Diffuse colonic ganglioneuromatosis in a Mastiff dog

By: Moira Kerr, Veterinary Pathologist, PDS

A six year-old, castrated male, Mastiff dog presented to its primary care veterinary for melena and weight loss of unknown duration. A colonic mass was identified when the patient was placed under general anesthesia and a colonoscopy was attempted. The mass was removed per rectum and submitted for histologic examination.

A 1.5 x 4.0 tan tissue was received. The section of the colonic mass was extensively ulcerated with accompanying marked collections of neutrophils, small lymphocytes, plasma cells and hemosiderophages that also occurred as a diffuse infiltrate in the lamina propria. Singleton and small groupings of neuronal ganglion cells were present throughout the lamina propria (see Fig 1).

The neuronal ganglion cells were polygonal with distinct cell borders and a moderate nuclear to cytoplasmic ratio. The nucleus was eccentric, round to oval with a finely stippled chromatin pattern and a single, prominent, round nucleus. The cytoplasm was moderate and there was a finely stippled to fibrillar, pale pink material (Nissl substance) placed eccentrically in the cytoplasm. There were accompanying haphazard to parallel arrays of spindled cells and thin collagen fibers within the lamina propria that extended through the muscularis mucosa, interpreted to be a schwannian stroma. The cell borders were indistinct and the nuclear to cytoplasmic ratio was high. The nucleus was centric, oval to oblong with a finely stippled chromatin pattern and one to three, small nucleoli. The cytoplasm was scant and pink. Mitoses and cellular features of malignancy were not present in the neuronal ganglion cells and schwannian stroma. Profiles of submucosal plexuses were increased in number and size. The muscularis mucosa and serosa were not present in the sections examined.

The neuronal ganglion cells stained positively with the neuron specific enolase (NSE; see Fig. 2) while Luxol fast blue staining failed to reveal the presence of myelin. Immunohistochemical staining for S-100 and glial fibrillary acidic protein (GFAP) and electron microscopy were not pursued.

The presence of a diffuse infiltrate of ectopic neuronal ganglion cells, a schwannian stroma and hypertrophied and hyperplastic enteric plexuses coupled with the location of the mass warranted a diagnosis of diffuse colonic ganglioneuromatosis (GN) in this dog.

GN is a rare disorder characterized by the abnormal, intramural to transmural, multinodular to diffuse, proliferation of nerve fibers and ganglion cells in a segment of the intestine.1,2 The affected segment of bowel is thickened and the lumen can be dilated or narrowed. Human GN can occur anywhere in the gastrointestinal tract but most reported cases involve the colon and rectum.1,2 Human GN may present as an acute gastrointestinal obstruction or motility disorder or incidentally, during investigations for other gastrointestinal diseases.1,2 Human hereditary intestinal GN commonly occurs in association with multiple endocrine neoplasia type IIB (MEN-IIb), neurofibromatosis 1 (NF1; von Recklinghausen’s disease) and Cowden’s disease.1,3 The pathogenesis of human GN remains undetermined. Surgical resection is recommended in human GN when lesions are confined to one section of the intestine.3 When surgical resection is not an option, symptomatic management is advocated (may include one or more of the following: adjustments, laxatives or enemas, fibre supplementation and gastrointestinal motility modifiers).3

In the veterinary literature, reports of GN have been limited to juvenile and adult dogs, a horse and a steer.4-9 Affected animals may present with gastrointestinal signs (e.g. vomiting, diarrhea or constipation, hematochezia, melena, tenesmus and abdominal pain) or they can be asymptomatic.4-9 Abdominal ultrasonography may reveal thickening of the affected segment of the intestine and loss of the normal layers of the intestinal wall.24 Histopathologic examination of full-thickness biopsies from or the surgically resected affected segment of the intestine is needed to establish the diagnosis. Immunohistochemistry for neuron specific...
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enolase, S-100 and glial fibrillary acidic protein (GFAP) will aid in the establishing the diagnosis.4, 5 There are too few reports in the veterinary literature to comment on the prognosis or behaviour. In two of the reports the affected dogs were euthanized due to the development of a postoperative septic peritonitis.4, 7 There is a single report of a successful outcome following surgical resection in a dog with small intestinal GN.8

The pathogenesis of GN in animals is also unknown. It has yet to be established if the genetic mutations that have been identified in human GN occur in animal cases of GN. There is a single report in the veterinary literature in which a duplication of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) was demonstrated, using a quantitative multiplex polymerase chain reaction, in a Great Dane puppy with concurrent colorectal hamartomatous polyposis and GN, implying a similar pathogenesis to human Cowden’s disease.3, 8

GN should be included as a differential diagnosis in dogs with intestinal thickening and gastrointestinal signs.

REFERENCES:
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Antimicrobial use (AMU) in animals —are you a good steward?

By: Dr. Betty Althouse, D.V.M., Saskatchewan Chief Veterinary Officer

Recently in the news there are reports of the global crisis with respect to antimicrobial resistance. The World Health Organisation (WHO) calls it “a problem so serious that it threatens the achievements of modern medicine” (WHO, 2014). In September, 2014, President Obama signed an Executive Order directing key Federal departments and agencies to take action to combat the rise of antibiotic-resistant bacteria. The order includes “taking steps to eliminate agricultural use of medically important antibiotics for growth-promotion purposes” and “develop alternatives to the use of antibiotics for some agricultural purposes”. It also speaks to strengthening national surveillance efforts for resistant bacteria and promoting new and next generation antibiotics and diagnostics.

In Canada, several initiatives are underway, including reviews by Health Canada both through the Communicable and Infectious Disease Steering Committee (CIDSC) and the Veterinary Drug Directorate (VDD). They are looking at options such as stopping over-the-counter sales of antibiotics, requiring import permits for own-use importation and adding restrictions on Active Pharmaceutical Ingredients (API’s). The Council of Chief Veterinary Officers is examining options to collect reliable antimicrobial use data and looking at the possible effects of prescription-only antimicrobial use.

Use of antimicrobials for growth promotant purposes is being phased out in North America. The VDD announced earlier this year that, in harmony with similar changes in the US, and working with the Canadian Animal Health Institute (CAHI), there will be removal of growth promotion and/or production claims of medically-important antimicrobial drugs over the next three years. As well, they are looking at options to strengthen veterinary oversight, such that medically important antibiotics would be used in food animals only under the direction of a veterinarian for a specific disease challenges.

In many cases, voluntary stewardship actions are being taken by animal agriculture to limit use of antimicrobials to that required to protect animal health and welfare. In May, 2014, the Chicken Farmers of Canada (CFC) voluntarily banned injection of hatching eggs with cefotiofur. Other on-farm food safety programs stipulate that any extra-label drug use (ELDU) requires a veterinary prescription to assure proper dosage and withdrawal times.

Everyone involved with animal agriculture should be aware of initiatives that are occurring in Canada. Currently the Canadian Veterinary Medical Association (CVMA) is having consultations on AMU. It would be beneficial to participate and keep current on the issues. Are you in favour of prescription-only antibiotic use? If that leads to decoupling of prescribing and dispensing as well, what effects would that have on your practice?

Agriculture is under pressure from many fronts to reduce or remove AMU in animals. Many times emotion trumps science. It is a good exercise to examine your own practices. Are you comfortable being open and transparent about prescribing and administering activities? Vets and producers should have nothing to hide and if it needs to be hidden perhaps you should re-think what you are doing.

Antimicrobial stewardship has been described as the practice of minimizing the emergence of antimicrobial resistance by using antibiotics only when necessary and, if needed, by selecting the appropriate antibiotic at the right dose, frequency and duration to optimize outcomes while minimizing adverse effects (Do bugs need drugs, 2014). We, as veterinarians, must be good stewards in order to maintain our social license to use these drugs.
Is it possible to get rid of Equine Infectious Anemia in Western Canada?

By: Anatoliy Trokhymchuk, Disease Surveillance Veterinarian, PDS

From January to October, 2014, twelve hundred and seventy one voluntary tests for Equine Infectious Anemia (EIA) were performed at Prairie Diagnostic Services. From the perspective of close to 600,000 horses in Western Canada, this is a very small number. Voluntary testing and Canadian Food Inspection Agency (CFIA) investigating positive cases have resulted in the detection of 60 positive animals across 11 rural municipalities in Saskatchewan and one jurisdiction in Alberta.

EIA is a viral disease of horses that is difficult to deal with. Although it is uncommon to see a horse become clinically ill and drop dead of eIA, this retrovirus is of concern for a variety of reasons. First of all, there is no cure nor is there a vaccine that will prevent an animal from becoming infected. Infection can take an acute fatal form, a recurring milder disease, or it can have no apparent clinical signs at all. As with all retroviruses, the immune system is the target of EIA, and this defines the danger of ignoring this disease. Animals with compromised immunity become very susceptible to any kind of infection and are not able to mount a good immune response to vaccines; death from secondary infections is a common sequela. The life-long carrier status of surviving horses poses a hazard for herdmates and other horses in its vicinity. EIA is on the list of important animal diseases of the World Animal Health Organization and it is federally reportable disease in Canada.

EIA status is a consideration for international trade and animal movement. To ensure the health of Canadian equines and to secure access to international markets, there is a national EIA control program administered by the CFIA. This program consists of two components: voluntary screening and mandatory response actions. Horse owners are free to choose whether their horses are tested and must pay for testing. However, if a positive animal is detected in a herd, federal regulations mandate a full investigation, including complete herd testing and the removal of positive reactors.

The aggressive testing and culling program appeared to be gaining success, with no positive animals found in Saskatchewan in 1993. The virus was detected again in 1994, with positive cases again found in Saskatchewan every year thereafter until 2005. Testing numbers increased, and EIA was brought under control with no positive horses detected from 2005 to 2009. Naturally, animal owners, boarding facilities, and veterinarians alike started to feel that EIA was a thing of the past. With the emergence of new horse diseases like West Nile and herpes viruses, priorities shifted and the number of EIA tests decreased significantly. Even the “Coggins certificate” requirement was dropped by most equestrian events organizers and boarding facilities operators. Unfortunately, the virus is present and well-established in some parts of the province. We are witnessing an outbreak in Western Canada which has been ongoing since 2010, with positive horses found mainly in northern parts of Saskatchewan, Alberta, British Columbia, and in the Yukon. This unwelcome comeback is partially explained by the nature of the virus. EIA is caused by a retrovirus that belongs to Lentivirus family and is closely related to other lentiviruses such as human immunodeficiency virus and feline immunodeficiency virus, among others. All equines are susceptible. The virus, residing in lymphocytes, can be transmitted by contaminated needles, during breeding, from pregnant mare to her fetus and, most importantly, by large blood sucking insects that tend to attack multiple animals within short periods of time. In many cases, infected animals may be asymptomatic. Once infected, horses become life-long carriers. Infection can spread unnoticed in a herd and can affect many animals if testing is not done in a timely and systematic fashion.

Unfortunately, there are no good options for a horse that tests positive for EIA: it either needs to be placed in a life-long quarantine or euthanized. It can be very hard for animal owners and veterinarians alike to cope with a situation where an intelligent companion animal must be euthanized despite showing no outward signs of illness. This may, in many cases, explain an owner's reluctance to have their horses tested; however, choosing not to test is a precarious move. If present within a herd, and if not controlled, the virus will inevitably spread to other horses creating an even more devastating situation. There is still a fresh memory of a situation from 2011, where 40 out of 80 horses on a single farm in Clarksville, Arkansas, were euthanized as a result of prolonged reluctance to do EIA testing; similar situations have been found in the current outbreak on Saskatchewan farms. There are a number of factors that may be contributing to the current outbreak:

- decline in the horse meat trade has resulted in fewer animals crossing borders, and consequently, less EIA testing performed during the last 10 years;
- diminished awareness of EIA among horse owners, veterinarians, equestrian events organizers, and horse boarding facilities managers which has led to relaxed testing requirements; and
- the increased presence of semi-feral or abandoned horses, creating potential infection reservoirs.
Is it possible to get rid of Equine Infectious Anemia in Western Canada? continued...

EIA eradication is possible. Iceland and Japan are free from the infection, and eradication has been achieved in some North American jurisdictions – for example, the province of Ontario in Canada and the state of California in the USA. The key elements necessary for EIA eradication are:

- regular testing of all equines at risk (all animals participating in organized events, all animals travelling, all animals newly introduced into herd, animals residing in a proximity to territories known to be affected by EIA);
- use of disposable needles and syringes;
- use of strict hygiene practices; and
- good insect control program for the stable and property.

Eradication of equine infectious anemia from Western Canada is a difficult but not impossible task. There are many things that need to be done, but most importantly, everyone involved in the equestrian community must commit to the goal.

If you have any questions regarding EIA testing please contact Prairie Diagnostic Services either by telephone (306) 966-7316 or e-mail: pds.info@usask.ca.

If you require further assistance in advising your clients on issues related to EIA please contact Dr. Wendy Wilkins at the Saskatchewan Ministry of Agriculture either by telephone (306) 798-0253 or e-mail: wendy.wilkins@gov.sk.ca.

Tips for submission of portions from field necropsy cases

By: Yanyun Huang, Veterinary Pathologist, PDS

In a prior issue of the Animal Health Perspectives I encouraged the submission of a variety of tissues for histological examination from field necropsies, even when gross lesions were not evident (please see: The value of ‘normal’ in postmortem specimens: my thoughts; AHP, May 2014, Volume 10, Issue 2; available at www.pdsinc.ca). The work involved in preparing these samples for submission and the cost of shipping these specimens to the laboratory may be increased. So, I would like to provide some tips on the submission of fixed and fresh tissues for histologic examination and adjunct laboratory tests. Hopefully these will be useful to you and help in establishing a diagnosis.

1. Size of fixed tissues:
In most cases, 3cm3 or equivalent of parenchymal tissues (e.g. liver, spleen, kidney, etc.); 3-5 cm of tubular organs (e.g. intestines, esophagus, etc.) and half of the brain (should include cerebrum, cerebellum and brain stem) are sufficient for histologic examination and adjunct laboratory tests. Hopefully these will be useful to you and help in establishing a diagnosis.

2. Size of fresh tissues:
The same sizes indicated above are also ideal for most of adjunct tests, including detection of minerals from the liver (2 -5 grams). Five grams of feces are recommended for parasitology.

3. Fixation of tissues:
It is generally recommended that tissue to formalin volume ratio be 1:10. However, if tissues are collected on one day and are to be shipped the following day, then the fixed tissues can be transferred to a smaller container, with less formalin, for shipment. Tissues, especially gastrointestinal tract, need to be fixed as soon as possible. Gastrointestinal tract begins to undergo autolysis in 15 minutes! This is particularly important in diarrhea cases. Optimal tissue preservation is one of the keys in the success of achieving a diagnosis.

4. Containers and bags:
For fixed tissues, leak-proof containers are to be used. Laboratory film (e.g. Parafilm, www.parafilm.com) can be used to seal the lids of containers. The use of tape (eg. black electrician tape, clear plastic, tape, zinc oxide tape and duct tape) to seal the lids should be discouraged for two reasons. First, the tape does not prevent leakage; and second, it is very time-consuming for people who receive the samples to remove the tape. Fixed tissues can be pooled together and need not be put in many small containers, because pathologists are able to identify the tissues histologically. The exception is when there is need for identifying a specific location of the tissue. In that case, separate containers with clear labeling are needed.

For fresh tissue, separate whirl-pak bags for each tissue with a clear legible label on the bags are recommended. This can prevent cross contamination between tissues and facilitate quick identification of the tissue for adjunct tests.

5. Please don’t hesitate to call us first, before you proceed with the field necropsy (1-306-966-7316)!
Communication is important. You can talk to our reception staff about how to properly ship specimens. You can also ask to talk to a pathologist before you plan to do a necropsy for advice on sample collection and things to look for during the necropsy.

All the above are meant to be helpful suggestions and not stringent rules. Diagnostic investigation requires teamwork. Mutual consideration and support is fundamental to the success of this teamwork. Let’s work together!