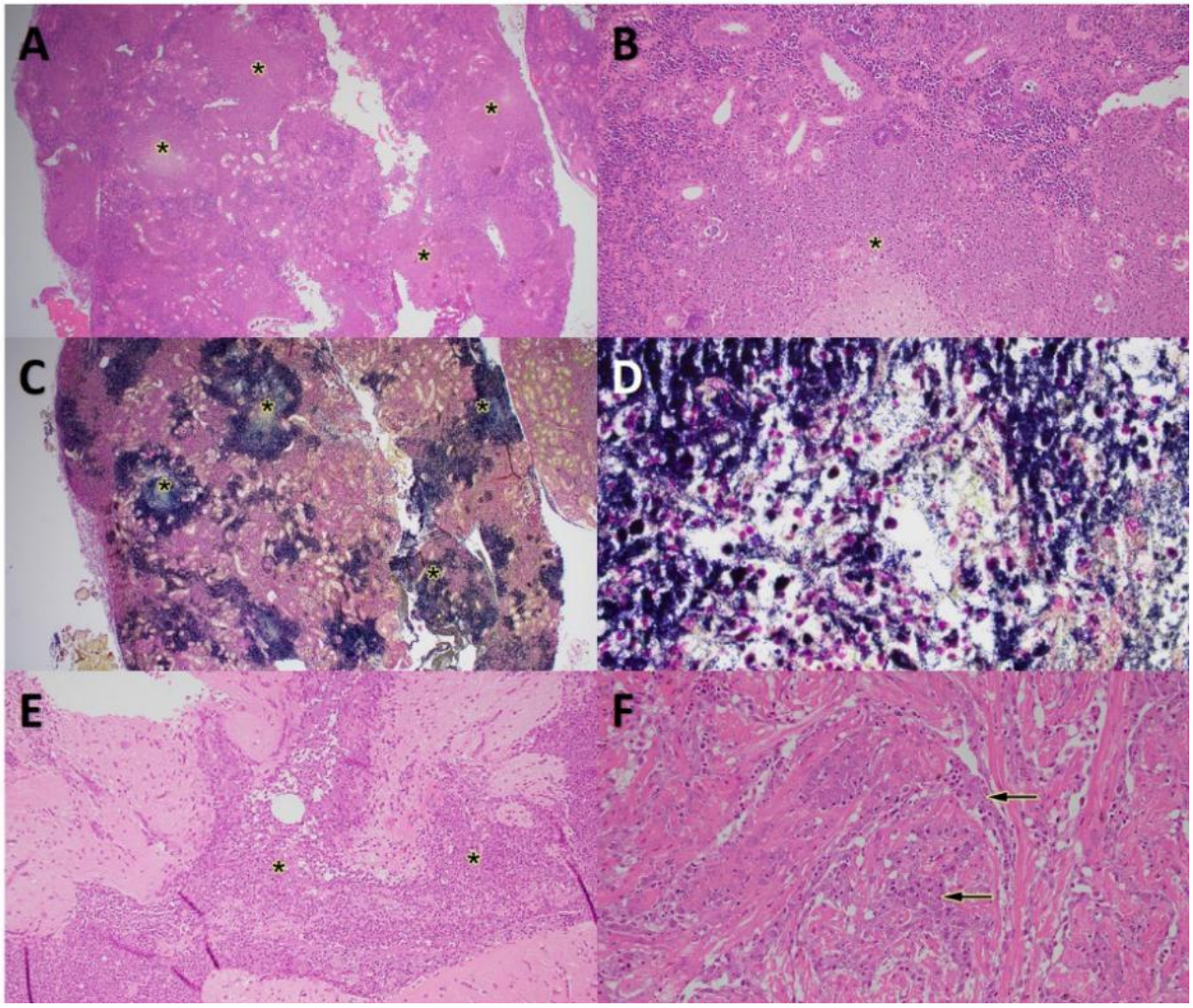

Figure 1. Bacterial kidney disease in farmed rainbow trout. **A&B.** Trunk kidney with disseminated coalescing interstitial histiocytic aggregates (asterisks). **C&D.** Lesions contain abundant intra-histiocytic gram-positive bacilli. **E.** Meninges are expanded by the same dense histiocytic infiltrates (asterisks). **F.** The heart contains widespread endocardial cell hypertrophy (arrows) with myofiber degeneration and necrosis, and intracellular bacilli.

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Mast cell tumor in a Dutch Warmblood horse

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AHL Newsletter 2020;24(2):19.

A biopsy was submitted to the AHL from a 3-year-old female Dutch Warmblood horse that presented with a firm painful cutaneous swelling on the back. Histopathology of the lesion revealed a subcutaneous mass composed of a monotypic population of round cells (**Fig. 1**). The round cells were arranged in densely cellular sheets and aggregates that were interspersed with eosinophils, necrotic tissue and fibrous stroma. The cells had uniform oval nuclei, abundant cytoplasm, and cytoplasmic granules that stained metachromatically with toluidine blue. No mitotic figures were observed in ten-400X high-powered fields. The round cells extended to the margin of the biopsied tissue. Immunohistochemistry for the tyrosine kinase receptor KIT (CD117) revealed positive cell membrane staining of the round cells (**Fig. 2**). The cell morphology, metachromatic granules and positive immunostaining for KIT supported a diagnosis of mast cell tumor.

Mast cell tumors (MCT) in horses (also called equine cutaneous mastocytoma or mastocytosis) are uncommon and comprise between 2-10% of equine cutaneous and mucocutaneous neoplasms. A search of the AHL database over the past decade identified 20 equine pathology submissions with a diagnosis of MCT. These involved mostly Warmblood (n=8) and Arabian horses (n=6). Age at the time of diagnosis ranged from 3 to 24 years and the majority were geldings (n=14). Clinical descriptions were incomplete for most submissions, but many of the horses had solitary tumors involving the skin or subcutaneous tissue. Tumor location included the neck (n=5), limbs (n=5), trunk (n=3), nostril (n=2), anus (n=1), and lymph node (n=1). Some tumors located on the distal limbs contained mineralized foci. Two horses had multiple MCTs. One horse with a MCT on the limb also had a lymph node metastasis and developed another MCT on the limb a few years after the initial diagnosis.

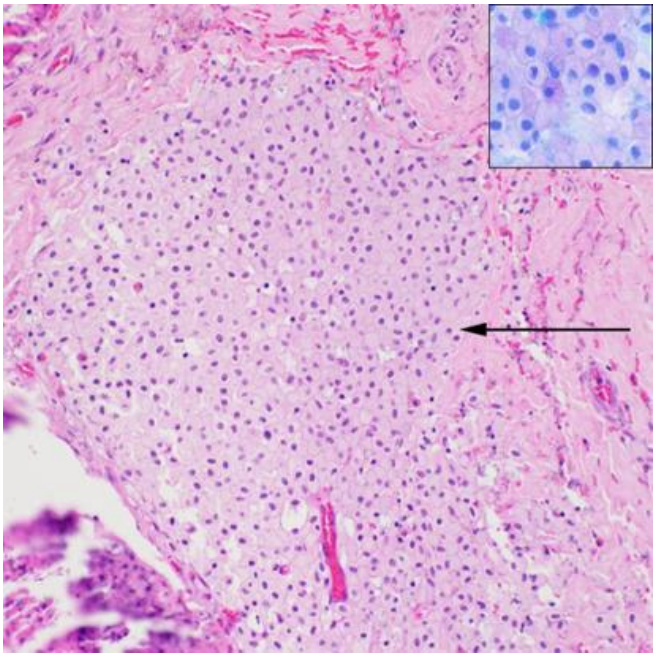

Figure 1. The subcutaneous mass is composed of a uniform population of round cells which exhibit metachromatic cytoplasmic granules in the toluidine blue stain (inset).

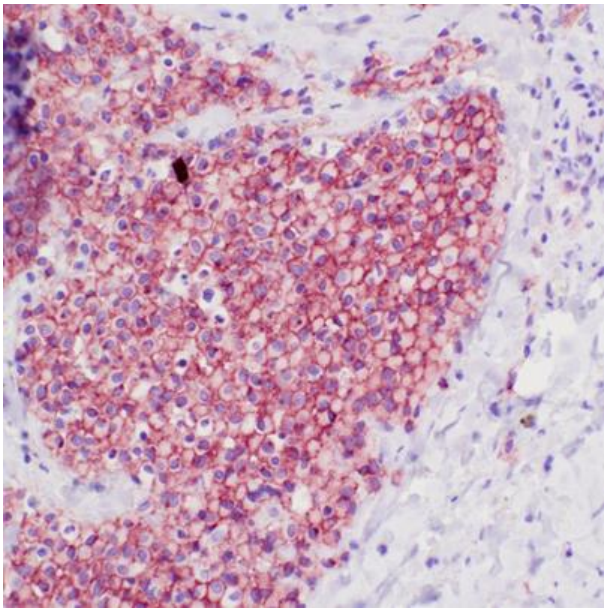

Figure 2. The round cells exhibit positive immunostaining for KIT (CD117).

The majority of equine cutaneous MCTs are benign and respond well to complete excision; however, there are reports of more aggressive tumors that may be recurrent or multicentric. In some cases, it is uncertain whether equine MCTs represent a true neoplasm or a reactive process. Histologically, these tumors are often characterized by eosinophil infiltrates, necrosis, fibrosis and mineralization, which requires differentiation from equine collagenolytic granuloma. MCTs are reported more often in male horses and usually present as a solitary skin mass on the head, neck, trunk or limbs. There does not appear to be a breed predilection, but Arabian horses are overrepresented in some surveys. Diagnosis of mast cell tumor in horses can be obtained by cytology or histopathology. *AHL*

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Fungal and mixed bacterial infection of the frontal sinus in a Hanoverian mare

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AHL Newsletter 2020;24(2):20.

A 10-year-old Hanoverian mare presented with a clinical history of chronic sinusitis with right-sided nasal discharge. Endoscopy revealed plaques of purulent material, and samples from the right frontal sinus were submitted for cytological examination and bacterial culture.

The cytological preparations contained hemorrhagic backgrounds with large numbers of markedly lytic neutrophils. Numerous extracellular bacteria composed of both small cocci and variably sized rod-shaped organisms were noted. Large numbers of extracellular, small, round, slightly refractive structures consistent with conidia were found throughout the slides as well as in clusters in association with stalks, or conidiophores **(Fig. 1)**. Also present were numerous septate, non-pigmented fungal hyphae which were intermixed with inflammatory cells **(Fig. 2)**.

Culture revealed growth of a mixed bacterial population along with 4+ growth of fungus consistent with *Aspergillus* spp.; fungal speciation was not requested.

Aspergillus species are saprophytes that are widely distributed in the environment, being found in soil, decaying vegetation, and organic debris. These organisms are opportunistic, and healthy animals are typically resistant to infection unless exposed to massive numbers of conidia or mycelia. Transmission is usually by aerosol. Conidia can remain in suspension in air for a long time due to their small size and hydrophobicity and are ubiquitous in the environment of horses. Typically, mucociliary clearance and phagocytosis by macrophages are effective in clearing inhaled organisms. The risk of infection may be increased in the presence of severe concurrent illness, or with immunodeficiency. This mare did not have any reported evidence of illness or other predisposing factors, and unfortunately was lost to follow up, so response to therapy cannot be reported.

Because of the universal exposure of horses to *Aspergillus* and other fungi, it is important to demonstrate the presence of conidia or hyphae within diseased tissue. This may be done via cytological evaluation of affected tissue or exudates, or biopsy of affected tissues, along with culture of that material. *Aspergillus* grows well on most fungal culture media. *AHL*

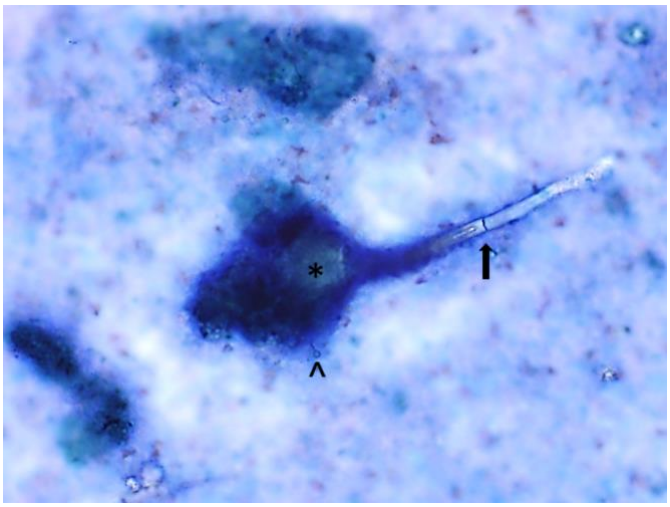

Figure 1. Aggregates of conidia (asterisk), individual conidia (open arrow head), and conidiophore (arrow) from the right sinus. (Photo: R. Egan, AHL).

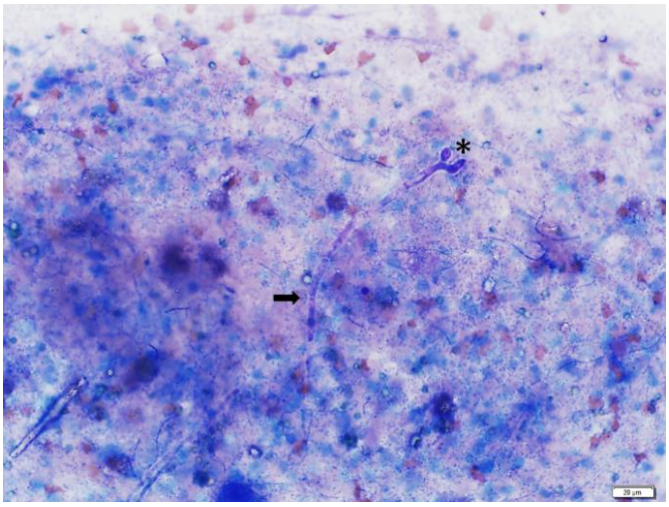

Figure 2. Fungal hyphae (arrow) with budding (asterisk). The slide background contains abundant lytic neutrophils and extracellular bacteria. (Photo: R. Egan, AHL).

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Canine mixed mammary tumor with extramedullary hematopoiesis and bone formation

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AHL Newsletter 2020;24(2):22.

Direct smears prepared from a fine needle aspirate of a 1 cm mass associated with the right caudal mammary gland in a 9-year-old intact female, mixed breed dog were submitted for cytological evaluation. The dog was otherwise clinically well.

The slides contained hemorrhagic backgrounds with limited numbers of epithelial cells present in small cohesive uniform clusters. Limited numbers of plump stromal cells were also present and were occasionally found embedded within lightly eosinophilic extracellular matrix. More intensely-staining eosinophilic matrix associated with low numbers of osteoclasts and osteoblasts, thus suggestive of bone, was also found. Foci of lightly basophilic material consistent with collagen were evident. The most prominent feature of this sample was the presence of numerous hematopoietic precursors. All stages of erythroid and granulocytic precursors were identified, and rare megakaryocytes were noted **(Fig.1)**.

These cytological features were consistent with a mixed mammary tumor with evidence of extramedullary hematopoiesis and osseous tissue.

As with all mammary masses, excision and histological evaluation were recommended. Histology confirmed the diagnosis of a mixed mammary tumor containing cartilage, bone, and limited numbers of hematopoietic cells **(Fig. 2)**. Epithelial cells appeared uniform and exhibited tubular formations with no evidence of invasion into surrounding tissues; thus this tumor was characterized as benign.

Mixed mammary tumors represent up to 50-65% of canine mammary tumors and contain epithelial, fibroblastic, cartilaginous, osseous and rarely, hematopoietic components. Mixed mammary carcinomas are rare. Of 384 mixed mammary tumors examined in one study, only 4% exhibited both extramedullary hematopoiesis of all three cell lineages, as noted in this case, as well as the presence of bone. The underlying cause of mammary extramedullary hematopoiesis (EMH) is not known. Several suggested hypotheses include: disruption of the bone marrow (for example related to drug therapy, myelofibrosis, marrow neoplasia) which stimulates circulating stem cells to find a favorable environment and differentiate into hematopoietic cells; and focal tissue insults which might induce the production of growth factors or cytokines that activate stem cells already present in these tumors.

Cytological evaluation of masses within the mammary chain can be used to rule out inflammation or the presence of other masses of non-mammary origin. However, histological evaluation of all mammary tumors is strongly recommended. Accurate diagnostic cytology of mammary tumors is associated with many challenges. Stromal mammary tumors may exfoliate poorly. The heterogeneous nature of some tumors may result in sampling or exfoliation of only one cell type. Mammary hyperplasia, dysplasia, benign epithelial cell tumors, and well-differentiated carcinomas form a continuum of morphologic appearance, often making cytological differentiation difficult. Inflammation may result in cytological changes which mimic malignancy. Finally, it should be noted that the presence of tissue, lymphatic, or blood vessel invasion by tumor cells, which has been identified as one of the most significant histological criteria of malignancy in canine mammary tumors, cannot be evaluated by cytology. *AHL*

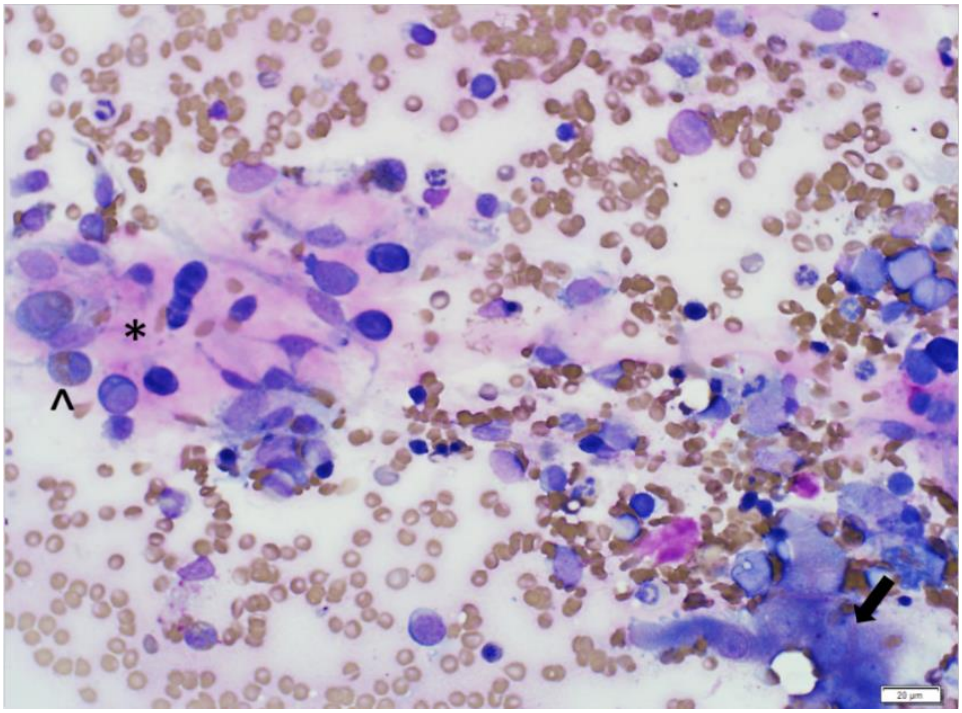

Figure 1. Cytological features of a canine mixed mammary tumor with evidence of hematopoietic cells (open arrow head), extracellular matrix (asterisk) and an osteoclast (arrow). (Photo: R. Egan, AHL).

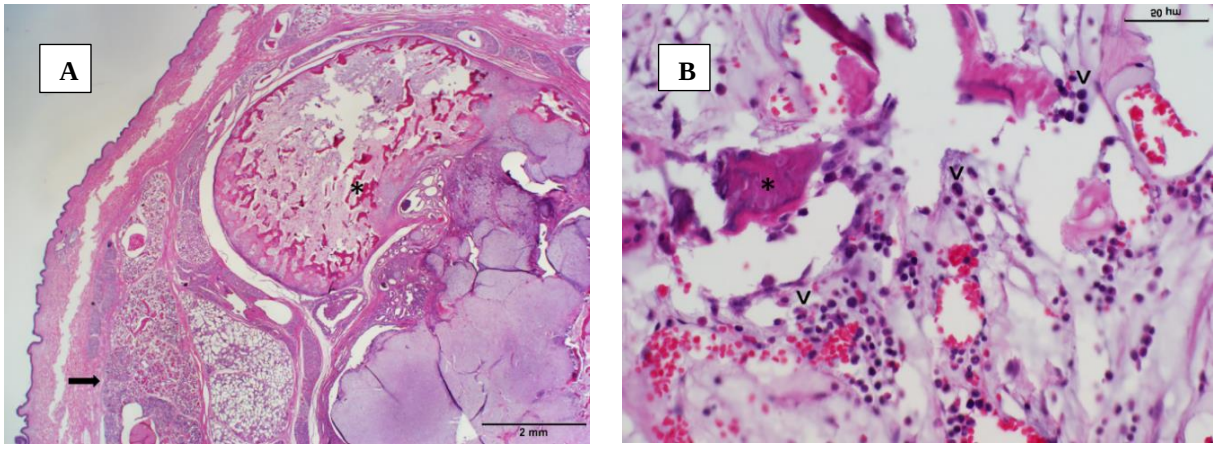

Figure 2. Histological sections of a benign canine mixed mammary tumor with mammary lobules (arrow, Figure 2A), and marrow amongst trabecular bone (asterisk, Figure 2A). Marrow (open arrow heads, Figure 2B) amongst trabecular bone. (Photos: R. Egan, AHL; L. Tatiersky, Vetpath Canada).

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