Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

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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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Welcome Dr. Spinato!

Dr. Maria Spinato has been appointed Director, Animal Health Laboratory, and Co-Executive Director, Laboratory Services Division, effective September 1, 2019.

Dr. Spinato graduated with DVM and DVSc degrees from the Ontario Veterinary College, is an Anatomic Pathology diplomate of the American College of Veterinary Pathologists, and completed an MBA at the University of Regina. She brings 20 years of relevant management / supervisory experience to the role, as well as having deep knowledge of the Animal Health Laboratory. In her current role, Maria has managed a considerable pathology caseload, as well as supervising the postmortem laboratory section. She has special interests in gastrointestinal and small ruminant pathology, and has organized emergency exercises involving foreign animal disease suspects for the AHL. As a member of the Accreditation Committee of the American Association of Veterinary Laboratory Diagnosticians, Maria has also gained insight into animal health laboratory operations across North America.

"Dr. Grant Maxie has established the AHL as a top-level, high-quality organization that serves the testing needs of animal health industries, livestock producers, and animal owners. My goals are to support AHL staff and clients as we continue to work together in a rapidly evolving sector that will require innovative testing capabilities and adding value to laboratory data." *AHL*

LabVantage LIMS update coming September 7-8, 2019

The AHL is currently using LabVantage 6 as our Laboratory Information Management System (LIMS). Many of you are familiar with our LIMS from using online e-forms or online submission result look-up.

From 6 PM on Friday September 6th to finish on Sunday September 8th we will be upgrading LabVantage 6 to the latest version - LabVantage 8.

There are several reasons for this upgrade:

- ✓ Modern look and feel with simple, clear start-up screen, enhanced usability and efficiency
- ✓ Improved navigation with the ability to track actions
- ✓ Mobile friendly
- ✓ Browser independent
- ✓ Responsive menu and configurable tool-bars
- ✓ Dashboards to view KPI

In order to do the upgrade, **our LIMS will be completely off line during this time.** AHL will have NO access as well. Those trying to work in LIMS or access cases, including AHL personnel, will be unable to do so.

AHL will be able to receive normal Saturday cases and provide testing in Clinical Pathology and Bacteriology as usual. As AHL will have NO access to client information – please make sure that you put full clinic contact information on the submission form if submitting during that period.

AHL will manually be emailing/faxing results produced by the laboratory equipment.

For e-form users – LIMS will be completely unavailable. If submitting, you will need to use paper forms and add email or fax information.

For our Client Portal users. Clients will be able to enter the submissions, but will have to save it and send it when the LIMS is back on-line. Client Portal persons can view results for anything resulted by 5:00 pm on Friday.

Once LIMS is back on-line on Monday, all of the data from the weekend cases will be entered into the new LIMS and resulted.

Thanks for your patience and continued support as we work through this upgrade. *AHL*

OAHN update – September 2019

Michael Deane, Kate Todd

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

AHL Newsletter 2019;23(3):3.

It's been a busy summer for the Ontario Animal Health Network, with the conclusion of multiple research projects, publishing many veterinary, producer, and owner reports, creating a small flock poultry video series, and continuing with publishing disease alerts, infographics, and information sheets. Read on to find out what we've been up to.

New infographics and info sheet

The OAHN Companion Animal network has created a new infographic on Bacterial Cystitis in Cats vs Dogs and an information sheet covering an overview, transmission, clinical signs, diagnosis, precautions, and additional resources on *Brucella canis*. Please find it here: https://oahn.ca/resources/brucella-canis-factsheet/.

Additionally, the network put together a **Rabies Resource page for Veterinarians**, containing links to resources, how-to videos for rabies testing sample collection, infographics, flow charts, and more. If you are a veterinarian, sign up to OAHN.ca, log in, and view it here: https://oahn.ca/resources/rabies-resource-page-for-veterinarians/.

The OAHN Equine Network also created a new infographic, which is focused on how veterinarians can reduce antimicrobial use in their equine practice. View the infographic here: https://oahn.ca/resources/infographic-how-you-can-help-reduce-antibiotic-use/.

OAHN small flock poultry veterinary 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. We have completed our respiratory disease video series; neurologic disease and enteric disease series are coming soon! Veterinarians can access these videos by first logging into the OAHN site (https://oahn.ca/log-in/)and following these links:

- https://oahn.ca/resources/respiratory-diseases-of-small-flock-poultry/
- https://oahn.ca/resources/neurologic-diseases-of-small-flock-poultry/
- https://oahn.ca/resources/enteric-diseases-of-small-flock-poultry/

Completed research projects

Each OAHN network has embarked on one or more research initiatives related to disease surveillance for their specific species. Recently, the poultry network published summary results of two projects, and the bee network published results from their research project as well. Find details below:

- OAHN Bee Research Project: Surveillance on resistant *Varroa destructor* mite population to 3 synthetic acaricides in Ontario—https://oahn.ca/resources/surveillance-on-resistant-varroa-destructor-mite-population-to-three-synthetic-acaricides-in-ontario/.
- OAHN Poultry Research Project: Antimicrobial resistance in fecal *E. coli* and *Salmonella* isolates of small poultry flocks in Ontario, Canada—https://oahn.ca/resources/antimicrobial-resistance-in-fecal-e-coli-and-salmonella-isolates-of-small-poultry-flocks-in-ontario-canada/.
- OAHN Poultry Research Project: Small flock poultry medicine workshop for Ontario veterinarians—https://oahn.ca/resources/small-flock-poultry-medicine-workshop-for-ontario-veterinarians/.

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

Alopecia areata in a Holstein bull

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AHL Newsletter 2019;23(3):4-5.

A 4-y-old Holstein bull was presented to the herd veterinarian because of multifocal-to-coalescing areas of hair loss. The lesions were localized in black-haired regions, were variably sized (2-10 cm diameter), and would frequently coalesce (**Fig. 1**). Eventually, the hair would re-grow but would be white where it was previously black (**Fig. 2**). The lesions were non-pruritic, non-painful, and the underlying skin appeared grossly normal. None of the other animals in the breeding facility were affected, and apart from the skin lesions, the bull was otherwise clinically normal.

The veterinarian collected two 6-mm skin punch biopsies, choosing one from a more recent lesion and one from an older lesion. Histologically, the findings in both sections were similar. Hair follicles were present and comprised of both anagen and telogen follicles. Most hair follicle bulbs contained degenerate matrix cells and abnormally clumped pigment contained within presumptive macrophages and multinucleate cells (**Fig. 3**). There was frequent peribulbar melanin incontinence, and a subtle increase in perifollicular connective tissue. There were very low numbers of CD3+ T lymphocytes within the follicular bulb and the surrounding dermis (**Fig. 4**). Superficial dermal vessels were surrounded by moderate numbers of eosinophils.

Adult-onset alopecia with subsequent leukotrichia has been described as the clinical presentation of alopecia areata in Angus, Holstein, and Eringer cattle. This rare condition is a non-scarring, idiopathic dermatosis that is presumed to be caused by autoimmune reaction to hair bulb antigens. In humans, rodents, dogs, and horses with the condition, circulating IgG antibodies directed against trichohyalin have been implicated. For this condition, the gross lesions, pigmentary incontinence, and melanophagia with a lymphocytic mural folliculitis are considered characteristic. Identification of CD3+ T cells within and surrounding the hair bulbs is considered diagnostic. *AHL*

Figure 1. Alopecia areata in a Holstein bull. Multifocal to coalescing, well-demarcated regions of alopecia and leukotrichia located in regions of pigmented hair.

Figure 2. Focus of alopecia with localized leukotrichia.

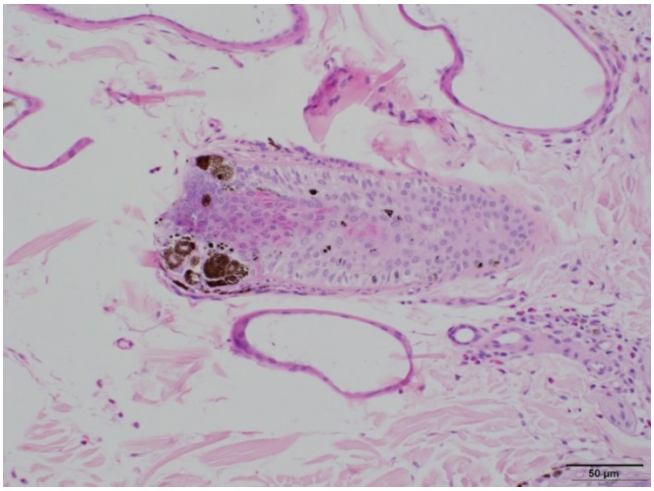

Figure 3. Hair follicle with abnormal pigment distribution and mural lymphocytes. H&E, 20X.

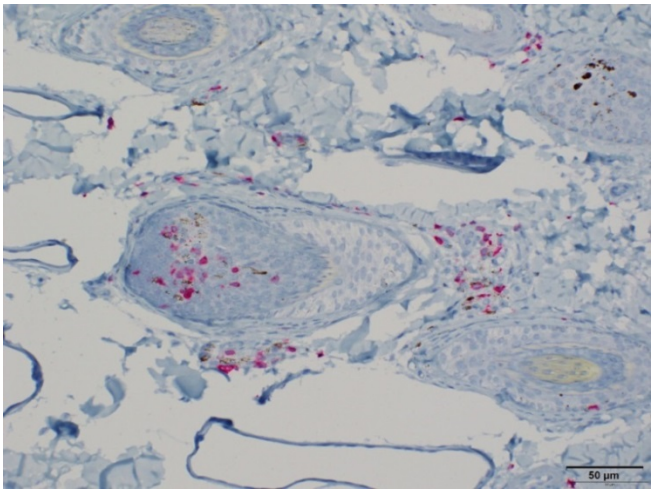

Figure 4. T cells within the hair bulb and the surrounding dermis. CD3 immunohistochemistry, 20X.

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Slobbering sheep: *Bibersteinia trehalosi* septicemia in lambs

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AHL Newsletter 2019;23(3):6-7.

In May 2019, three 4-mo-old weaned meat lambs were submitted for postmortem to the Animal Health Laboratory, Kemptville. Of ~100 at risk, 13 had died within 2 wk. Lambs had acute onset of drooling while continuing to try to eat and drink, but over a 24-36 h period became recumbent and died. Neither lameness nor stiffness was observed. Lambs were kept in a multi-age group pen, with no additions to the flock within 6 mo. Only lambs had clinical signs.

Gross postmortem examination revealed multifocal erosion and ulceration of the digestive tract of all 3 lambs, specifically, erosions on the dorsum of the tongue and hard palate of lamb 1, and a jejunal ulcer in lamb 2. Mucosal ulcers covered by plaques of fibrin were in the proximal 1/3 of the esophagus in lambs 1 and 3 (**Fig. 1**). All 3 lambs had numerous petechiae throughout the lungs, and diffuse hyperplasia of mesenteric, bronchial, and retropharyngeal lymph nodes. There were no gross lesions of the lips, muzzle, dental pad, or hooves, and vesicles were not observed.

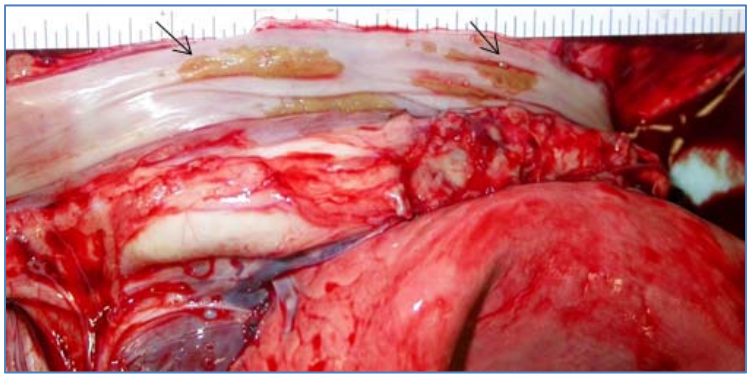

Figure 1. Mucosal ulcers covered by plaques of fibrin in the proximal 1/3 of the esophagus (arrows).

Histologically, mucosal erosions and ulcers were covered with fibrin, enmeshed neutrophils, caseous debris, and large numbers of intralesional bacteria. Lamina propria was congested and some blood vessels had thrombi. In lung, lymph nodes, liver, spleen, and brain, bacterial colonies and fibrin thrombi were seen in blood vessels, and bacterial colonies were in parenchyma, sometimes accompanied by caseous necrosis. The diagnosis of acute bacterial septicemia was supported by isolation of *Bibersteinia trehalosi* from lung, liver, and spleen.

***Bibersteinia trehalosi* is a sporadic cause of septicemia in sheep, usually occurring in weaned lambs.** *B. trehalosi* normally resides in the tonsils and nasopharynx of apparently healthy animals, but can progress from the tonsils to the lungs and bloodstream to cause systemic disease under exposure to various stresses, such as inclement weather, change of pasture or feed, overcrowding, poor ventilation, handling, and transport. Clinical signs may include fever, anorexia, lethargy, or sudden death with no signs. Common postmortem lesions are petechial and ecchymotic hemorrhages in subcutis, intermuscular fascia, pleura, lungs, epicardium, and mesentery, and swollen lymph nodes. Pneumonia is also frequently seen, and in many cases *B. trehalosi* is isolated in mixed culture with *Mannheimia haemolytica, Pasteurella multocida,* or *Trueperella pyogenes.*

Erosion and ulceration of the oral cavity, tongue and esophagus are not consistent lesions of *Bibersteinia trehalosi* septicemia, but when they occur, some owners report drooling as the prominent clinical sign. Observation of these lesions prompted consultation with CFIA regarding reportable disease differential diagnoses such as foot-and-mouth disease, bluetongue, peste des petits ruminants, and vesicular stomatitis. More common etiologies include parapoxvirus infection (orf), oral trauma, and bacterial infections.

Ovine herpesvirus type 2 (OHV-2) was also detected in lamb tissues by PCR. OHV-2 is believed to be endemic, and usually asymptomatic, in Ontario sheep flocks. However, experimentally, it may cause MCF-like mucosal disease in sheep. The possibility that OHV-2 could cause similar disease under field conditions, and the significance of OHV-2 in this case is unknown. *AHL*

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Bovine herpesvirus 4-associated ulcerative mammary dermatitis

Josepha DeLay, Kalie Bernardo, Cynthia Miltenburg, Betsy Elsmo

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AHL Newsletter 2019;23(3):7-8.

Two lactating cows in a 200-cow free-stall Holstein dairy herd developed ulcerative, exudative teat skin lesions that spread over a period of several days to other teats (**Fig. 1**). Skin biopsies taken at the margin of affected and unaffected skin demonstrated segmentally ulcerated epidermis covered by thick plaques of necrotic cellular debris and clusters of bacterial cocci. Eosinophilic intranuclear inclusions were evident in a few epithelial cells of intact epidermis adjacent to the cutaneous ulcers (**Fig. 2**).

The intranuclear inclusions were suspicious for viral involvement (specifically herpesviruses), and other histologic lesions indicated secondary bacterial dermatitis. Bovine herpesvirus 2 (BoHV-2), the agent of bovine herpes mammilitis, was the main etiologic differential diagnosis, however the virus was not detected in skin biopsies by PCR. **Subsequent PCR testing identified bovine herpesvirus 4 (BoHV-4) nucleic acid in the samples.** This virus also produces intranuclear inclusions in infected epithelial and other cells.

BoHV-4 is gammaherpesvirus. The virus has been associated with many disease conditions in cattle including pneumonia, metritis, abortion, and dermatitis, although direct causation of these diseases by the virus has not been confirmed. **BoHV-4 has also been associated with ulcerative dermatitis with vasculitis and pyrexia in haired, non-mammary skin of cattle.** A potentially synergistic effect of bovine leukemia virus infection with BoHV-4-associated disease has been suggested. Further research is required to confirm BoHV-4 causation of mammary dermatitis and potentially of other diseases in cattle, and to determine the pathogenesis of skin lesions.

The original 2 affected cows were euthanized or culled given the severity and persistence of teat lesions. A third cow in the herd subsequently developed teat lesions that were successfully treated with topical antibiotic preparations targeting presumed secondary bacterial dermatitis. Other measures were also taken to reduce any potential effects of teat trauma, including proper milking machine function. *AHL*

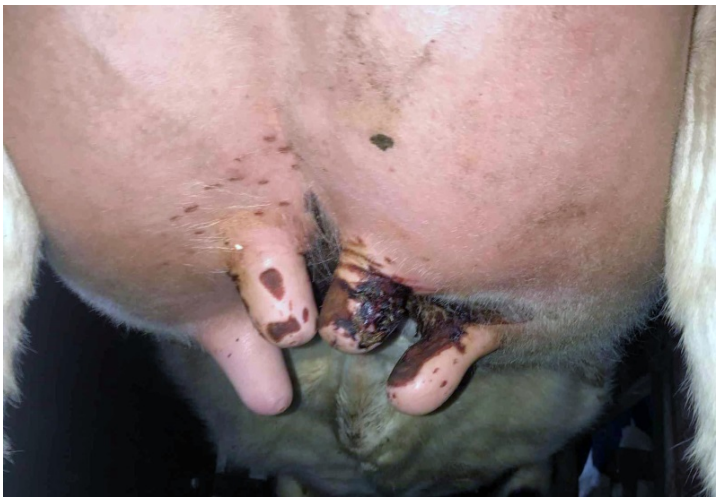

Figure 1. Ulcerative, exudative skin lesions involving multiple teats.

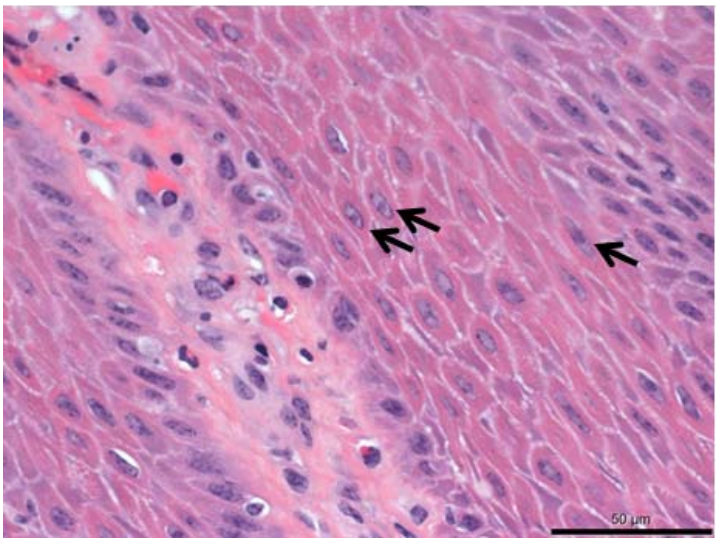

Figure 2. Intact epithelial cells with eosinophilic intranuclear inclusion bodies and peripheralized nuclear chromatin (arrows) in teat epidermis adjacent to ulcer.

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Acute nitrate toxicosis in Holstein heifers

Margaret Stalker, Clark Sinclair, Felipe Reggeti, Nick Schrier, Kalie Bernardo

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AHL Newsletter 2019;23(3):9.

Three 12-15 mo-old Holstein heifers were found dead overnight in 2 adjacent pens in a freestall barn. **A recent feed change to sorghum silage had occurred one week ago**, and 60 heifers were being fed the silage as the main source of roughage along with a small amount of corn and access to a protein lick tank. Feed intake was considered normal. No clinical signs were observed the previous evening. The animals were found dead in lateral recumbency in the morning. One heifer was sent to the AHL for postmortem examination. The animal was in good body condition, oral mucous membranes were slightly pale, and ocular mucous membranes were slightly dusky. No significant gross abnormalities were found on postmortem examination. The rumen was full of silage, with a small gas cap, and rumen pH was 6.5-7.0. Based on the history of multiple animals affected following a recent feed change to sorghum silage, the silage was removed and replaced with corn silage. A silage sample, as well as postmortem ocular fluid from the heifer, was submitted to the AHL Toxicology laboratory for nitrate analysis.

The sorghum silage nitrate analysis was 40,000 mg/kg dry weight (40,000 ppm or 4%). In general, feed with <5,000 ppm nitrate is considered safe to feed under all conditions, 5,000-9,999 ppm is considered safe for non-pregnant animals but should be limited to 50% of the ration for bred cows, 10,000-17,000 ppm should be limited to 35-40% of the total dry matter in the ration and should not be fed to pregnant animals; **feeds >17,000 ppm nitrate are considered to be toxic and should not be fed to cattle.**[1] Ocular fluid nitrate analysis was 52 mg/l. Nitrate values in excess of 10 ppm are suspicious for, and in excess of 20 ppm are considered indicative of toxicity[2].

Fast-growing forage crops such as sorghum may accumulate toxic levels of nitrates under stressful growing conditions, for example periods of dry weather followed by a rain, or after heavy applications of nitrogen fertilizers. Although ensiling can reduce nitrate levels by 25-65%, the crop must be at the correct moisture level for proper fermentation to occur. Baleage is generally too dry to ferment completely, and nitrate levels are stable in dry hay.[3]

Nitrates in forage are reduced to nitrite in the rumen, then quickly converted by rumen bacteria to ammonia, which is absorbed and excreted into urine. **High levels of nitrate ingestion and subsequent nitrite production overwhelm conversion to ammonia, and nitrite is absorbed directly, oxidizing hemoglobin to methemoglobin (MHb) which is incapable of oxygen transport.** Clinical signs of hypoxia can be subtle, and include increased respiratory rate, apprehension, weakness, depression and recumbency. Pregnant animals may abort. If MHb reaches 75-80%, animals begin to die. If the accumulation is not lethal, MHb is slowly reduced back to Hb over a 12-24 hour period. At autopsy, few distinctive lesions are evident. Blood and tissues may be dark or "chocolate brown" colored, but this is not a consistent feature. **Forage testing of composite samples is the only method to evaluate risk for nitrate toxicosis, prior to feeding**. *AHL*

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2. Burrows and Tyrl. Toxic Plants of North America, 2nd ed. Wiley-Blackwell, 2013.
3. Crop Report – August 1, 2019, OMAFRA Field Crop News http://fieldcropnews.com/2019/08/crop-report-august-1-2019/ .

SWINE

Clostridium septicum dermatitis, cellulitis, and myositis (malignant edema) in swine

Josepha DeLay, Margaret Stalker, Đurđa Slavić, Clint Lichty, Greg Wideman

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AHL Newsletter 2019;23(3):10.

Sudden (unexpected) death was reported in several grow-finisher pigs from 2 unrelated herds. Most of the affected pigs had well-demarcated 30-50 cm diameter raised purple skin lesions involving mainly the ears, neck, and ventral trunk. Subcutaneous emphysema was palpable in some lesions. Similar skin lesions in a few live pigs expanded rapidly and the pigs' clinical condition deteriorated, culminating in death or euthanasia. Those pigs treated very early in the disease course, with clinical signs of lethargy and ≤5 cm skin lesions, did survive.

A range of tissue samples, including samples from the margin of affected and non-affected skin and underlying skeletal muscle, were examined histologically, and significant lesions were confined to skin. Epidermis was necrotic (infarcted); dermis and subcutis were expanded by edema, fibrin, and neutrophils; and myocyte bundles were degenerate and surrounded by fibrin and neutrophils. Many long bacilli compatible with *Clostridium* spp. were visible among the inflammatory debris. *Clostridium septicum* was cultured in low-to-moderate numbers from skin swabs from each of 4 sampled pigs. **Malignant edema caused by *C. septicum* was confirmed based on the combination of clinical history, gross appearance of skin lesions, histologic lesions, and isolation of the causative bacterium from lesions.**

Infection with the anaerobic bacillus *C. septicum* is an uncommon cause of disease and death in swine, and literature reports of this condition are sparse. *C. septicum* cellulitis and myositis is known as malignant edema, gas gangrene, or 'pseudo-blackleg' due to the clinical and pathologic similarity of the condition to blackleg (clostridial myositis, *C. chauvoei*) in ruminants. Clostridial exotoxins, particularly alpha toxin (ATX), cause localized tissue necrosis and inflammation, and systemic toxemia results in death. Fibrinohemorrhagic peritonitis and pulmonary edema are present in some animals. Wound infection is considered the main portal of entry for bacteria, although activation of dormant intramuscular clostridia and bacteremia secondary to gastrointestinal mucosal injury are also possible. Concentration of lesions in the head and neck area of these pigs supports contamination of fighting wounds in this case. Lesions may also be associated with injection sites**. Both histopathology and bacterial culture are important for confirmation of a diagnosis of *C. septicum* cellulitis and myositis,** because the organism may also be present in tissues as a postmortem invader unassociated with lesions. **Penicillin is the antibiotic of choice for treatment of animals with suspected *C. septicum*-associated disease.** *AHL*

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Acute ionophore toxicosis in pigs

Felipe Reggeti, Nick Schrier

Animal Health Laboratory – Toxicology section, University of Guelph, Guelph, ON

AHL Newsletter 2019;23(3):11-12.

Acute deaths were reported in a pig farm after feeding a new diet for 12-24 hours. A non-specified number of animals weighing 35-60 kg died within 24 hours, while sublethally affected animals showed clinical signs consisting of incoordination, stumbling, hind-end weakness, and ataxia. Some tail biting and ear tip necrosis were also noted. Most animals improved upon removal of the diet; however, some were euthanized because of welfare reasons.

Blood work on 1 severely affected animal showed the following biochemical changes: CK: 217,940 U/L (RI: 50 – 3,531), AST: 4,561 U/L (RI: 16 – 65), K: 9.9 mmol/L (RI: 2.1 – 7.1) and P: 3.74 mmol/L (RI: 1.16 - 2.97). Of 7 blood samples tested, 4 consistently showed elevated serum CK and AST activities, compatible with ongoing muscle injury (rhabdomyolysis). Considering clinical presentation, laboratory test results, and history of recent diet change, feed samples were submitted to the Toxicology section of the Animal Health Laboratory (AHL) for ionophore quantification. **Samples from 2 different bins contained salinomycin at 320 and 390 µg/g (ppm), respectively;** monensin and narasin concentrations were below the limit of quantitation (< 1.0 ppm). **The target dose of salinomycin in the feed was 25 ppm, as recommended,[1] however, a mistake was made on the prescription** and the final concentration was high enough to cause acute toxicity (**reported lethal doses in pigs: 166-720 ppm of feed[2,3]**). Most survivors had recovered one week after the incident; however, a few animals still showed hind-end weakness and lameness.

Ionophores are feed additives used as coccidiostats or to improve feed efficiency; however, overdose or inadvertent exposure of non-target species can cause toxicity. Clinical signs of salinomycin toxicosis in pigs are typically related to the gastrointestinal and muscular (skeletal more than cardiac) systems. Affected animals commonly have anorexia, lethargy, stiffness, tremors, recumbency, and may be reluctant to move. They may also have diarrhea. Clinicopathologic changes are related to degenerative myopathy consisting of elevated serum CK and AST activities, as well as some electrolyte changes, in particular hyperkalemia. Rhabdomyolysis can also cause myoglobinuria and possibly renal disease as a result of acute tubular damage. Clinical signs may appear within 24 hours, and some animals will continue to show problems after several days or weeks. Prognosis is guarded when the intoxication has caused significant heart damage. Animals surviving the acute phase may recover but weight gain and other parameters might be compromised.

Table 1. Cases of ionophore toxicosis confirmed at the AHL in recent years.

Date	Province	Type of contaminant	Level in feed (µg /g)	Species	Exposed animals	Dead animals
Jun 2019	ON	narasin	33	turkeys	5,000	300
May 2019	MB	salinomycin	390	porcine	N/A	unspecified number of sudden deaths and euthanized
Jan 2019	SK	monensin	93	ovine	160	7
Dec 2018	AB	monensin	76	bovine	N/A	unspecified number of deaths

Date	Province	Type of contaminant	Level in feed (µg /g)	Species	Exposed animals	Dead animals
Aug 2018	SK	monensin	700	ovine	N/A	5 dead 1 euthanized (for PM)
Feb 2018	SK	monensin	850	bovine	200	4
Jun 2016	ON	narasin	220	porcine	800	not available
Dec 2015	AB	monensin	470	bovine	350	4
Jan 2015	ON	monensin	240	bovine	240	4
Aug 2014	ON	monensin	250	ovine	400	10
Jul 2014	AB	monensin	3,600	bovine	600	unspecified number of deaths
May 2014	AB	monensin	2,200	bovine	N/A	10
Sep 2013	ON	monensin	1.1	equine	N/A	2

The AHL Toxicology section offers a "feed additive" screen to quantify levels of monensin, salinomycin, and narasin in feed and GI contents by HPLC, with a limit of quantification of 1.0 µg/g (ppm). For questions on submissions, please contact AHL client services (ahlinfo@uoguelph.ca) or visit our website: https://www.uoguelph.ca/ahl . *AHL*

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Diagnosing respiratory disease in backyard chickens

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AHL Newsletter 2019;23(3):13-14.

Veterinarians who treat backyard flocks are often faced with respiratory signs that can be the result of different etiologies. Respiratory signs can include rales (fine crackles), snicking (sneezing), watery eyes, fluid or mucus from nostrils, swollen head, gaping (open-mouth breathing), gasping, or head shaking. The birds may or may not be depressed but if they are, they will be down on the ground with their feathers fluffed up and their eyes closed. Morbidity and mortality will be variable. How do you approach diagnosing these flocks? Your first consideration should be diagnostic testing prior to treatment. Treatment prior to testing will compromise lab results and the ability to obtain a diagnosis.

If there is **no mortality,** you could consider drawing blood from a few birds (if the birds can tolerate handling) and run ELISAs (**Table 1**). In unvaccinated flocks, any rise in titer will indicate exposure and is the least expensive testing. Swabs (gel or viral transport medium) of the choanal slit and/or trachea could also be collected to run any of the PCR tests for specific diseases.

If there is **mortality,** then there is an opportunity for postmortem examination and more intensive sampling. Postmortem examination could be done at a clinic or birds could be shipped to AHL. If birds are shipped, please refer to the AHL website for a PM submission form (https://www.uoguelph.ca/ahl/submissions/submission-forms) and packaging instructions (https://www.uoguelph.ca/ahl/submissions/ahl-labnote-27-submission-instructions).

Clinic postmortem: If the head is swollen, you can cut across the beak and then cut the skin under one of the eyes to **access the infraorbital sinus**. Swabs can be taken for bacteriology or mycoplasma testing.

Once you **open the oral cavity,** you can look for oral plaques and examine the laryngeal opening for yellow caseous material. Rule outs include wet pox or ILT. Collect pieces of the larynx/trachea for pox and/or ILT PCR as well as a piece of larynx/trachea (eyelid, lung) for histology given that both tests are needed to confirm ILT. ILT is an immediately notifiable disease that requires OMAFRA and CFIA notification. These organizations may call to ask a few questions regarding the affected flock.

Cut down the trachea to look for worms (gapeworm is not commonly diagnosed). Open the birds to examine the lungs. Lung changes could include congestion, edema, yellow caseous material filling airways or over the pleural surface, and nodules. Yellow caseous material could indicate bacterial involvement (i.e. *Staphylococcus aureus, E. coli*). It is always a good idea to divide at least one lung in half and put one half in formalin and keep the other half for other testing. Both fungus and bacteria can create nodules in the lungs.

Two final areas to check are air sacs (normally clear like plastic wrap) that could be clouded or filled with fluid and/or caseous material. **Air sacs could be swabbed for bacterial culture**. Also **check the syrinx** as this is a key place for fungal organisms to settle out, grow, block the airway, and result in death by asphyxiation.

For more information on respiratory diseases in chickens, please refer to the OAHN website: https://oahn.ca/resources/respiratory-diseases-of-small-flock-poultry/
and:
https://oahn.ca/resources/oahn-small-flock-poultry-veterinary-resources-2019/.

Table 1. List of chicken respiratory disease tests available at the AHL.

Disease (by lab section)	PCR	ELISA	Culture
Virology lab			
Newcastle/APMV-1 (avian paramyxovirus 1)	X	X	
IBV (infectious bronchitis virus)	X	X	
ILTV (infectious laryngotracheitis virus)	X		
Avian influenza/influenza A	X		
Poxvirus	X		
Mycoplasma lab			
Mycoplasma (*M. gallisepticum, M. synoviae*)	X (MG or MS)	X (MG/MS)	X
Bacteriology lab			
Bacterial culture			X
Mycology/fungal culture			X

Sampling summary:

NOTE: If screening a flock for disease, up to 5 swabs or tissues can be pooled for PCR testing. PCRs are generally the preferred test as they target specific diseases and can be done quickly.

Live bird testing:

- Blood for ELISAs - limited number of tests available, but lower cost.
- Choanal slit/tracheal swab (gel swab or viral transport medium) for PCR (wider selection of tests.)
- Photos of affected birds can be submitted to AHL along with the case history.

Dead bird testing:

NOTE: If a postmortem is declined, swabs from the choanal slit/trachea could still be collected for PCR.

- **Postmortem** - key test for lesion identification and sample collection.
- **Histology** - an option to screen tissues for lesions or determine the etiology of specific lesions such as nodules (bacterial, fungal, neoplasm). Collect a wide variety of tissues to place in formalin, including all major organs: lung, liver, spleen, and kidney.
- **PCR tests** can be run on swabs or tissues. If you are sending tissues, please remember to package each tissue type separately and do not mix intestinal tissue with organ tissue. PCR is a fast test to screen quickly for multiple diseases.
- **Bacterial/fungal culture** can be run on swabs or tissues. Culture will screen for multiple bacterial organisms and our MALDI-TOF MS system is used for identification.
- **Mycoplasma** can be run on swabs or tissues. PCRs are fast and will test for the key species. Culture is available but results take more than a week.

If high mortality and/or clinical signs lead you to suspect avian influenza or Newcastle disease – phone CFIA! *AHL*

HORSES

Equine penile squamous cell carcinoma

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A search of pathology records since 2007 yielded a total of 67 lesions involving the penis or prepuce that were considered benign (fibropapilloma, squamous papilloma) or malignant neoplasia (squamous cell carcinoma) (**Table 1**). As expected, the peak number of submissions was in late summer – likely the result of UV exposure on lightly pigmented mucosa, and perhaps increased fly activity spreading virus (**Fig. 1**). Smegma is also considered to be an irritant that may promote neoplastic transformation. AHL data shows that tumors of the male equine genital tract comprise 6.3% of all tumors reported in this species (78 of 1,240) since 2007; 55 of these were squamous cell carcinomas.

Table 1. Benign and malignant lesions of the equine penis or prepuce, 2007-2019.

Diagnosis	**Age (average y)**	**Number of cases**	**Time of year (peak submission month)**
Papilloma	18.7	9	NA
Squamous cell carcinoma	18.8	56	August
Other tumors*	9	2	NA

*melanoma, giant cell sarcoma

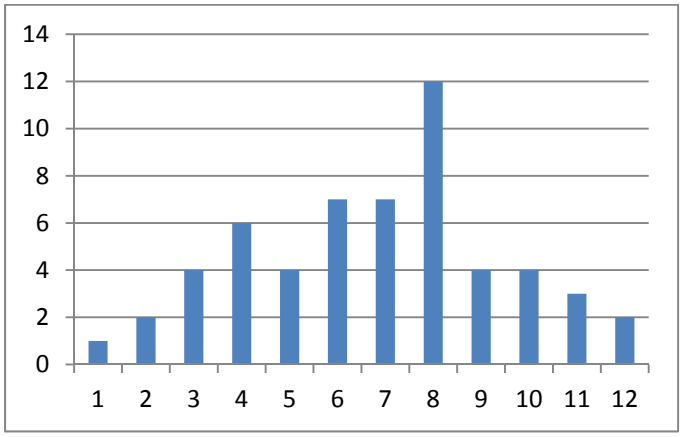

Figure 1. Number of cases of penile SCC (y axis) by month (x axis).

In a recent study, **equine papilloma virus 2** DNA was identified in 45% of penile squamous cell carcinomas.[1] A subsequent paper found virus in both papillomas and carcinomas and proposes that a transition may occur.[2] **Figure 2** shows this **proposed continuum**, starting with a squamous papilloma with probable viral cytopathic effect, and progressing to squamous cell carcinoma.

Differentiating between benign and malignant penile tumor histologically can be challenging for pathologists. **Benign squamous papilloma** is comprised of hyperplastic or papillated keratinized squamous epithelium overlying sparse fibrous stroma. The absence of stromal invasion by epithelial cells usually differentiates benign papilloma from squamous cell carcinoma. However, an intermediate stage exists: **squamous cell carcinoma in situ** refers to a tumor that contains sufficient cellular atypia to warrant a diagnosis of malignancy. **Overt squamous cell carcinoma** is associated with invasion of

neoplastic squamous epithelial cells into the subjacent stroma. Metastatic spread to superficial and deep inguinal lymph nodes is reported in a small proportion of cases. *AHL*

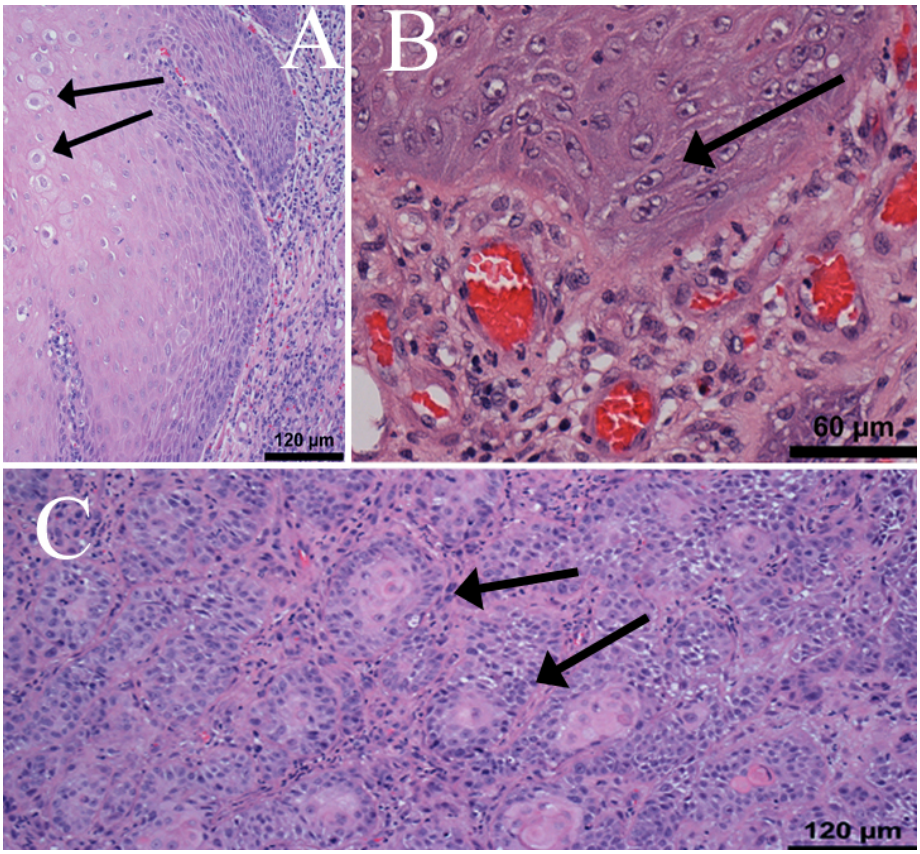

Figure 2. Equine penile squamous cell carcinoma. **A.** Squamous papilloma showing what likely represents viral cytopathic effect (pale cells at ends of arrows). **B.** Lesion with large open-faced irregular nuclei = epithelial dysplasia progressing to carcinoma-in-situ. **C.** Squamous cell carcinoma with islands of invasive epithelial cells (arrows). These islands often keratinize centrally.

References

1. Knight CG et al, Equine penile squamous cell carcinomas are associated with the presence of equine papillomavirus type 2 DNA sequences. Vet Pathol 2011;48:1190-1194.
2. Lange CE et al, ECPV2 DNA in equine papillomas and in situ and invasive squamous cell carcinomas supports papillomavirus etiology. Vet Pathol 2013;50:686-692.

COMPANION ANIMALS

Companion animal histopathology test codes explained

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Histopathology tests offered by the AHL for companion and non-food animals are arranged in 3 price categories that reflect the **number and/or size of the biopsies** or postmortem samples. The categories translate into the number of slides required for the case, which in turn reflects the cost (for reagents and supplies, and technical and professional time) for each case.

Companion animal histopathology test codes and criteria are as follows:

- **histcm1**: Histopathology, companion / other, 1-2 biopsies or tissues < 6 cm, OR multiple (6 or fewer) punch, trucut, or endoscopic biopsies.
- **histcm2:** Histopathology, companion / other, 3-6 biopsies or tissues, OR biopsy 6-10 cm.
- **histcm3**: Histopathology, companion / other, 7 or more biopsies or tissues, OR biopsy >10 cm.

Additional charges also apply to those samples **from any species** requiring the following special procedures for histologic processing:

- Decalcification, examples: digit amputations, bone biopsies.
- Hoof or nail softening.
- Tumor margin evaluation (for biopsies >2 cm): Please inform us at the time of sample submission if margin evaluation is required.
- Complex cases requiring pathologist intervention at the trimming stage, examples: limb amputations, splenectomies, large mammary resections.

Please see the online AHL Fee Schedule for pricing.
https://www.uoguelph.ca/ahl/

When necessary, AHL histotechnologists and pathologists will adjust requested tests and associated fees to reflect the samples that we receive. We will make an effort to inform clients whenever these changes must be made. However clients must be aware of the test criteria described above and choose the appropriate histopathology test for individual cases submitted to the AHL.

Please contact Dr. Josepha DeLay with any questions about histopathology submissions: 519-824-4120 ext 54576 or jdelay@uoguelph.ca *AHL*

Disseminated mycosis in a German Shepherd dog

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A male 3-y-old German Shepherd was euthanized because of renal failure, vomiting, and uveitis. The dog had developed hind-end weakness over the previous 2 weeks. The dog was autopsied at the clinic and selected tissues (liver, kidney, spleen, small intestine) were submitted for histology. In the section of kidney, there was a large zone of infarction with mineralization and fibroplasia. Several other smaller infarcts were seen. There were scattered multinucleate giant cells present, and objects suspicious for fungal hyphae could be seen; these were verified as fungal hyphae with a silver stain of the section (**Fig. 1**). Morphologically they were suspected to be *Aspergillus* spp. Testing of formalin-fixed paraffin-embedded tissue scrolls for fungal PCR products was unfruitful, and the fungus could not be further identified.

A search of pathology cases since 2007 for fungal lesions in dogs reveals most are either associated with aspergillosis (7 cases) or blastomycosis (37 cases). Two other cases of disseminated mycosis likely associated with *Aspergillus* spp. were both in German Shepherds (**Table 1**).

Most cases of disseminated aspergillosis occur in German Shepherd dogs.[1] Common clinical signs are vertebral pain, anorexia and wasting. CNS involvement, lymphadenopathy, and endophthalmitis are also reported manifestations. *AHL*

Table 1. Mycoses in AHL canine pathology submissions.*

Case	Fungus*	Breed	Age (y)	Location
1	*Aspergillus*	German Shepherd	3	Renal
2	*Aspergillus*	German Shepherd	2.6	Pulmonary
3	*Aspergillus*	German Shepherd	7.4	Brain
4-8	Nasal aspergillosis	1 German Shepherd, 1 Golden Retriever, 1 Standard Poodle, 2 mixed breed	1-9	Nasal
Blastomyces – all cases	*Blastomyces dermatitidis*	37 cases (12 disseminated or pneumonic)		

*Diagnosis usually based on fungal morphology in histologic sections.

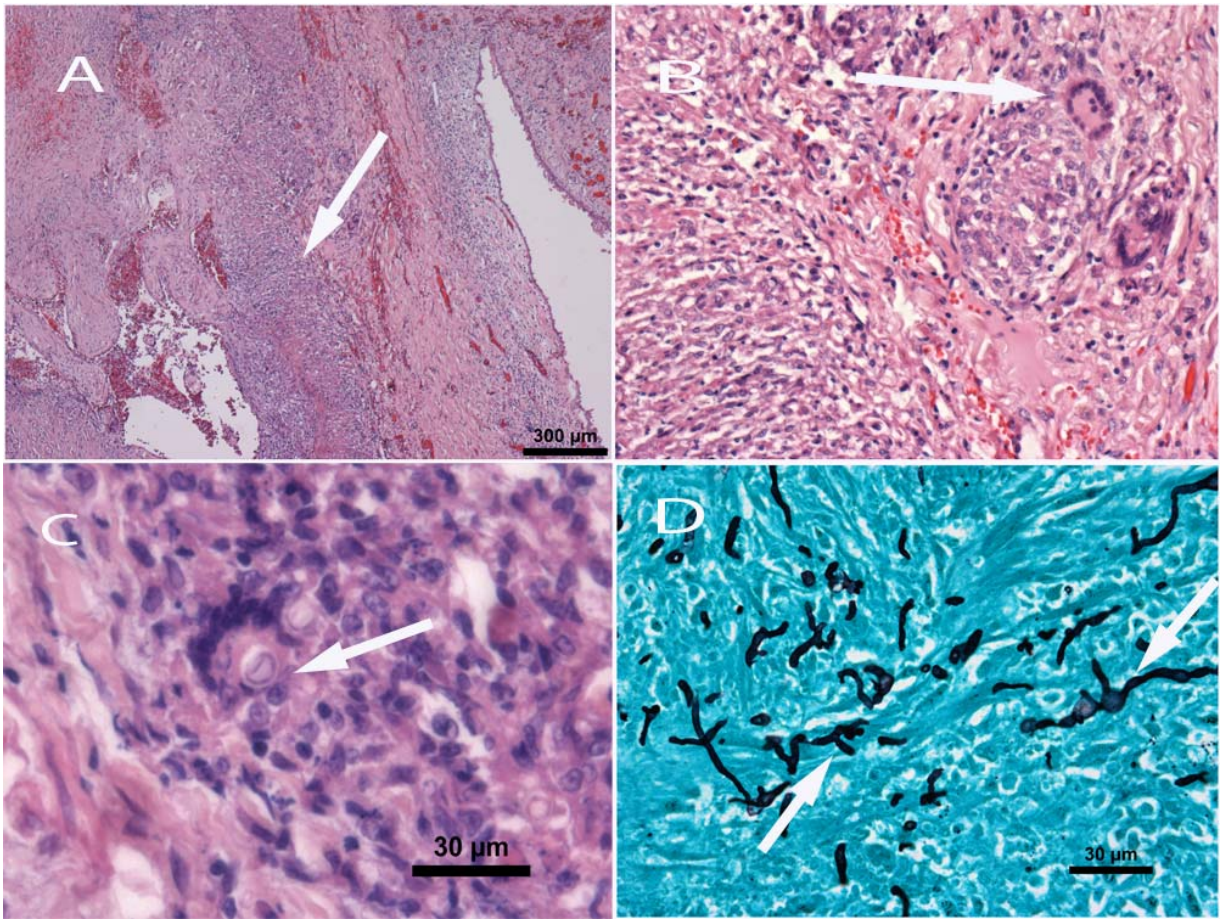

Figure 1. Disseminated mycosis in a German Shepherd dog. **A.** Loss of renal parenchyma with an intense infiltrate of mixed inflammatory cells (arrow). H&E. **B.** Areas of granulomatous inflammation with multinucleate giant cells (arrow). (H&E). **C.** Suspicious fungal hyphae in H&E section (arrow) confirmed with silver stain. **D.** Methenamine silver stain.

References

1. Day MJ. Canine disseminated aspergillosis. In: Green, Infectious Diseases of the Dog and Cat, 3rd ed. 2006:620.