Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

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

Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

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

Update from the Director

The view from the Director's office

Checking in with our clients is an important means of ensuring that we continue to satisfy your testing needs. AHL does this by circulating a survey consisting of 27 questions that examine issues such as timeliness and accuracy of test results, responsiveness of staff, preferred method of communication, among others. We hope that you had an opportunity to respond to the recent survey. Thanks to all of you who took the time to complete the survey. The results are summarized and compared graphically to previous years' results, and we'll be reporting on these in a future newsletter. In addition, we read all of the comments provided, and our management team will discuss how we can best address the concerns and criticisms that are inevitable in any service industry such as ours.

Not only is it important to seek out opportunities for continuous improvement, but also it is a requirement of our accreditations that we conduct and document these activities. We are thankful to the members of our Quality Assurance (QA) team for the hard work they have put in this year in preparing for the biennial ISO/IEC 17025 audit which took place in October. More details and a photo of our stellar QA team are available in the staff highlights section.

Thinking back over the past few years, one cannot but be grateful that this holiday season is looking much more promising in many ways. Despite ongoing staffing and supply challenges, most businesses have recovered to pre-pandemic levels. For the first time in 4 years, AHL staff will be sitting together to enjoy a holiday lunch in person. We hope that you are also able to celebrate the season with your colleagues, families and friends. Best wishes for the holidays, happiness, and peace in 2024.

Maria Spinato, Director

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL holiday hours 2023/2024

Except for Mon. Dec. 25 (closed – no service), AHL-Guelph is open every day from Fri. Dec 22nd, 2023 until Tues Jan 2, 2024 with limited services. The University of Guelph is officially closed during this period.

Thurs. Dec. 21	Guelph and Kemptville - All laboratory sections open with full service
Fri. Dec. 22	Guelph and Kemptville - All laboratory sections open with full service
Sat. Dec. 23	Guelph: specimen receiving, emergency mammalian postmortems, full bacteriology set-up, as well as clinical pathology testing; Kemptville closed
Sun. Dec. 24	Guelph: specimen receiving, emergency mammalian postmortems; Kemptville closed
Mon. Dec. 25	Guelph and Kemptville laboratories closed
Tues. Dec. 26	Guelph: specimen receiving, emergency mammalian postmortems; Kemptville closed
Wed. Dec. 27	All laboratory sections open with limited services (Guelph - 9:00 am to 5:00 pm; Kemptville 8:30-4:30 pm)
Thurs. Dec. 28	All laboratory sections open with limited services (Guelph - 9:00 am to 5:00 pm; Kemptville 8:30-4:30 pm)
Fri. Dec. 29	All laboratory sections open with limited services (Guelph - 9:00 am to 5:00 pm; Kemptville 8:30-4:30 pm)
Sat. Dec. 30	Guelph: specimen receiving, emergency mammalian postmortems, full bacteriology set-up, as well as clinical pathology testing; Kemptville closed
Sun. Dec. 31	Guelph: specimen receiving, emergency mammalian postmortems; Kemptville closed
Mon. Jan. 1	Guelph: specimen receiving, emergency mammalian postmortems; Kemptville closed
Tues. Jan. 2	All laboratory sections open with limited services (Guelph - 9:00 am to 5:00 pm; Kemptville 8:30-4:30 pm)
Wed. Jan. 3	Guelph and Kemptville - All laboratory sections open with full service

Guelph **drop box and fridges available 7AM to 10PM** and Kemptville **drop box and/or fridges are available 365/24/7** for specimen drop off.

For full details, please see our website – www.ahl.uoguelph.ca

Note: Last day for system-generated invoicing is Tuesday December 19th, 2023.

Client Services update

Tim Pasma

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(4):4.

Purolator adding an extra day to transit times

Purolator has informed us that they will be adding an extra day of transit time to their Purolator Ground Service. This change will be in effect from October 29, 2023, until Spring of 2024 and will account for increased volumes during the holiday season and occurrences of inclement weather. The standard transit time for shipments by Ground Service will be 2 days or more.

Please ensure that you package materials to prevent freezing of samples due to the extra transit time and colder weather at this time of year. To prevent formalin from freezing, add 1 mL of ethanol to 10 mL of formalin. Specimens such as EDTA tubes should be packaged in an insulated container along with room temperature ice packs.

Postmortems protocols for OVC-HSC cases

Our clients may not know that our facilities are shared between the AHL and the Department of Pathobiology at OVC. For clients that submit cases to OVC, please note that if the patient dies while at OVC-HSC, the case can be sent for a postmortem conducted by graduate students and pathologists in the Pathobiology department. This postmortem is done at no charge to the client or owner and enables OVC-HSC to follow up on cases from its hospital, and to meet its mandate of education, research and training opportunities for graduate students.

If AHL receives a submission that has previously been a case at OVC-HSC, our staff will contact the submitting veterinarian to confirm whether they want the postmortem to be conducted by AHL pathologists (standard fees apply) or the Department of Pathobiology.

Submission and humane transport of live animals for postmortem

Andrew Brooks, Tim Pasma, Hein Snyman

Animal Health Laboratory, University of Guelph, Guelph ON

AHL Newsletter 2023(4):5.

The AHL encourages submission of live animals for immediate euthanasia and postmortem to promote a high rate of diagnostic accuracy in poultry submissions, and in outbreaks of diarrhea in young pigs, ruminants and agricultural rabbits.

Please note that only livestock weighing less than 50 kg can be euthanized at AHL-Guelph. Livestock weighing greater than 50 kg will be transferred to the OVC-Large Animal Hospital to be euthanized by a large animal clinician and extra fees will apply. **At AHL-Kemptville, euthanasia of animals weighing above 50 kg is not available**, and all submissions of live animals must be coordinated in advance with the Kemptville pathologists.

We encourage all submitters to **please contact the AHL in advance** to confirm the arrival time, and to facilitate preparations for receiving live animals. Note that AHL euthanasia fees apply, and additional fees may be charged depending on the species, number of animals, and the size.

We remind submitters that live animals must be transported humanely. The Provincial Animal Welfare (PAW) Act requires that veterinarians report all suspected cases of animal abuse and AHL veterinarians will confer with PAWS inspectors to determine if an investigation is warranted in suspected cases of inhumane transport.

Examples of inappropriate restraint and transportation methods include:

- hog-tying calves, adult sheep or goats;
- baby pigs or chickens placed in sealed plastic tubs or styrofoam containers;
- pigs or chickens submitted in tied feed sacks;
- compromised animals in open air (back of a truck) in any type of weather.

Examples of acceptable transport containers include:

- dog or cat kennels for small pigs;
- cardboard boxes of appropriate size with ventilation holes for baby pigs or chickens;
- poultry crates;
- large dog kennels or enclosed bedded truck cabs for larger pigs, calves, small ruminants.

The transport containers should be of sufficient size to avoid crowding or smothering, and animals should be protected from extreme temperatures. **Animals that exceed the weight limits or that cannot be transported humanely must be euthanized on the farm**, and the practitioner is encouraged to collect appropriate samples from a field postmortem for diagnostic testing. For guidance with field postmortems, please refer to AHL LabNote 42: *Field and clinic postmortems* and AHL LabNote 2: *Tips for practitioners for field postmortems* (AHL LabNotes | Animal Health Laboratory (uoguelph.ca)).

Please contact the AHL to consult with a pathologist if assistance is required with sample and test selection when performing on-farm postmortems. *AHL*

Detection of *Clostridium botulinum* toxin genes by PCR: New test now available at AHL to diagnosis botulism

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AHL Newsletter 2023; 27(4):6.

Botulism is a frequently fatal paralytic disease of mammals and birds caused by the toxins produced by *Clostridium botulinum*, an anaerobic bacterium that persists in the environment in the form of dormant spores which can remain viable for years. *C. botulinum* produces seven distinct types of toxins, designated A through G. Two of these toxins, types C and E, most often cause disease outbreaks in animals. Type C affects filter-feeding birds, waterfowl, shorebirds, and domestic birds, as well as cattle, horses, and ranch mink. Type E, on the other hand, is relatively common in wild birds of the Great Lakes Region, and does not occur elsewhere in Canada.

Currently, no veterinary diagnostic lab in Ontario or Canada offers testing for *C. botulinum*. To replace the mouse inoculation test (MIT) used in the past, AHL verified a PCR-based detection of 6 *C. botulinum* toxin types A, B, C, D, E, and F. Whereas MIT is used for detection of *C. botulinum* toxins primarily in serum samples, PCR is used for detection of toxin genes and has been validated using liver and cloacal swabs from birds. There were no differences in detection observed between these two sample types, indicating that both are acceptable for PCR testing. As serum contains only *C. botulinum* toxin, not bacteria, it is not an acceptable sample for the *C. botulinum* PCR test. Furthermore, as this PCR is validated only for avian samples, samples submitted from mammals will be tested as non-routine (unvalidated) testing.

Funding for this project was provided by the Ontario Animal Health Network (OAHN) and supported by OAHN – Wildlife (https://www.oahn.ca/network/wildlife/).

To submit samples, collect liver or gastrointestinal content in sterile, leak-proof containers and refrigerate immediately. Ship to the lab in insulated container with freezer packs. The test code is ‘bot’ and the current fee is $120 per sample.

Staff highlights

AHL recently underwent another successful ISO/IEC 17025:2017 audit by Standards Council of Canada. This 4-day audit involved evaluation of multiple laboratory sections by a team of 9 highly-skilled external auditors. The auditors were very complimentary of AHL processes and procedures, a testament to the hard work that the QA (quality assurance) team members accomplished not only in preparation for the audit, but also throughout the year as their activities support the quality objectives of the AHL.

ISO/IEC 17025:2017 accrediation is an internationally-recognized quality standard that is required for veterinary diagnostic laboratories involved in regulatory and export testing. It also provides assurance for all clients of the accredited laboratory that their samples are tested under the most rigorous scientific requirements. A comprehensive listing of AHL's accreditations can be located on our website at: https://www.uoguelph.ca/ahl/about-us/accreditation

Congratulations QA team on another successful ISO/IEC 17025:2017 audit!

Michael Morrison, Shennell Savoury, Elizabeth King, Ashley Lacey, Liz Karn
Laboratory Services Division (AHL) Quality Assurance team

OAHN update – December 2023

Mike Deane, Tanya Rossi

Animal Health Laboratory, University of Guelph, Guelph, ON

This fall, the OAHN networks worked on research projects and held multiple species-specific network meetings. We are also planning our 2024 Annual General Meeting for February of next year – this meeting allows our species networks to take stock of the previous year, share their successes and challenges with their peers, and plan for veterinary projects and resources in 2024. To view any of our network reports and research projects, go to OAHN.ca and navigate to the species in which you are interested.

A message from the OAHN Bees Working Group: Varroa and Hive Health

As we head into the end of the beekeeping season, it is important to remember that colony health and winter survival depend on proper colony management. Varroa mites are the primary cause of colony mortality in Ontario. While beekeepers are treating for varroa mites, they must recognize that all treatments have limitations. Beekeepers should not assume their varroa levels are under control simply because a treatment has been applied. Beekeepers are strongly encouraged to take the extra steps to check their varroa levels, the efficacy of treatments and take further action if needed.
We are also reminding beekeepers to use the 2023 Ontario Varroa Monitoring Campaign questionnaire to share their data. These data are important to capture the reality of what is occurring with varroa and support future projects addressing this very serious pest.
https://www.oahn.ca/resources/varroa-monitoring/

OAHN 2023 Public Health Update

The OAHN Companion Animal Network has created the 2023 Public Health Update. This annual update was created especially for public health professionals in Ontario to highlight pertinent topics from the last 12 months from the OAHN companion animal and other species networks, and to help strengthen the link and communication between animal health and public health networks. This year's update includes information about: HPAI in dogs and cats, H5N1 in feral cats, echinococcosis, Lyme disease, blastomycosis, *Salmonella* and raw food diets, piscine lactococcosis, wildfire smoke and equines, *Chlamydia* and pet birds, rabies titres in imported dogs, rabies updates, and more! Find it here:
https://www.oahn.ca/resources/2023-oahn-public-health-update/

New Reports
Most OAHN networks create reports once per quarter. To view any of the veterinary reports below, please click on the OAHN icon for each network, or go to OAHN.ca and navigate to the species in which you are interested.

- OAHN survey: CIRDC, *Anaplasma*, HW
- OAHN project: H5N1 HPAI – feral cats
- Resistant hookworm detections, Canada
- RHDV2 subsidized testing still available!
- Rabies update, rabies titre project results

- Ensuring optimal test results from your diagnostic laboratory: Garbage in…garbage out
- Poultry veterinary survey highlights – Q2 2023
- Events and news

- Bits ‘N Snips
- Colic due to moldy food
- Network member reports
- Syndromic and lab surveillance dashboard

- Interesting cases from the Animal Health Lab
- Bovine provincial slaughter condemnation data
- Interactive Animal Pathogen Dashboard (IAPD) project update
- Identification of *Culicoides* species found in selected areas of Ontario from Jun – Sep 2022.

- Disease surveillance discussion
- Animal Health Laboratory reports
- Ontario slaughter statistics
- CanSpot ASF surveillance update
- International disease surveillance topics

RUMINANTS

Japanese yew intoxication in a herd of cattle

Dominque Comeau

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AHL Newsletter 2023;27(4):10.

In a herd of ten Shorthorn cattle, there were three sudden deaths within 12 hours. Two of the affected animals were 4-year-old cows, one of whom had calved in the previous month. The third animal was a 4-year-old bull. All animals showed no preceding clinical signs, were noted to have no lameness or abnormal behavior prior to death, and were seen eating well and chewing cud just before being found dead. They had been in the same field for one month. The field was walked, and no debris or material of concern was noted at that time. The most recent sudden death was submitted to the Animal Health Laboratory for postmortem examination.

On external examination, there were external injuries consistent with scavenging; the wounds were postmortem and considered unrelated to the cause of death. The rumen was filled with abundant moist green fibrous digesta. There were scattered twigs, as well as multiple symmetrical elongated flat leaves with a defined, slightly curved point at the tip. These were determined to be leaves from the Japanese yew plant (**Figs. 1**, **2**). There were also fragments of woody stems and rare small red-brown berries/seeds in the rumen.

Japanese yew is an ornamental shrub that grows well in colder climates, and thus is very common in Canada and the northern United States. All parts of the leaves and seeds are highly toxic to cattle, as well as other grazing animals such as goats, sheep, and horses. Exposure is most often due to grazing in areas where the shrubs grow, or by feeding landscaping trimmings to livestock. The plant is toxic year-round, and maintains its toxicity when dried. It contains alkaloids called taxines which are highly cardiotoxic; they act as cardio-depressants by inhibiting sodium and calcium conduction within the muscles of the heart, leading to diastolic cardiac arrest. Exposure to even a small amount of the plant is dangerous, as ingestion of only 1-10 grams per kilogram body weight is sufficient to cause fatal toxicity. The toxins are rapidly metabolized and cannot be analyzed in tissue or digesta, and intoxication causes no specific gross lesions. Diagnosis depends on the identification of the plant material in the mouth, esophagus, or forestomaches.

Following identification of the plant in this case, it was confirmed that the owner had cut the shrubs around his home and thrown the trimmings into the pasture with the cattle. This case highlights the importance of careful monitoring of feed/plants/water that livestock have access to, and of considering all potential sources of exposure in cases of suspected toxicity. The latter is especially relevant in this case, as the trimming pile was not considered in the initial walkthrough of the field, and was not included in the original history. The cattle were removed from the pasture and no further losses were reported.

Figure 1. A selection of the leaves, seeds, and stems from the rumen of the affected cow.

Figure 2. Reference photo for the appearance of Japanese yew.
http://www.bio.brandeis.edu/fieldbio/pkenlan/HTML/Taxaceae/taxus_cuspidata.html

References

1. Wilson CR, et al. Taxines: A review of the mechanism and toxicity of yew (*Taxus* spp.) alkaloids. Toxicon 2001;39 (2-3):175-185.
2. Sula JM et al. Characterization of cardiac lesions in calves after ingestion of Japanese Yew (*Taxus cuspidata*). J Vet Diagn Invest 2013;25(4):522-26.

SWINE

Make the change to *E.coli* genotyping

Đurđa Slavić, Josepha DeLay

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AHL Newsletter 2023;27(4):12.

AHL will discontinue agglutination serotyping for identification of F4/K88 and F5/K99 *E.coli* fimbrial antigens as of December 2023 because reagents for this test method are no longer commercially available. In place of agglutination serotyping, veterinarians are encouraged to request genotyping of *E.coli* isolates from young animals with diarrhea. This includes ruminants ≤ 5 days of age and pigs ≤ 14 weeks of age. Results will determine if the isolate is an enterotoxigenic strain of *E.coli* (ETEC). PCR-based ETEC genotyping provides more information about a broader range of fimbrial and toxin genes. The test is also more sensitive and more accurate than serotyping. There are certain toxin/fimbrial genes that are important only in swine, and a subset that are important in both swine (S) and ruminants (R) (**Table 1)**. All swine F18 positive isolates will also be automatically checked for the presence of the *stx2e* gene to exclude/confirm edema disease-causing isolates.

Fimbrial genes	**Toxin genes**
F4 / K88 (S)	STa (S/R)
F5 / K99 (S/R)	STb (S)
F6/987P (S)	LT (S)
F18 (S)	STX2e (S)
F41 (S/R)	

Table 1. Fimbrial and toxin genes targeted by ETEC genotyping. S=swine, R=ruminants.

When requesting bacterial culture from intestine or feces of young animals within the above-specified age groups and for which *E.coli* enteritis or edema disease is a differential diagnosis, please also request ETEC genotyping (**Fig. 1**).

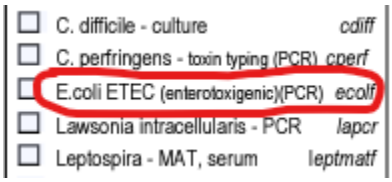

Figure 1. AHL submission form listing *E.coli* ETEC genotyping (PCR) test.
This test is included on ruminant and swine submission forms.

Only *E.coli* isolates that are positive for both toxin and fimbrial genes are designated by the lab as enterotoxigenic *E.coli* (ETEC). These *E.coli* types are considered pathogenic when found in conjunction with other clinical and morphologic evidence of colibacillosis.

References
1. Fairbrother JM. Nadeau E. Colibacillosis. In: Diseases of Swine, 11th ed. Zimmerman JJ et al, eds. Wiley-Blackwell, 2019:807-834.
2. Izzo M, et al. Neonatal diarrhea. In: Large Animal Internal Medicine, 5th ed. Smith BP, ed. Elsevier, 2015:324.

Just how significant is that pathogen?

Josepha DeLay

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AHL Newsletter 2023; 27: (4):13.

The use of new techniques such as high throughput sequencing (HTS) in disease investigation can lead to identification of many potential pathogens in a given clinical scenario. Meeting the criteria of a modified version of Koch's postulates establishes causation between a pathogen and a disease condition. However, this is complicated by subclinical infections and multifactorial disease conditions which do not necessarily follow direct rules of cause and effect.

Disease causation is straightforward for some swine pathogens, such as influenza A virus and transmissible gastroenteritis virus (TGEV). Detection of these pathogens in pigs is associated with specific disease conditions, and these pathogens aren't normally detected in healthy animals. Other pathogens may not have such a clear cut, consistent role in disease causation in every clinical situation, and infection may not always result in disease. PCV-2 is an example of this more complex scenario.

Correlating PCR or culture results with results of other test methods, especially histopathology and related direct detection tests such as immunohistochemistry (IHC) and *in situ* hybridization (ISH), is very helpful in assessing a pathogen's role in individual clinical cases. PCR and culture are important to assess the presence and quantity of a pathogen. Histopathology and direct detection techniques (IHC, ISH) allow visualization of lesions potentially caused by the pathogen, and determine if the pathogen is present in association with the lesion of interest. Including formalin-fixed histopathology samples with a case can dramatically strengthen the certainty of a diagnosis. **In diagnostic pathology, seeing really is believing.** Tissues are assessed for presence of lesions indicative of infection with various pathogens. Where available, direct detection tests are used to confirm presence of the pathogen in association with the characteristic microscopic lesion. For some disease conditions such as PCV2 and PCV3-associated disease, confirmation of the diagnosis is dependent on identification of characteristic histologic lesions and co-localization of virus in lesions.

As an example, interpreting the clinical significance of a moderately high Ct value for porcine rotavirus can be problematic in young pigs with mild diarrhea. In some pigs, rotavirus may be present in the intestine with a low to moderate viral load, but may not necessarily be causing disease. If histologic sections from intestine of these same PCR-positive pigs have evidence of intestinal villus atrophy with attenuated enterocytes (i.e., lesions typical of viral enteritis), presence of these compatible lesions adds support to the clinical significance of the positive PCR result. If rotavirus antigen is also detected in these lesions using IHC, then we can be confident that rotavirus is contributing to diarrhea in the group. Conversely, if rotavirus is detected by PCR but these pigs lack histologic lesions of atrophic enteritis, the contribution of rotavirus to clinical enteritis is less likely. As always, selecting the 'right' pigs to test is an important factor in correct interpretation of test results, and should focus on acutely affected, untreated animals.
The same approach is also very useful in the investigation of emerging pathogens, where we might not yet have a complete understanding of the prevalence and significance of a pathogen in various

populations and geographic areas. Relying on a single test method can lead to misinterpretation of results, and the use of multiple test methods in conjunction with histopathology is encouraged. Omitting histopathology is a missed opportunity. Finally, circling back to correlate test results with clinical history and gross postmortem lesions is vital to the understanding and final interpretation of diagnostic testing.

Congenital tremors in piglets infected with atypical porcine pestivirus

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AHL Newsletter 2023;27(4):14.

Two sucking piglets from a farrow-to-finish operation were submitted to the AHL for postmortem following a one-month history of an increased prevalence of piglets developing full body shaking/tremoring and splayleg within the first week of age. Approximately 70-80% of litters were affected, with some litters having 2-3 animals affected, and others involving all littermates. Affected piglets were farrowed from both gilts and multi-parity sows. The majority of the piglets survived with eventual resolution of clinical symptoms. There were no other obvious health concerns (e.g., scours, coughing, wasting) involving these piglets, and no sows were affected.

Prior to postmortem, piglets were bright and alert, but aggressively shaking/tremoring (link to video posted below). Postmortem findings revealed the piglets were in good body condition, and there were no visible gross lesions in the brain, spinal cord, or any other organ system. Microscopic changes were very subtle and consisted solely of patchy subtle cerebral and cerebellar neuronal degeneration, and mild multifocal white matter vacuolation in the brain and spinal cord. Sections of brain were sent to the Iowa State Veterinary Diagnostic Laboratory, and were PCR positive for the detection of atypical porcine pestivirus (APPV).

APPV was first identified in the United States in 2015 and has a global distribution; however, its clinical relevance is poorly understood. APPV has been associated with congenital tremors (CT) in newborn pigs. Clinical signs include muscle spasms, posterior paresis and splayleg. Litters from gilts are most commonly affected. There are no gross lesions associated with infection, and hypomyelination or demyelination of the brain and spinal cord can sometimes be seen microscopically. Experimental infection of gilts/sows with APPV during gestation can result in transplacental (vertical) transmission with development of CT in offspring. Horizontal transmission can also occur, but infection seems to be transient, and piglets do not develop clinical signs. In litters infected with APPV, morbidity is highly variable and ranges from 0–100%. There is no treatment for APPV infection; therefore, strategies for control often include acclimatization of replacement gilts to ensure APPV exposure before breeding, testing of semen for APPV RNA before insemination, and feedback on farms with clinical CT cases.

Link for the video: https://www.youtube.com/shorts/iBw-udZ54FI

References

1. Hause BM, et al. Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. *J Gen Virol.* 2015;96:2994–98.
2. Buckley AC, Falkenberg SM, Palmer MV, et al. Distribution and persistence of atypical porcine pestivirus in experimentally inoculated pigs. J Vet Diagn Invest. 2021;33(5):952-955.
3. de Groof A, et al. Atypical porcine pestivirus: a possible cause of congenital tremor type A-II in newborn piglets. *Viruses.* 2016;8:271.

Streptococcus gallolyticus in poultry

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AHL Newsletter 2023;27(4):15.

Since the introduction of MALDI-TOF MS (mass spectrometry) for bacterial identification, there is an increased number of bacterial species reported as being associated with a variety of clinical conditions in birds. Starting in 2021, *Streptococcus gallolyticus* has been routinely identified from cases of meat turkeys, chicken broilers, chicken layers and waterfowl in mixed culture, and occasionally in pure cultures in meat turkey cases.

Streptococcus gallolyticus is a Gram-positive coccus that is arranged singly, in pairs, or short chains. It is part of the Lancefield group D streptococci having the subclassification of *S. gallolyticus* subspecies *gallolyticus* (formerly *Streptococcus bovis* biotype I). The Lancefield group D streptococci are considered part of the intestinal microbiota of wild animals, companion animals, farm animals (including poultry) and humans. They are also occasionally associated with opportunistic infections in humans and animals.

Turkey poults, broiler chickens, ducks and layer chickens are the avian species most commonly infected with *S. gallolyticus*. Turkeys and waterfowl are affected in the first 3 weeks post hatch, while chickens tend to be affected between 5-7 weeks or 44-55 weeks of age. The most common **clinical signs** are depression, listlessness, pasted vents, watery droppings, and dehydration. There is poor growth performance with increased culls and increased mortality in commercial flocks. In ducks, there is a drop in egg production and development of neurologic signs (i.e., fine head tremors, disorientation, dorsal recumbency, leg paddling). Chickens can also have lesions of vegetative valvular endocarditis, subclinical infection, or increased mortality with increased feed conversion.

The most common **postmortem** lesions include enlarged, congested, friable livers and spleens. The liver and spleen have multifocal necrotic foci (**Fig. 1**) and a marbled appearance. There is polyserositis as well as pale intestines that are segmentally distended with watery contents, and the cecal content appears frothy. The lymphoid organs are atrophied in individual birds that have not reached sexual maturity. Rule-outs for liver necrosis include *E. coli* septicemia, blackhead (histomoniasis), salmonellosis, pasteurellosis, clostridia-induced cholangiohepatitis, erysipelas, viral tumors, and tuberculosis.

There are multiple **risk factors** associated with development of *S. gallolyticus* infection, including immunosuppressive diseases (FAdV, IBDV, ALV-J-virus, HEV, circovirus), skin wounds, and other conditions causing loss of gastrointestinal tract integrity that can result in bacterial overgrowth, migration of bacteria, and septicemia. Environmental factors such as poor litter conditions, high stocking density, insufficient numbers of feeders and waterers, and inadequate lighting programs can result in competition, aggressive behavior, and stress which can all impair the immune response, resulting in a variety of infections, including *S. gallolyticus*. Poor hygiene levels and water quality can also play a role in the pathogenesis of disease.

As *S. gallolyticus* often presents in combination with other bacterial, viral and protozoal infections, its virulence potential remains to be established. AHL now routinely monitors all clinical cases for the presence of this bacterium, and reports it when isolated.

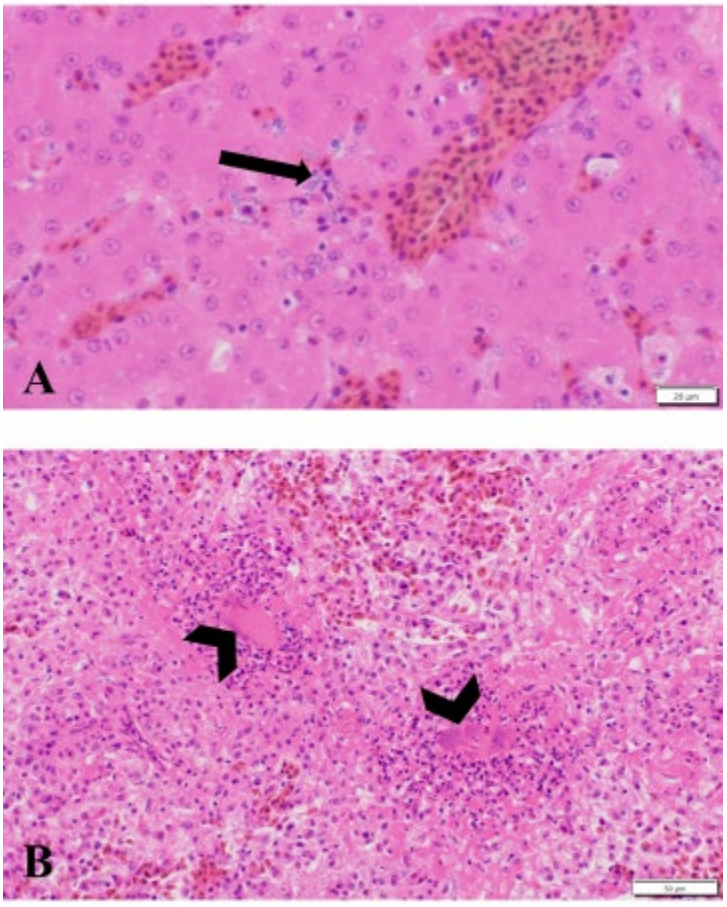

Figure 1. *S. gallolyticus* septicemia in 12-day-old meat turkeys.
A: Liver has numerous intravascular bacteria (basophilic stippling at arrow). H&E stain, 60x.
B: Spleen contains intravascular bacteria (arrow heads) with perivascular necrosis and fibrin deposition. H&E stain, 40x.

References:
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Intestinal T-cell lymphoma in an African pygmy hedgehog

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An approximately 2-year-old female African pygmy hedgehog (*Atelerix albiventris*) stopped eating over a 3-week period, developed mucoid, dark green-black tarry stool, and progressively became more listless and weaker. No other abnormalities were detected on physical examination and whole-body radiographs and ultrasound did not detect any abnormalities or obvious signs of a tumour. CBC showed a moderate leukocytosis with a mature neutrophilia, suspected toxic changes, and a moderate regenerative anemia. No abnormalities were detected on standard biochemistry profile. Given the neutrophilia and tarry stool, a possible bacterial infection was considered, and a course of antibiotic therapy (enrofloxacin and amoxicillin/clavulanic acid) as well as prednisone was prescribed.

The hedgehog's clinical condition deteriorated, and she was humanely euthanized with pentobarbital in the caudal jugular vein. An in-clinic postmortem identified hepatic lipidosis with the liver being grossly enlarged and yellow, and cut sections floating in formalin. The intestinal tract appeared thickened and was diffusely distended with clear, sometimes green, mucoid luminal fluid. Representative formalin-fixed tissue samples were taken and submitted to the Animal Health Laboratory for further analysis.

Histological sections of the intestinal tract revealed some segments with large, dilated villus tips typified by interstitial edema of the lamina propria and prominent lacteal lymphangiectasia (**Fig. 1A & inset**). Other segments contained marked expansion of the lamina propria of individual villi and mucosal folds by a dense infiltrate of small neoplastic lymphocytes (**Fig. 1B**). Neoplastic lymphocytes often extended into the adjacent and overlying mucosa, forming small clusters of intra-epithelial lymphocytes (**Fig. 1C**). Infiltration of the lamina proprial gut-associated lymphoid aggregates with crowding and compression of the deep mucosal crypts was also present (**Fig. 1D**). These findings were consistent with a diagnosis of intestinal lymphoma with lymphangiectasia occurring as a secondary consequence due to regional obstruction of villus lacteals. Additional histological findings included anorexia-associated hepatic lipidosis, and rare foci of villus tip mucosal erosion explaining the neutrophilia and tarry feces.

Hemolymphatic tumours are considered the second-most common neoplasm in hedgehogs (~ 11% of all neoplasms), with the intestinal tract being frequently involved. A retroviral cause has been suggested for hedgehog lymphoma; however, this has not yet been proven. A T-cell phenotype is most common for intestinal lymphomas in other domestic animals; however, this has not been particularly investigated in this species. As such, additional immunohistochemical (IHC) stains for T-lymphocyte (CD3) and B-lymphocyte (CD20, PAX5 and CD79a) markers were performed to further classify the lymphoma in this case (**Fig. 2)**. Neoplastic lymphocytes exhibited positive immunoreactivity to CD3 antibodies and were negative for CD20, PAX5 and CD79a, consistent with a T-cell phenotype in this hedgehog. Regional gut-associated and visceral lymphoid tissues acted as positive internal controls. As they exhibited the expected staining patterns, these results further confirmed the utility of these markers in this species.

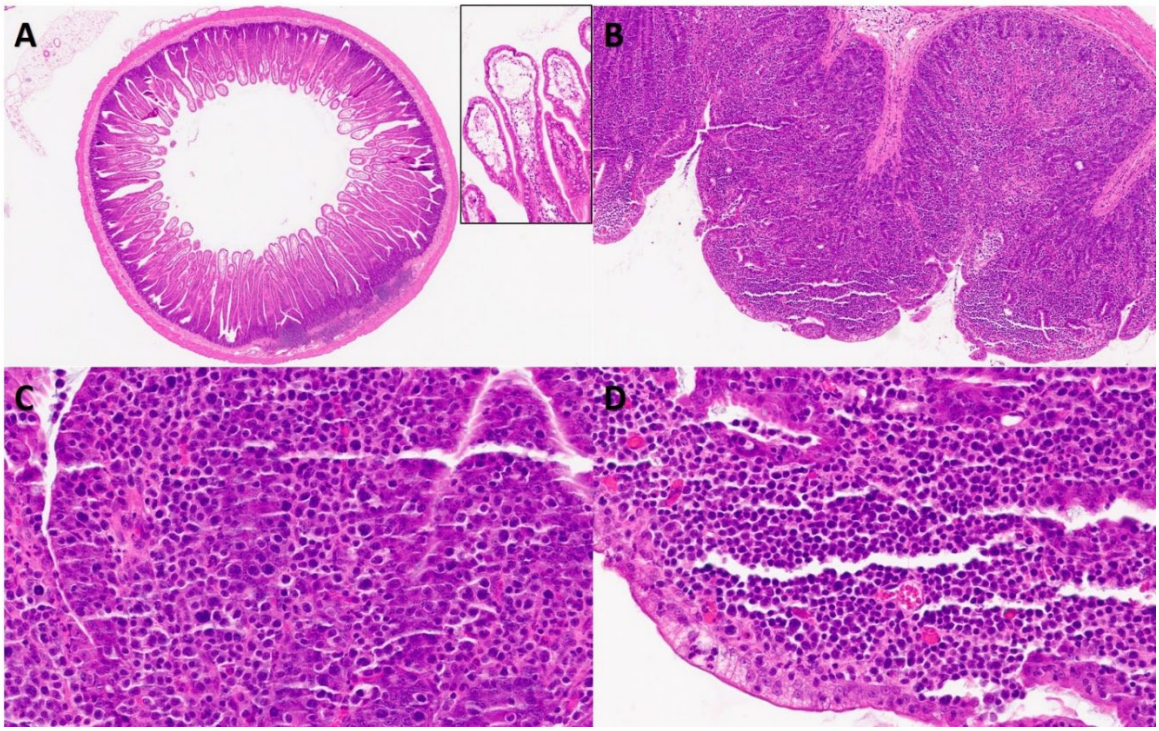

Figure 1. Histological findings in a 2-year-old African pygmy hedgehog with intestinal T-cell lymphoma. **A. Intestine.** Villus tips contain prominent dilated lacteals that contain fine flocculent proteinaceous fluid (inset). H&E stain. **B. Intestine.** There is diffuse neoplastic lymphocyte infiltration of the lamina propria that fills and obscures the villus outlines. H&E stain. **C and D. Intestine.** Neoplastic lymphocytes extend into the surface mucosal layer forming jumbled clusters while lamina propria is also expanded by the same neoplastic lymphocytes. H&E stain.

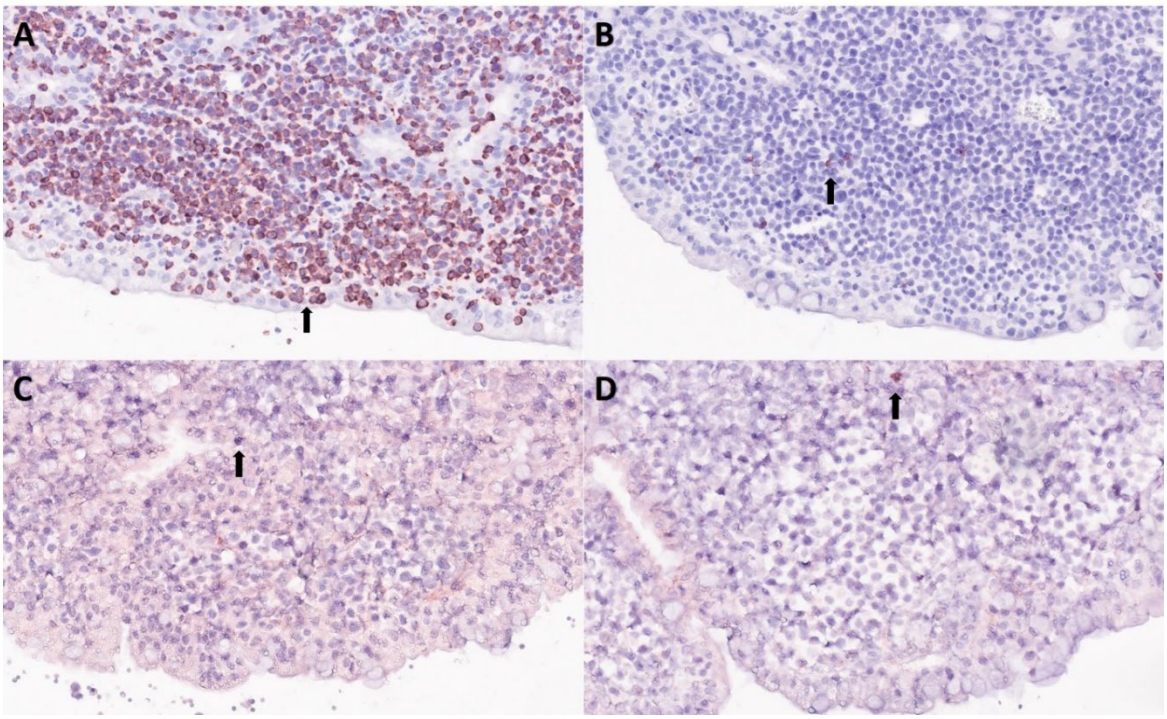

Figure 2. Immunohistochemical staining of intestinal sections in a 2-year-old African pygmy hedgehog with intestinal T-cell lymphoma. **A.** Neoplastic lymphocytes within the lamina propria as well as intra-

epithelial lymphocyte clusters (arrow) show strong positive membranous immunoreactivity, consistent with T-cell lineage. CD3 stain. **B.** CD20, **C.** CD79a, and **D.** PAX5 stains. Neoplastic lymphocytes fail to show immunoreactivity with few to rare individual immunoreactive B-lymphocytes (arrows) being scattered in the background.

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Demodex in horses

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Due to concern for eyelid squamous cell carcinoma, surgical biopsies of three 2-3 mm nodules at the eyelid margin of a 15-year-old female Welsh pony were submitted to AHL for histological diagnosis (**Fig. 1**). The nodules were situated on non-pigmented skin and had been present for one year with very slow growth.

Microscopic review of the nodules revealed a hyperplastic conjunctival and epithelial surface with hyperplastic sebaceous/meibomian glands forming expansile nodules interspersed with ectatic ducts. Within these ducts, and occasionally in acini, were numerous *Demodex* mites (**Fig. 2**). Surrounding inflammation was relatively mild.

Demodectic mange in horses is caused by infestation with *Demodex equi* or *Demodex caballi*. *D. caballi* is often restricted to the eyelids and muzzle, whereas *D. equi* infestation occurs over the body. *Demodex* mites infest hair follicles and sebaceous glands (as seen in this case).

Equine demodicosis can manifest as nodules or patchy alopecia and scaling. It has been reported in association with pituitary pars intermedia dysfunction or chronic corticosteroid treatment (neither of which were associated with this case). Demodectic mange is rare in horses. This is the only case of equine *Demodex* that has been diagnosed at the AHL in a data search spanning back to 2010.

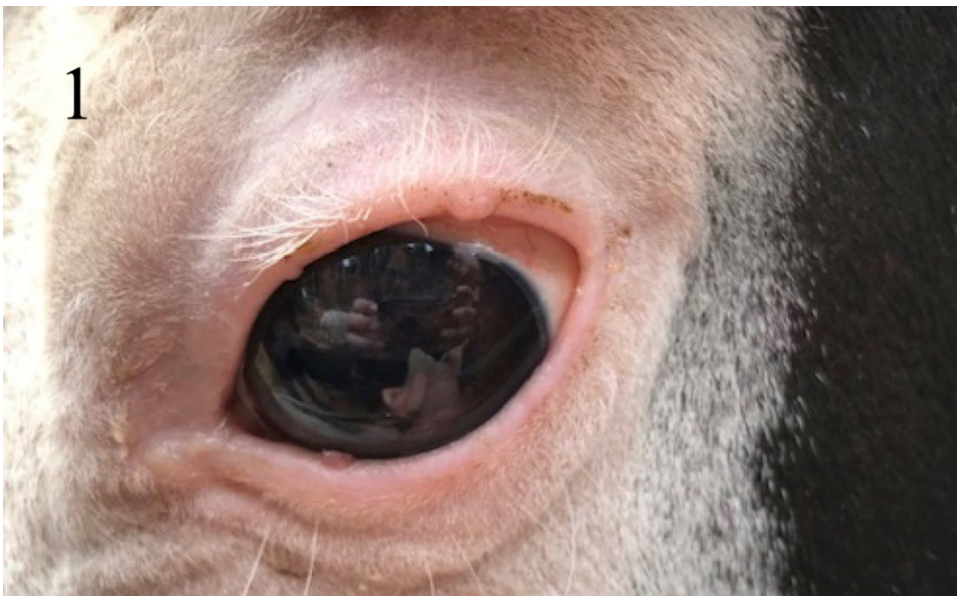

Figure 1. Equine *Demodex*. Multiple small, raised, tan/pink nodules on the eyelid margins.

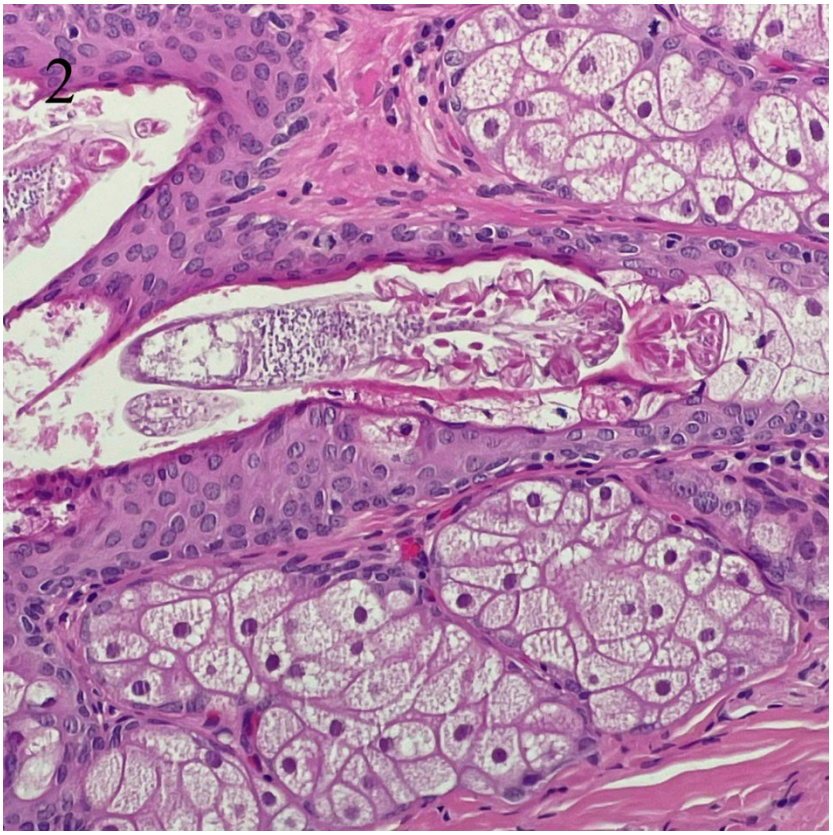

Figure 2. Equine *Demodex*. H&E stain. *Demodex* mite in cross-section within a duct of the sebaceous (meibomian) glands of the eyelid. 40x.

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Hepatocutaneous syndrome/superficial necrolytic dermatitis (SND) in dogs

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A 9-year-old male Shiba Inu presented to the AHL for postmortem with a history of chronic hepatic disease. On external examination, the dog was very thin with generalized mild icterus of the skin and mucous membranes. Multiple roughly symmetrical, large regions of alopecia were situated on the hind end (around the tail, hips and caudal aspect of the thighs), the ventral abdomen and thorax, and the muzzle (**Fig. 1A**). Affected skin appeared wet/exudative or crusted, thickened and flakey. All paw pads were diffusely and markedly hyperkeratotic (**Fig. 1B**). Internally, the liver was very small and had a generalized "cobblestone" appearance of the capsular surface. There was bridging and coalescing fibrosis intersecting between variably-sized nodules of hepatocellular hyperplasia (**Fig. 1C).**

Microscopic review of the liver confirmed the marked hepatic fibrosis and parenchymal collapse with multinodular hepatocellular regeneration. Hepatocellular vacuolation, portal vascular and biliary proliferation, and cholestasis were also present (**Fig. 2B**). Sections of affected skin showed the classic "red, white, and blue" pattern of superficial necrolytic dermatitis (SND) characterized by a thick overlaying parakeratotic hyperkeratosis (red), ballooning degeneration, epithelial necrolysis and spongiosis of the underlaying layers of epithelium (white), and a deeply basophilic hyperplastic epithelial basal layer (blue) (**Fig. 2A**).

In dogs, hepatocutaneous syndrome or SND is a rare, chronic, and progressive syndrome most commonly associated with severe liver disease, such as severe vacuolar hepatopathy, idiopathic hepatocellular collapse, and hepatopathy secondary to anti-convulsant drug administration. More rarely, it can accompany cases of glucagonoma and gastric carcinoma.

Classic skin lesions consist of roughly bilaterally symmetrical, erythematous erosive to ulcerative and crusted lesions of the muzzle, lips, periocular skin, edges of the pinnae, distal extremities, ventrum, points of pressure or friction, and the external genitalia. Oral and mucocutaneous lesions are occasionally reported. The footpads are markedly hyperkeratotic.

This condition is very rarely reported in cats without the associated footpad hyperkeratosis. A similar syndrome has been described in horses with erosive, ulcerative, and exudative coronitis and concurrent liver pathology.

The pathogenesis of SND is unknown, but hepatic dysfunction and derangement of glucose and amino acid metabolism are thought to be implicated, most likely involving abnormal or impaired ability to properly use nutrients.

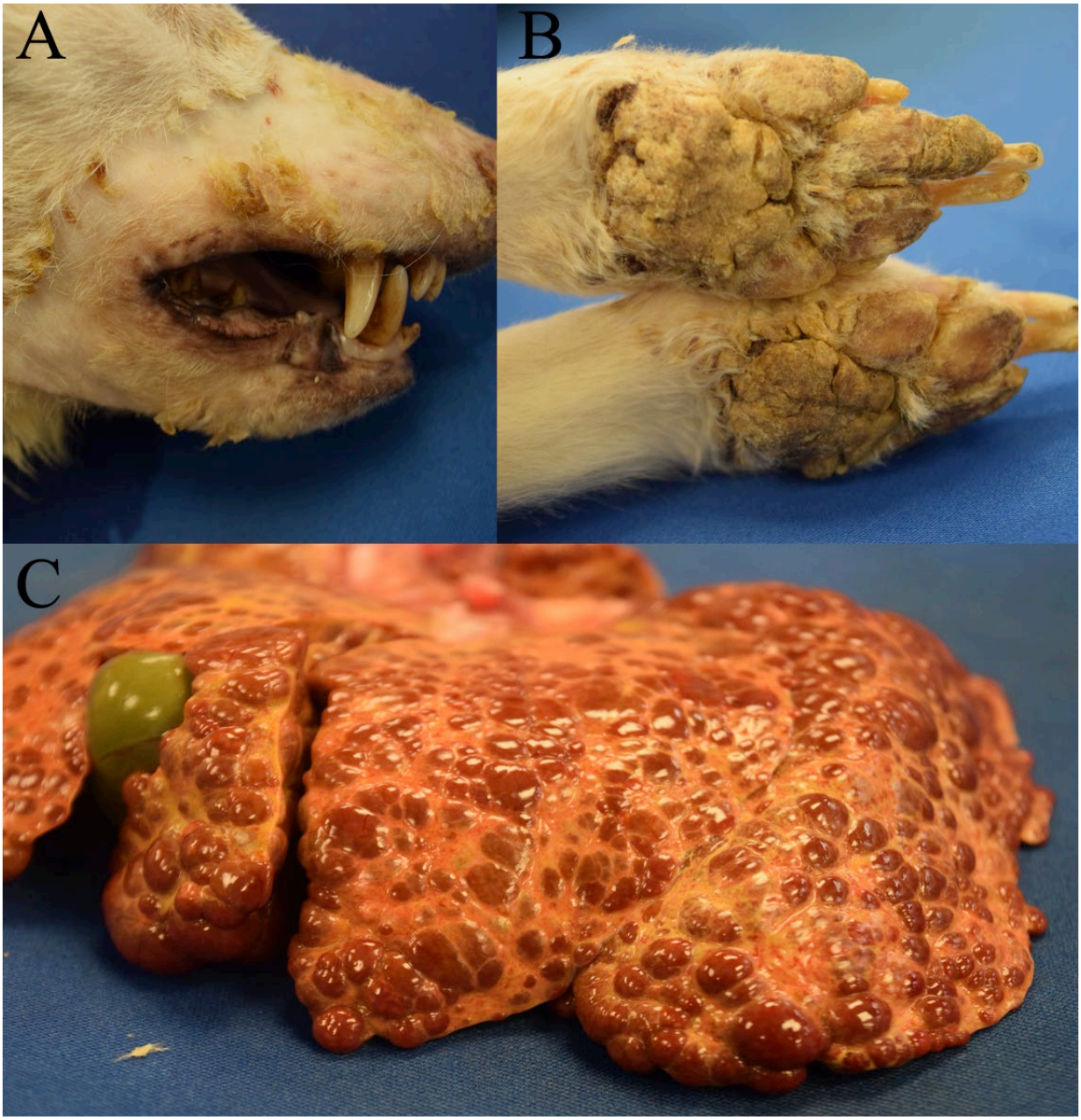

Figure 1. Canine hepatocutaneous syndrome. **A.** Alopecia with erosive, ulcerative, and crusted lesions of the muzzle. **B.** Marked footpad hyperkeratosis. **C.** Microhepatica with severe hepatic fibrosis, parenchymal collapse and multinodular hepatocellular regeneration.

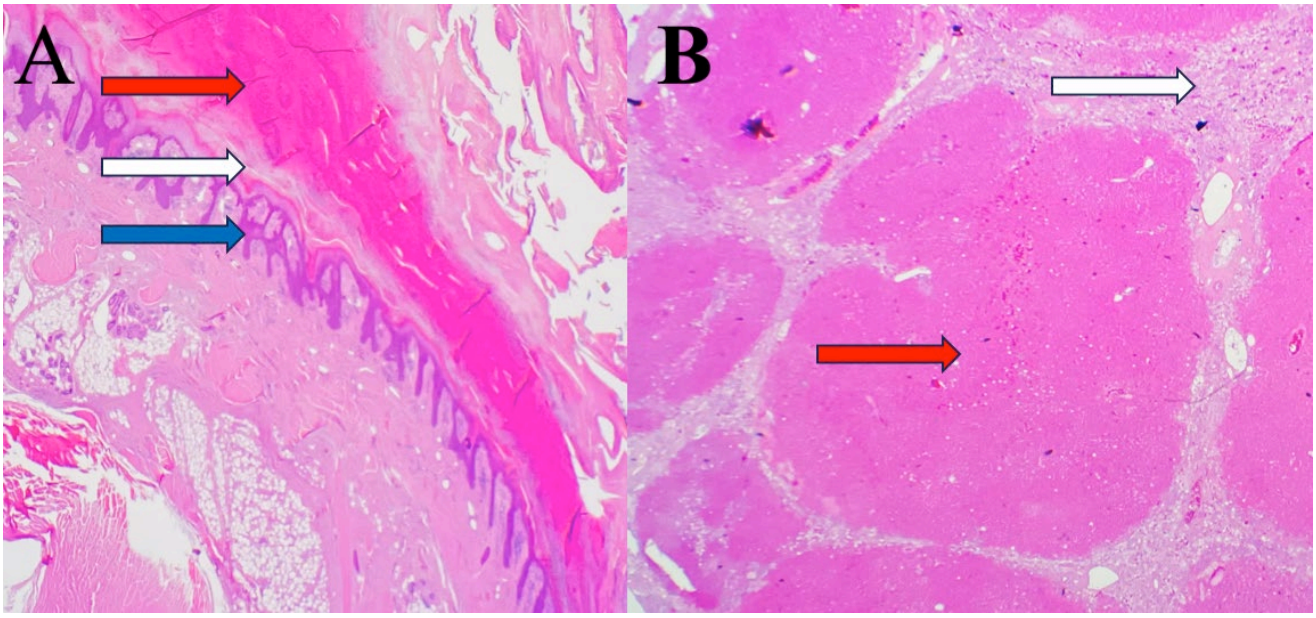

Figure 2. Canine hepatocutaneous syndrome. H&E stains. **A.** Footpad skin with the classic "red, white and blue" lesions of superficial necrolytic dermatitis. Thick parakeratotic hyperkeratosis (red arrow), ballooning degeneration, epithelial necrolysis and spongiosis of the epithelium (white arrow), and the hyperplastic epithelial basal layer (blue arrow). H&E stain, 2x. **B.** Severe bridging hepatic fibrosis and parenchymal collapse (white arrow) with multinodular hepatocellular regeneration (red arrow). H&E stain, 2x.

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