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339 rue Booth Street
Ottawa, Ontario K1R 7K1
Telephone: (613) 236-1162
Fax: (613) 236-9681
E-mail: hbroughton@cvma-acvm.org
Website/Site Web: www.canadianveterinarians.net
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One health and a new Canadian vet school?
Une seule santé et une nouvelle école de médecine vétérinaire au Canada?

This issue of The Canadian Veterinary Journal carries a concise but powerful commentary by Dr. Ole Nielsen who proposes that we consider a 6th Canadian veterinary school, a One Health track in the DVM curriculum, and limited licensure of veterinarians in specified fields. These are issues of enormous importance to practicing veterinarians, the profession at large, veterinary schools, and society. Some of the ideas expressed in this commentary are presented in greater detail in an article in the Journal of the American Veterinary Medical Association in September of this year (1). It is well worth reading.

Dr. Nielsen makes a strong argument for a 6th veterinary school in Canada. He notes that over the past 53 years 3 new veterinary schools were established at approximately 20-year intervals in response to, among other things, growth in the population of people and pets, student demand and increasing importance of new areas such as aquaculture. He argues that these factors are still relevant and they indicate that another veterinary school would be appropriate around 2025, 20 years after the most recent Canadian veterinary school. An additional factor cited in support of the 6th veterinary school is expansion needed to provide veterinarians with satisfactory training in One Health.

The One Health theme is a major feature of Dr. Nielsen’s vision for the future of veterinary medicine. He notes the increasing connectivity within ecosystems involving animals, humans and the environment and the associated profound impacts on health. He reasons that these developments demand a One Health approach and veterinary medicine is the discipline best suited to take up the challenge. If veterinarians are to take a leadership role in this endeavor then they need to be adequately educated. Dr. Nielsen’s proposal is that there be a One Health track within the DVM curriculum. This track would provide increased emphasis on subjects such as comparative medicine, ecology, ecological medicine, computer modeling and social sciences (1).

L e présent numéro de La Revue vétérinaire canadienne publie un commentaire concis mais puissant rédigé par le Dr’ Ole Nielsen qui propose que nous considérons la création d’une sixième école de médecine vétérinaire au Canada, d’un volet d’Une seule santé dans le curriculum du programme de D.M.V. et de permis restreints pour les médecins vétérinaires dans des domaines particuliers. Il s’agit là d’enjeux d’importance considérable pour les médecins vétérinaires en exercice, la profession dans son ensemble, les écoles de médecine vétérinaire et la société. Certaines des idées exprimées dans ce commentaire sont présentées plus en détail dans un article publié dans le numéro de septembre de cette année de la revue Journal of the American Veterinary Medical Association (1). Cet article mérite certainement d’être lu.

Le Dr’ Nielsen présente un argument solide pour la création d’une sixième école de médecine vétérinaire au Canada. Il signale que, au cours des 53 dernières années, trois nouvelles écoles de médecine vétérinaire ont été établies à des intervalles d’environ 20 ans en réponse, entre autres, à la croissance de la population d’humains et d’animaux de compagnie, à la demande étudiante et à l’importance grandissante de nouveaux domaines comme l’aquaculture. Il fait valoir que ces facteurs sont toujours pertinents et qu’ils indiquent qu’une autre école de médecine vétérinaire serait appropriée vers 2025, 20 ans après l’établissement de la plus récente école de médecine vétérinaire canadienne. Un autre facteur cité en support de la sixième école de médecine vétérinaire est l’expansion requise pour offrir aux médecins vétérinaires une formation satisfaisante dans le domaine d’Une seule santé.

Le thème d’Une seule santé est l’un des principaux éléments de la vision du Dr’ Nielsen pour l’avenir de la médecine vétérinaire. Il signale la connectivité croissante au sein des écosystèmes qui relie les animaux, les humains et l’environnement et ses profondes répercussions connexes sur la santé. Il raisonne que ces progrès exigent l’approche d’Une seule santé et que la médecine...
There is also a proposal that regulations be changed to allow licensing of veterinary graduates in designated fields, including One Health. At present, licensing of veterinary graduates in North America requires competence in all fields, indicated by success in the comprehensive North American Veterinary Licensing Examination (NAVLE) and veterinary curricula prepare students to perform satisfactorily in the NAVLE. Limited licensure, in contrast to the existing comprehensive licensure, would allow veterinary schools to better prepare graduates in restricted areas of practice. Dr. Nielsen suggests that veterinary schools may be able to find funding for development of a One Health track by reducing in-house clinical training and involving private practices to a greater extent in this aspect of their curriculum.

Whenever Dr. Ole Nielsen speaks (or writes) it is always worth listening (or reading), because he has rich and valuable experience in veterinary education and is a thoughtful leader in the profession. Whether one agrees or disagrees with Dr. Nielsen’s vision, I suspect that we can all agree that the issues he has raised are worth considerable discussion by the profession, leading to possible action.

Establishment of a new veterinary school requires a long lead time and now would be the time to initiate discussion for a 2025 target date. Such action usually comes with much debate about the need for veterinary graduates, the location of the school, financial support at provincial and federal levels, the impact on local veterinary practices, the curriculum, accreditation, and faculty. There is also a requirement for a leader or leaders who are prepared to do the organization and lobbying for a new school.

The need for a One Health track within the DVM curriculum is an issue that does not have to be linked to a new veterinary school but the question of how best we prepare some veterinarians to take a leadership role in One Health is a topic that is ripe for discussion. An alternative approach would be to develop the One Health track within a post-DVM MPH program; this could be a program for MD and DVM graduates, offered as a collaborative effort between a veterinary school and a medical school.

I believe that provision for limited licensure among veterinary graduates is long overdue. This too can develop with or without a One Health track for veterinarians. A large number of veterinary curricula presently offer some degree of specialization along various clinical tracks, but the extent of specialization in a 4-year veterinary curriculum is constrained by the requirements of the comprehensive licensing examination. Limited licensure and changes in accreditation requirements could result in some veterinary schools opting to specialize in some areas of veterinary education and not in others. The changes in accreditation and licensing would require action on a North American basis, involving the American Veterinary Medical Association Council on Education.

Dr. Nielsen portrays a grand vision for veterinary medicine of the future. It includes a plan to make veterinarians leaders in One Health, which is seen as essential to understanding health and disease in the context of ecosystems involving interactions among animals, people, and the environment. These proposed veterinary is the discipline the most equipped to handle the defi. Si ce sont les vétérinaires qui assumeront un rôle de leadership dans ce domaine, ils devront alors être bien formés. Le Dr. Nielsen propose qu’il y ait un volet d’Une seule santé dans le curriculum du D.M.V. Ce volet permettrait d’approfondir des sujets comme la médecine comparée, l’écologie, la médecine écologique, la modélisation informatique et les sciences sociales (1).

On suggère aussi que la réglementation soit modifiée afin de permettre l’octroi de permis aux diplômés vétérinaires dans des domaines particuliers, notamment celui d’Une seule santé. À l’heure actuelle, l’obtention du permis d’exercice en médecine vétérinaire exige une compétence dans tous les domaines qui est confirmée par la réussite de l’Examen nord-américain d’agrément en médecine vétérinaire (NAVLE), qui est une évaluation complète, et le curriculum vétérinaire prépare les étudiants à un rendement satisfaisant au NAVLE. Par contraste au permis exhaustif actuel, un permis limité permettrait aux écoles de médecine vétérinaire de mieux préparer les diplômés dans des domaines d’exercice restreints. Le Dr. Nielsen suggère que les écoles de médecine vétérinaire pourraient être capables de trouver des fonds pour la création d’un volet d’Une seule santé en réduisant la formation clinique à l’interne et en invitant la collaboration des pratiques privées dans une plus grande mesure pour cet aspect de leur curriculum.

Quand le Dr Ole Nielsen parle (ou écrit) il vaut toujours la peine de l’écouter (ou de le lire) parce qu’il possède une expérience riche et utile dans l’enseignement de la médecine vétérinaire et que c’est un brillant leader de la profession. Que l’on soit en accord ou en désaccord avec la vision du Dr Nielsen, je soupçonne que nous pouvons tous convenir que les enjeux qu’il a soulevés méritent de faire l’objet de discussions considérables par la profession, ce qui pourrait se traduire par des actions futures.

L’établissement d’une nouvelle école de médecine vétérinaire exige de longs délais d’exécution et c’est maintenant que nous devrions entamer des discussions pour une date cible de 2025. Une telle action s’accompagnera de nombreux débats à propos du besoin de diplômés en médecine vétérinaire, de l’emplacement de l’école, du soutien financier aux paliers provinciaux et fédéral, de l’impact sur les pratiques vétérinaires locales, du curriculum, de l’agrément et des professeurs. Il existe aussi un besoin d’un leader ou de leaders qui sont prêts à travailler à l’organisation et aux efforts de lobbying pour une nouvelle école.

Le besoin d’un volet d’Une seule santé dans le curriculum du D.M.V. est un enjeu qui ne doit pas nécessairement être relié à une nouvelle école de médecine vétérinaire, mais la question de la meilleure façon dont nous pouvons préparer certains vétérinaires à assumer un rôle de leadership au sein d’Une seule santé est un sujet qui doit être abordé. Une autre approche consisterait à créer un volet d’Une seule santé dans un programme d’études de maitrise en santé publique enseigné après le D.M.V. Ce programme pourrait s’adresser aux diplômés en médecine humaine et en médecine vétérinaire et être offert dans le cadre d’une collaboration entre une école de médecine vétérinaire et une école de médecine.

Par ailleurs, je crois que les dispositions pour l’émission de permis restreints se font attendre depuis longtemps. Ce
developments are intended to enhance the contribution of our profession to society. I look forward to hearing the opinions of veterinarians in practice and in academia.

Reference

Carlton Gyles

(Opinions expressed in this column are those of the Editor.)

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Ethical question of the month — December 2017

The Health of Animals Animal Transport Regulations ensures that animals are transported humanely. Transporters, producers, and abattoirs have been charged by the Canadian Food Inspection Agency (CFIA) with not adhering to these regulations. Infractions include overcrowding, insufficient protection from the elements, improper segregation of animals, and improper protection of animals from injury. The routine shipment of Canadian horses to Japan by air for slaughter falls under this regulatory jurisdiction. The CFIA oversees the loading of these large breed horses into wooden shipping containers prior to the horses being loaded onto the aircraft. Horses are commonly shipped with 3 to 4 horses per container, where the larger horses’ heads come into contact with the tops of the crates. These events are in contravention of the animal transport regulations that require horses over 14 hands to be individually segregated and further require that animals’ heads do not come into contact with the tops of the shipping containers. In addition, the International Air Transport Association (IATA) Live Animal Regulations do not allow horses to be shipped in wooden containers. Documentation shows that horses have been injured and killed in the course of these shipments. The federal regulatory body effectively enforces the transport regulations when infractions by producers or transporters are noted. How can infractions by the regulatory body itself be addressed?

Submitted by Maureen Harper, Brampton, Ontario

Responses to the case presented are welcome. Please limit your reply to approximately 50 words and forward along with your name and address to: Ethical Choices, c/o Dr. Tim Blackwell, 6486 E. Garafraxa, Townline, Belwood, Ontario N0B 1J0; telephone: (519) 846-3413; fax: (519) 846-8178; e-mail: tim.e.blackwell@gmail.com

Suggested ethical questions of the month are also welcome! All ethical questions or scenarios in the ethics column are based on actual events, which are changed, including names, locations, species, etc., to protect the confidentiality of the parties involved.

Les dispositions relatives au transport du Règlement sur la santé des animaux veillent à ce que les animaux soient transportés de manière non cruelle. Des transporteurs, des producteurs et des abattoirs ont fait l’objet d’accusations de la part de l’Agence canadienne des inspections des aliments (ACIA) pour avoir enfreint ces règles. Les infractions incluent la surpopulation, une protection insuffisante contre les intempéries, une ségrégation inadéquate des animaux et une protection inappropriée afin de prévenir les blessures chez les animaux. L’expédition routière par avion de chevaux canadiens vers le Japon aux fins d’abattage est soumise à cette réglementation. L’ACIA supervise le chargement de ces chevaux de grande race dans des conteneurs d’expédition avant qu’ils ne soient placés dans l’avion. Les chevaux sont habituellement expédiés en plaçant 3 ou 4 chevaux dans un conteneur où la tête des grands chevaux touche le plafond des caisses à claire-voie. Ces instances contreviennent au Règlement sur le transport des animaux qui exigent que les chevaux de plus de 14 mains soient séparés individuellement et aussi que la tête des animaux n’entre pas en contact avec le plafond des conteneurs d’expédition. De plus, la Réglementation du transport des animaux vivants de l’Association du transport aérien international (IATA) n’autorise pas l’expédition dans des conteneurs en bois. La documentation montre que des chevaux ont été blessés et tués lors de ces expéditions. L’organisme fédéral de réglementation applique les règles sur le transport lorsqu’il observe des infractions par les producteurs ou les transporteurs. Comment peut-on aborder les infractions commises par l’organisme de réglementation lui-même?

Soumise par Maureen Harper, Brampton (Ontario)

Les réponses au cas présenté sont les bienvenues. Veuillez limiter votre réponse à environ 50 mots et nous la faire parvenir par la poste avec vos nom et adresse à l’adresse suivante : Choix déontologiques, a/s du D’ Tim Blackwell, 6486, E. Garafraxa, Townline, Belwood (Ontario) N0B 1J0; téléphone : (519) 846-3413; télécopieur : (519) 846-8178; courriel : tim.e.blackwell@gmail.com

Les propositions de questions déontologiques sont toujours bienvenues! Toutes les questions et situations présentées dans cette chronique s’inspirent d’événements réels dont nous modifions certains éléments, comme les noms, les endroits ou les espèces, pour protéger l’anonymat des personnes en cause.

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L’usage du présent article se limite à un seul exemplaire pour étude personnelle. Les personnes intéressées à se procurer des réimpressions devraient communiquer avec le bureau de l’ACMV (hbroughton@cvma-acmv.org) pour obtenir des exemplaires additionnels ou la permission d’utiliser cet article ailleurs.
Ethical question of the month – September 2017

Groups defending current livestock production practices claim that videos showing abusive handling procedures in livestock facilities are the exception rather than the norm. If this is true, then animal activists have an unexpectedly high success rate videotaping farms with substandard animal husbandry practices. It can be argued that activists are more likely to be hired by large facilities due to staff turnover and therefore animal welfare problems are more likely to be documented on large facilities. Animal agriculture proponents argue that animal welfare problems are not related to farm size. Large facilities can dedicate staff to particular animal care responsibilities, which is a luxury that smaller facilities cannot afford. Should veterinarians consider farm size as a significant risk factor for animal welfare problems?

An ethicist’s commentary on the connection between farm size and animal welfare

This is an issue that cannot be answered in an unequivocal, straightforward manner. There is no question that erosion of good stockmanship and care that is based in acknowledgment of farm animals’ needs and natures is historically associated with the industrialization of animal agriculture. As we have often remarked in this column, good husbandry was the key to success in animal agriculture from its inception until very recently.

Domestication of animals is a key feature in the development of civilization. Some 12 000 years ago, animals congenial to human society were domesticated and, by selective breeding, were further bound up with humans. This resulted in what has been called the ancien contract between humans and animals, wherein both sides, i.e., humans and animals, were able to live an improved life. Humans utilized animals for food, fiber, locomotion, and power. Animals depended on humans for providing environments that suited their biological natures, food during famine, water during drought, protection from predation, help in birthing, and such medical attention as was available.

If the animals’ welfare was not protected, and their needs not met, they failed to produce, which ultimately harmed the farmers. Thus, self-interest, the ultimate motivation for people, drove the perpetuation of good husbandry. The guarantee of a secure and predictable food supply in turn made possible the development of civilization. One of the great ironies in human history is that part of the development of civilization was the rise of agricultural technology, which undercut the very husbandry whose care and treatment of their animals is abysmal. Inevitably, the farmers. Thus, self-interest, the ultimate motivation for people, drove the perpetuation of good husbandry. The guarantee of a secure and predictable food supply in turn made possible the development of civilization. One of the great ironies in human history is that part of the development of civilization was the rise of agricultural technology, which undercut the very husbandry that made it possible! As I have repeatedly written, husbandry was about putting square pegs in square holes, round pegs in round holes, and creating as little friction as possible doing so. But the rise of “technological sanders” such as antibiotics, vaccines, air handling systems, allowed us to, as it were, force square pegs into round holes, round pegs into square holes, resulting in significant loss of animal welfare without concomitant loss in productivity, thereby breaking the ancient contract.

Associated with the industrialization of agriculture and the major truncation of the space in which animals were kept came a related loss of good husbandry and what has been called “animal smart labor.” As one industrialized hog production manager said to me, “the intelligence is in the system.” We are all aware that “systems” do not display intelligence. Furthermore, confinement feeding, rather than pastoral grazing, allowed large numbers of animals to be produced in small spaces. In essence, capital replaced labor, with industrialized operations being highly capitalized and growing very large.

Perhaps inevitably, with concerns for individual animals tending to be replaced by concern for profit, welfare further suffered. This is clearly evidenced by the history of confinement agriculture. Having said that, however, there is no necessary, logical connection between large operations and poor husbandry. There are in fact good examples of very large operations paying a great deal of attention to the well-being of the animals; these exist, but are regrettably scarce. These constitute exceptions, rather than the rule. It is perfectly conceivable, albeit very difficult, to create large operations that excel in husbandry. On the other hand, my animal agricultural colleagues assure me that they have seen small, even family-owned and family-run operations, whose care and treatment of their animals is abysmal. Inevitably, of course, such operations will collapse under their own weight.

And herein lies the crux of my answer to the question posed. It is certainly conceivable that large industrialized operations operate with a strong commitment to husbandry and thus to good welfare. But it is also unlikely, given the nature of these...
operations as profit-making organizations. It is equally conceivable that small family units operate against their own interest, and fail to provide good husbandry. But, again, that is unlikely.

As I have argued before in this column, good welfare for farm animals is inexorably emerging as a major societal concern that must be addressed by virtue of producers needing to bend to consumer demand. And such demand will inevitably push in the direction of creating better animal husbandry even in large corporate entities. Indeed, given the significant resources of such corporate entities, they are well situated to effect changes quickly that benefit animal welfare, as occurred in 2007 when Smithfield Farms committed to eliminating gestation crates. Such changes will of course not be easy, but will inevitably occur. And the self-interest that historically dictated good husbandry will now be guided not by the historical connection between productivity and welfare, but rather by equally profound ethical concerns in society, demanding a return to husbandry that will determine the playing field on which animal agriculture plays out.

*Bernard E. Rollin, PhD*
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Have Another Look at CJVR
Avez-vous consulté la RCRV dernièrement?

Members of the CVMA are entitled to receive the Canadian Journal of Veterinary Research (CJVR) at no additional charge. The CJVR, in the form of an interactive (portable document format) pdf, can be found on the CVMA member-only website (www.canadianveterinarians.net/publications-research-issue.aspx).

Published by the CVMA, this quarterly, peer-reviewed journal is Canada’s only national veterinary research publication.

Articles from the October 2017 issue of CJVR that might be of interest to practitioners include:

Evaluation of a welfare assessment tool to examine practices for preventing, recognizing, and managing pain at companion-animal veterinary clinics on page 270

Identification of Mycobacterium avium subspecies paratuberculosis strains isolated from dairy goats and dairy sheep in Ontario, Canada on page 304

Histological and functional characterizations of the digital cushion in Quarter horses on page 285

The CJVR, along with the monthly Canadian Veterinary Journal, is also archived on PubMed Central (www.pubmedcentral.com) 6 months after publication. An interactive pdf of The CVJ is also available on the member-only section of the CVMA website.


Publiée par l’ACMV, cette revue trimestrielle évaluée par les pairs est la seule publication nationale de recherche vétérinaire au Canada.

Les articles suivants du numéro d’octobre 2017 de la RCRV pourraient intéresser les praticiens :

Evaluation of a welfare assessment tool to examine practices for preventing, recognizing, and managing pain at companion-animal veterinary clinics à la page 270

Identification of Mycobacterium avium subspecies paratuberculosis strains isolated from dairy goats and dairy sheep in Ontario, Canada à la page 304

Histological and functional characterizations of the digital cushion in Quarter horses à la page 285

La RCRV, avec La Revue vétérinaire canadienne qui est publiée mensuellement, est aussi archivée sur PubMed Central (www.pubmedcentral.com) six mois après la publication.

Un pdf interactif de La RVC est aussi disponible dans la section réservée aux membres du site Web de l’ACMV.
1. Concerning cat heartworm disease, which of the following statements is NOT CORRECT?
   A. Heartworm infections in cats may spontaneously resolve due to their strong immune response.
   B. Microfilaria are transient, seen 20% of the time, and last approximately 1 month in the cat.
   C. Cats are less susceptible to infection than dogs.
   D. Heartworms live longer in the cat than the dog.
   E. The lungs of cats are usually the organ with the greatest pathology, whereas the lungs and heart are affected most often in dogs.

2. Which of the following statements does NOT describe a benefit of cytology over histology?
   A. Cytology is generally much faster from sample acquisition to evaluation.
   B. The details seen with cytology are generally finer than with histology.
   C. Acquiring samples of cytologic specimens often induces less morbidity than obtaining samples for histologic evaluation.
   D. The amount of materials consumed and therefore the price of cytology is often less than that for histology.
   E. Cytology allows for the examination of tissue architecture.

3. A horse is being evaluated for generalized lesions, described as nonpruritic nodules that rapidly appear and disappear. Which of the following is the most appropriate diagnostic test to confirm the cause of these lesions?
   A. Skin biopsy of the nodules
   B. Skin scrapings of the nodules
   C. Fungal culture of hair over a nodule
   D. Fine-needle aspirate of a nodule
   E. Bacterial culture of material aspirated from a nodule
4. A patient under anesthesia suddenly has an increase in body temperature. Malignant hyperthermia is suspected. Which of the following drugs is recommended for treatment of this anesthetic complication?
   A. Dantrolene
   B. Flumazenil
   C. Atipamazole
   D. Nalbuphine

5. Which of the following lab tests is most appropriate in following a dog treated with erythropoietin?
   A. A liver biochemistry panel
   B. Oxygen saturation
   C. Complete blood (cell) count (CBC)
   D. Chest radiograph
   E. Neurologic examination

(See p. 1331 for answers./Voir les réponses à la page 1331.)
Animal Welfare: Safeguarding the Five Animal Freedoms
2017 Animal Health Week Wrap-Up

The Canadian Veterinary Medical Association (CVMA) celebrated another successful Animal Health Week in October. With over 200 clinics and animal hospitals participating, thousands of animal families from coast to coast were introduced to

One of the Lessard Callingwood Veterinary Hospital’s (Edmonton, Alberta) RVTs, Janelle, put her creative skills to work and drew the Five Animal Freedoms on the clinic chalkboard — what talent! The Hospital was the winner of our Animal Health Week early bird merchandise order prize and received a $100 Subway gift card from the CVMA. Some of the staff’s Subway treats snuck into the photo.

Check out the Northumberland Veterinary Services staff (Colborne, Ontario) modelling this year’s amazing Animal Health Week T-shirts with the help of some furry friends! The clinic provided free treats (for pets and people!), pet insurance information, safety flashers, collapsible bowls, and more during the week! They also offered daily door prizes and a children’s coloring contest.

The Canadian Food Inspection Agency (CFIA) invited Dr. Shane Renwick, CVMA manager, National Issues and Animal Welfare, to talk about Animal Health Week on October 4 and 5. The CFIA is always a strong supporter of Animal Health week as evidenced by the photos! Dr. Renwick (left) is pictured with Dr. Mohit Baxi, director, Animal Import/Export, CFIA.

L’Association canadienne des médecins vétérinaires (ACMV) a célébré une Semaine de la vie animale réussie en octobre. Grâce à la participation de plus de 200 cliniques et hôpitaux vétérinaires, des milliers de familles d’animaux d’un océan à l’autre ont été introduites au thème de cette année de Protéger les cinq libertés afin d’assurer le bien-être animal.

Merci aux vétérinaires, aux équipes vétérinaires et aux étudiants qui ont contribué aux célébrations. Nous espérons que vous avez

Regardez le personnel de la clinique Northumberland Veterinary Services (Colborne, Ontario) qui porte les spectaculaires t-shirts de la Semaine de la vie animale de cette année avec l’aide d’amis à fourrure! La clinique offrait des gâteries gratuites (pour les animaux et les humains!), des renseignements sur l’assurance maladie pour animaux, des clignotants de sécurité, des bols escamotables et plus encore durant la semaine! Elle a aussi offert des prix de participation quotidiens et tenu un concours de coloriage pour les enfants.

L’Agence canadienne d’inspection des aliments (ACIA) a invité le Dr Shane Renwick, gestionnaire des Enjeux nationaux et bien-être animal, pour parler à propos de la Semaine de la vie animale. L’ACIA est toujours un solide partisan de la Semaine de la vie animale, comme en témoigne d’ailleurs les photos! Le Dr Renwick (à gauche) avec le Dr Mohit Baxi, directeur, Importation et exportation des animaux, ACIA.
this year’s theme of Animal Welfare: Safeguarding the Five Animal Freedoms.

Thank you to all veterinarians, veterinary teams, and students who helped to celebrate. We hope you were able to engage with your clients and disseminate information about animal welfare that will help animals to lead healthy and happy lives, so they not only survive, but thrive.

We are thrilled with the success of this year’s Animal Health Week. Check out clinic celebrations by looking up #AnimalHealthWeek on Facebook and Twitter.

Mark your calendar for the 2018 Animal Health Week campaign, which will take place from September 30 to October 6 next year.

We could not have achieved this success without the support of our generous sponsors. We would like to graciously thank our 2017 Animal Health Week sponsors: Principal Plus Sponsor, Boehringer Ingelheim, Principal Sponsor, Petsecure Pet Health Insurance, and Program Sponsors, iFinance Petcard and Elanco.

Please visit the website (www.canadianveterinarians.net) to learn more about the Canadian Veterinary Medical Association and Animal Health Week.

Hold on Tight for the Most Unique CVMA Convention Experience Ever

Vancouver, July 5–8, 2018

CVMA member, Dr. Lynn Morgan, was featured on Rogers Daytime Ottawa speaking about the Five Animal Freedoms. She was joined by her adorable border collie, Fox!

La Dʳ Lynn Morgan, qui est membre de l’ACMV, a été invité à Rogers Daytime Ottawa pour parler à propos des Cinq libertés animales. Elle était accompagnée de son adorable Border collie, Fox!

The Village Cat Clinic (Ancaster, Ontario) did not hold back when celebrating Animal Health Week this year! Check out their vibrant display illustrating the Five Animal Freedoms. And they made kitty cupcakes to boot!

La Village Cat Clinic (Ancaster, Ontario) a déploié les grands moyens lors de la célébration de la Semaine de la vie animale de cette année! Regardez leur exposition colorée illustrant les Cinq libertés animales. Et ils ont même préparé des petits gâteaux ressemblant à des chats!

Please visit the website (www.canadianveterinarians.net) to learn more about the Canadian Veterinary Medical Association and Animal Health Week.
Convention delegates will be housed at the JW Marriott, which provides access to the Parq Vancouver’s wide range of amenities, including downtown’s only casino, 8 diverse restaurants and lounges, a 30 000-square foot park with native trees located 6 floors above street level, a world class spa and more. The sessions and Exhibit Hall will be held in the 60 000 square feet of well-appointed, flexible meeting and event space featuring Vancouver’s largest hotel ballroom.

2018 CVMA Convention Local Chair, Dr. Sarah Armstrong, moved to Vancouver 8 years ago and she is still in awe of its beauty. “It has everything a big city has to offer in addition to mountains and the ocean at your fingertips,” she said.

As a destination, the majestic mountains, sparkling ocean, rainforests and beautiful foliage throughout all 4 seasons make Vancouver one of the most beautiful cities in the world. Dr. Armstrong said: “In one day I can go from hiking the North Shore, shopping on Robson Street, to dining out, and then watching the sunset over the beautiful mountain ranges. I highly recommend you attend the 2018 Convention and visit this wonderful city.”

Attend the Convention and then plan your gateway to adventure. With quick and easy access to Whistler, the Canadian Rockies, Victoria, other parts of Vancouver Island, and of course, endless year-round water and land sports, whether you’re looking for extreme sports or family fun, you’ll find your personal adventure here. Vancouver also is the home port for Alaska cruises May through October.
The Vancouver International Airport has consistently been rated as a top North American airport providing easy access from all over the world. The city has public transportation, but given how walkable Vancouver is, you just might never use it! A great walking city, Vancouver is clean, green, safe, and easily accessible.

For more ideas of what Vancouver has to offer, visit the website (www.tourismvancouver.com). We are waiting to welcome you in Vancouver!

### Planning to Hit the Slopes of Mont Tremblant this Winter?

**Vous planifiez dévaler les pentes du Mont-Tremblant cet hiver?**

It’s summer and it’s time to plan your winter activities. If you’re planning to ski or snowboard, the CVMA has negotiated the best rates to enjoy the slopes of Mont Tremblant through SkiMax.

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<th>Dates</th>
<th>CVMA Member Price</th>
<th>Regular Price</th>
<th>Savings</th>
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<tr>
<td>Until December 7, 2017</td>
<td>$56 per ticket</td>
<td>$89</td>
<td>36%</td>
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<td>As of December 8, 2017</td>
<td>$72 per ticket</td>
<td>$89</td>
<td>19%</td>
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Fully transferable.

In addition, if you do want to ski or snowboard during the holidays, you can save 46% on the ticket office rate (different from regular prices above) with Tremblant SkiMax tickets from December 27 to January 3, 2018.

To receive the best rate, simply follow these steps:

1. On the CVMA website (www.canadianveterinarians.net), under Value of Membership, click on the member benefits and services section and scroll down to Mont Tremblant SkiMax.
2. Have your CVMA member log-in ready and click on the “promo code” link.
3. Click on “Buy Now,” select the number of tickets you need and click “Add to basket.”
4. Proceed to checkout.
5. Log into your account or create one, then click “Continue.”
6. Enter your payment information and your promo code. Your discount will then be applied.
7. Place your tickets at the Customer Services Desk or at the ticket office, if you have not planned a delivery (valid photo ID required).

Hurry, take advantage of the best CVMA rates and make your winter a memorable one.

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### Save with the CVMA Discounts

If you’re planning to ski or snowboard, the CVMA has negotiated the best rates to enjoy the slopes of Mont Tremblant through SkiMax.

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<th>Dates</th>
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<tr>
<td>Jusqu’au 7 décembre 2017</td>
<td>56 $ le billet</td>
<td>89 $</td>
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<td>À compter du 8 décembre 2017</td>
<td>72 $ le billet</td>
<td>89 $</td>
<td>19%</td>
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Valable pour toute la saison 2017-2018 sans restrictions, sauf pour le congé de Noël (du 27 déc. au 3 janv. 2018)

Entièrement transférable.

De plus, si vous désirez faire du ski ou de la planche à neige durant le congé des Fêtes, vous pouvez économiser 46% sur le tarif des billets vendus à la billetterie (différent des tarifs ci-dessus) en achetant des billets auprès de Tremblant SkiMax du 27 décembre au 3 janvier 2018.

Pour obtenir le meilleur tarif, veuillez suivre les étapes suivantes :

1. Sur le site Web de l’ACMV (www.veterinairesaucanada.net), sous Value of adhesion, cliquez sur la section des avantages et des services aux membres et définissez le bas jusqu’à Mont Tremblant SkiMax.
2. Ayez vos données de connexion à portée de la main et cliquez sur le lien de « code promotionnel ».
3. Cliquez sur « Acheter en ligne », choisissez le nombre de billets dont vous avez besoin et cliquez sur « Ajouter au panier ».

(Veuillez noter que votre rabais SkiMax sera appliqué ultérieurement durant le processus.)

4. Choisissez « Continuer les achats » si vous desirez que vos billets soient livrés par Xpresspost.
5. Ouvrez une session de votre compte ou créez un compte, puis cliquez sur « Continuer ».

6. Entrez les données de paiement et votre code promotionnel.
    Votre rabais sera ensuite appliqué.
7. Allez ensuite au paiement.
8. Vous pouvez cueillir vos billets au comptoir du service à la clientèle ou à la billetterie, si vous n’avez pas choisi la livraison (pièce d’identité photo en règle requise). Dépêchez-vous, pour profiter du meilleur tarif et passer un hiver formidable!
CVMA Emerging Leaders Program
Programme des futurs leaders de l’ACMV

The CVMA Emerging Leaders Program (ELP) started in 2010 as a small full-day workshop with the objective of supporting and developing leadership skills in Canadian veterinarians, and to inspire leadership within the veterinary profession.

The 2017 CVMA Emerging Leaders Program was realized with the help of the provincial veterinary medical associations, and exclusive sponsor, Virox Animal Health. If you would like to learn more about the Emerging Leaders Program and how to participate please contact Sarah Cunningham (scunningham@cvma-acmv.org).

The spotlight on ELP series will highlight the experience of some past program participants. Let’s turn the spotlight on Dr. Marianne Parent, Petfocus Harbour Cities Veterinary Hospital in Dartmouth, Nova Scotia.

1. Why did you want to participate in the CVMA ELP?
I wanted to participate in the CVMA Emerging Leader Program because I had some previous leadership training and wanted to learn more about veterinary leadership. I also wanted to become an effective communicator to engage my veterinary team, clients, and people in my personal life in providing the best care for their companion animals.

2. What was the highlight of the program for you?
The highlight of the program was gaining the self-awareness that I was already employing some of the tips and tricks outlined by the instructor. For example, I am a positive person who takes the time to empathize with the veterinary team and clients before making any judgements. The instructor allowed me to realize that this is a great tool to use to keep a positive workplace environment, and that not everyone takes this approach on their own.

3. Describe one specific action that you are doing differently after participating in the ELP?
Since completing the CVMA ELP, I have begun to develop my life vision, mission and goals. I have tried to provide a positive experience for the clients, and a small thing I have started doing is offering water to clients if they have to wait for any period of time.

4. Do you think it is important for others within the veterinary community to become more active in leadership training? Why?
I think it is crucial for the veterinary community to embrace the tools provided during leadership training. We need to foster the bond between the veterinarian, client and patient to demonstrate to the community that we are not “about the money” and we have their animals’ best interest at heart.

Dr./Drs Marianne Parent

Le Programme des futurs leaders (PFL) de l’ACMV a débuté en 2010 sous forme d’un atelier d’une journée dans le but d’appuyer et de développer les compétences de leadership parmi les vétérinaires canadiens et d’inspirer le leadership au sein de la profession vétérinaire.

Le PFL 2017 de l’ACMV a été mis en œuvre grâce à l’appui des associations provinciales de médecins vétérinaires et le commanditaire exclusif, Virox Animal Health. Si vous aimeriez en apprendre davantage à propos du Programme des futurs leaders et sur la façon d’y participer, veuillez contacter Sarah Cunningham, (scunningham@cvma-acmv.org).

La série Pleins feux sur le PFL soulignera l’expérience de quelques-uns des participants au programme. Présentons maintenant la D° Marianne Parent, de Petfocus Harbour Cities Veterinary Hospital, à Dartmouth, en Nouvelle-Écosse.

1. Pourquoi désiriez-vous participer au PFL de l’ACMV?
Je désirais participer au Programme des futurs leaders de l’ACMV parce que j’avais déjà suivi une formation en leadership et que je désirais en apprendre davantage à propos du leadership vétérinaire. Je désirais aussi devenir une communicatrice efficace pour engager mon équipe vétérinaire, mes clients et les personnes dans ma vie personnelle afin de fournir les meilleurs soins à nos animaux de compagnie.

2. Quel a été le fait saillant du programme pour vous?
Le fait saillant du programme a été la réalisation que j’employais déjà des astuces et trucs présentés par l’instructeur. Par exemple, je suis une personne positive qui prend le temps d’écouter l’équipe vétérinaire et les clients avant de porter des jugements. L’instructeur m’a permis de réaliser que c’est un excellent outil à utiliser pour maintenir un lieu de travail positif et que ce n’est pas tout le monde qui adopte automatiquement cette approche.

3. Décrivez une action particulière que vous effectuez différemment après votre participation au PFL?
Depuis l’achèvement du PFL de l’ACMV, j’ai commencé à élaborer ma vision, ma mission et mes objectifs de vie. J’ai tenté de fournir une expérience positive aux clients et j’ai aussi commencé à offrir de l’eau aux clients s’ils doivent attendre pendant de longues périodes de temps.

4. Croyez-vous qu’il est important pour les autres participants à la collectivité vétérinaire de devenir plus actifs dans la formation au leadership? Pourquoi?
Je crois qu’il est crucial pour la collectivité vétérinaire d’adopter les outils fournis durant la formation de leadership. Nous devons favoriser le lien entre le vétérinaire, le client et le patient afin de démontrer à la collectivité que nous ne pensons pas seulement à l’argent et que l’intérêt supérieur des animaux nous tient à cœur.
2018 CVMA Awards
Nominations are Due January 31, 2018

Prix de l’ACMV 2018
Les mises en candidature doivent être soumises d’ici le 31 janvier 2018

Each year, through its awards program, the Canadian Veterinary Medical Association (CVMA) proudly recognizes individuals who have demonstrated significant accomplishments, exemplary leadership, and tireless commitment to Canada’s veterinary community. Nominations for the 2018 CVMA Awards are being accepted from now until January 31, 2018.

Award eligibility
Award nominees (excluding those nominated for Honorary Membership) must be current CVMA members to be eligible for nomination; however, they can be nominated by non-CVMA members. We invite you to consider nominating a deserving colleague for one of the following CVMA’s prestigious awards:

• CVMA Humane Award
• CVMA Industry Award
• Merck Veterinary Award
• Small Animal Practitioner Award
• CVMA Practice of the Year Award
• CVMA Life Membership
• CVMA Honorary Membership

Nomination package
Selection of award recipients is based solely on the information provided in the nomination package. Please follow these steps to ensure all required documents are included with your nomination package:

1. Submit a completed nomination form. The form is available for download under the CVMA Awards section of our website (www.canadianveterinarians.net).
2. Include the following supporting documents as part of the nomination package:
   • Outline of nominee’s key professional accomplishments (maximum of 1000 words)
   • Letters of support (maximum of 5 letters; each letter 500 words or less)
   • Newspaper articles (maximum of 2 articles written within the last 2 years)
   • Articles written by nominee (maximum of 3 web links to articles).

Nomination packages are due by January 31, 2018, via e-mail (communications@cvma-acmv.org) or fax to 613-236-9681, or by mail to the CVMA office at 339 Booth Street, Ottawa, ON K1R 7K1.

Please visit the CVMA Awards section of the website (www.canadianveterinarians.net) or contact Communications via e-mail (communications@cvma-acmv.org) or 1-800-567-2862, ext. 125 for further information.

Chaque année, dans le cadre de son programme de prix, l’Association canadienne des médecins vétérinaires (ACMV) reconnaît fièrement des personnes qui ont accompli des réalisations exceptionnelles et ont fait preuve d’un leadership exemplaire ainsi que d’un dévouement infatigable envers la collectivité vétérinaire du Canada. Les mises en candidature pour les Prix de l’ACMV 2018 seront acceptées jusqu’au 31 janvier 2018.

Admissibilité aux prix
Les personnes mises en candidature (sauf celles mises en candidature pour le titre de membre honoraire) doivent être membres en règle de l’ACMV pour être admissibles à la mise en candidature. Cependant, elles peuvent être mises en candidature par des non-membres de l’ACMV. Nous vous invitons à considérer la mise en candidature d’un collègue méritant à l’un des prestigieux prix de l’ACMV suivants :

• Prix humanitaire de l’ACMV
• Prix de l’industrie de l’ACMV
• Prix vétérinaire Merck
• Prix du praticien des petits animaux
• Prix de la pratique de l’année de l’ACMV
• Membre à vie de l’ACMV
• Membre honoraire de l’ACMV

Trousse de mise en candidature
Le choix des récipiendaires de prix se fonde uniquement sur les renseignements fournis dans la trousse de mise en candidature. Veuillez suivre les étapes suivantes afin de garantir l’inclusion des documents requis dans la trousse de mise en candidature :

1. Il faut soumettre un formulaire de mise en candidature rempli. Le formulaire peut être téléchargé dans la section des Prix de l’ACMV de notre site Web (www.veterinairesaucanada.net).
2. Inclure les documents à l’appui suivants avec la trousse de mise en candidature :
   • Décrire les principales réalisations professionnelles du candidat (maximum de 1000 mots)
   • Lettres d’appui (maximum de cinq lettres; chaque lettre compte 500 mots ou moins)
   • Articles de journaux (maximum de deux articles écrits au cours des deux dernières années)
   • Articles rédigés par le candidat (maximum de 3 liens Web vers les articles).

Les trousses de mise en candidature doivent être soumises d’ici le 31 janvier 2018 par courriel (communications@cvma-acmv.org), par télécopieur au 613-236-9681 ou par la poste au bureau de l’ACMV, 339, rue Booth, Ottawa (Ontario) K1R 7K1.

Veuillez visiter la section des Prix de l’ACMV du site Web (www.veterinairesaucanada.net) ou communiquez avec le Service des communications par courriel (communications@cvma-acmv.org) ou au 1-800-567-2862, poste 125, pour obtenir de plus amples renseignements.
Welcome to All First-Year Veterinary Students and Welcome Back All Returning Students!

Bienvenue à tous les étudiants de première année en médecine vétérinaire et bonne rentrée à tous les autres étudiants!

The first semester is a time for new beginnings, a time when excited students — old and new — swarm the halls in search of their respective classrooms and classmates, catching up on summer adventures and new projects undertaken. Welcome to everyone, I hope your time off during the summer was as thrilling and as inspiring as you set it out to be and your fall semester has flown by smoothly. Hard to believe exams are around the corner already!

This semester, just like every other semester in veterinary medicine, has without a doubt provided you with a large array of opportunities to get involved in student life, and I encourage you to do so! Getting involved on campus is a chance to grow not only as a student, but also as a person, helping you develop a skillset and mindset that will be useful throughout your life and your career.

There is a large selection of clubs, organizations, committees and teams needing the help of students like you to use their passions in creating projects that will improve hundreds of young adults’ university experiences.

As veterinary students, we are all Canadian Veterinary Medical Association (CVMA) members. The Students of the CVMA (SCVMA) Committee consists of one student representative from each of the 5 Canadian veterinary colleges, serving as president, Symposium coordinator, VetRap Student Newsletter coordinator, or CVJ Editorial coordinator like myself. The SCVMA Committee is behind activities such as the One Voice presentation and “hot topic” discussion, the Lab Coat Ceremony, and the famous annual SCVMA Symposium. The SCVMA Symposium consists of a long weekend in January when veterinary students from all over Canada are invited to improve their veterinary knowledge and meet other Canadians from their field! This year, the SCVMA Symposium will be held

Marie-Anne Sirois
le contact! Si vous avez besoin de votre mot de passe pour ouvrir une session sur le site Web des membres de l'ACMV et accéder à vos avantages en ligne pour les étudiants, dont des rabais sur les hôtels et la location de voiture, des tarifs de groupe pour le conditionnement physique, les rapports sur la rémunération et les avantages sociaux des vétérinaires salariés ainsi que des rabais sur l'assurance habitation et auto, communiquez avec l'ACMV à (admin@cvma-acmv.org).

Le trimestre d’automne est le moment des nouveaux débuts, je l’ai déjà dit et je le redirai de nouveau, pourquoi ne pas saisir l’occasion de ce nouveau trimestre en créant quelque chose pour votre collectivité afin d’exprimer ce qu’elle représente pour vous?

Mettez vos idées et votre énergie à contribution et n’arrêtez jamais de progresser.

(par Marie-Anne Sirois, représentante des ÉACMV, Faculté de médecine vétérinaire de l’Université de Montréal)
Viral enteritis in calves

Diego E. Gomez, J. Scott Weese

Abstract — A complex community of bacteria, viruses, fungi, protists, and other microorganisms inhabit the gastrointestinal tract of calves and play important roles in gut health and disease. The viral component of the microbiome (the virome) is receiving increasing attention for its role in neonatal calf diarrhea (NCD). Rotavirus and coronavirus have for a long time been associated with NCD and commercial vaccines have been produced against these agents. Recently, several other viruses which may play a role in diarrhea have been discovered in calf fecal samples, mostly by sequence-based methods. These viruses include torovirus, norovirus, nebovirus, astrovirus, kobuvirus, and enterovirus. Most studies have involved epidemiologic investigations seeking to show association with diarrhea for each virus alone or in combination with potential pathogens. However, determining the contribution of these viruses to calf diarrhea has been challenging and much uncertainty remains concerning their roles as primary pathogens, co-infection agents, or commensals.

Introduction

Diarrhea is the most important cause of disease in calves < 30 d of age and is a major cause of economic loss to cattle producers (1). The financial losses arise not only from mortality, but also from the cost of medication (especially antimicrobials), labor needed to treat sick calves, delayed growth of calves, and higher age at first calving (2,3). The 2007 National Animal Health Monitoring System for US dairy cattle stated that 57% of calf deaths before weaning resulted from neonatal calf diarrhea (NCD), with most cases occurring in calves < 1 mo of age (1). Similar mortality rates due to diarrhea in dairy calves were recently reported in Korea (53%) (4) and Iran (58%) (5).

The cattle industry has made great improvements with herd management, animal facilities and care, feeding and nutrition, and timely use of bio-pharmaceuticals; however, calf diarrhea is still problematic, likely because of the multi-factorial nature of the disease (6). Investigation of diarrhea has been focused on individual pathogens, namely Escherichia coli, Salmonella spp., rotavirus, coronavirus, and Cryptosporidium spp.; however,
recent studies in humans have suggested that co-infection (simultaneous infection of a host by multiple pathogens) might be important in the pathophysiology of gastrointestinal diseases (7).

A complex community of bacteria, viruses, fungi, protists, and other microorganisms inhabit the gastrointestinal tract of calves. Recent studies have demonstrated that this complex community (the microbiota) and its total genetic complement (the microbiome) play important roles in gut health and disease. While the bacterial component of the microbiota is the most abundant, the viral component of the microbiome (the virome) is receiving increasing attention. Various pathogenic viruses have been well-characterized in cattle, causing a range of diseases via acute, persistent, or latent infections (8). Viruses that infect animal cells represent a small proportion of the gut virome when compared to bacteriophages (viruses that infect bacteria), but animal viruses are among the most important etiologic agents of acute NCD. This review focuses on animal viruses and their role in NCD.

Viral enteritis: A brief history

*Escherichia coli* was considered to be the main cause of NCD during the first decades of the 20th century (9). In 1943, a filterable virus was suspected to cause diarrhea and pneumonia in calves younger than 1 mo (10). Soon after this, viruses such as bovine viral diarrhea virus (11), adenoviruses (12), parvoviruses (13), and enteroviruses (13) were also suggested as possible causes of this syndrome. In 1970, 2 viruses were isolated from cases of NCD on Nebraska ranches. The first one was a reovirus-like virus that induced disease generally within the first 96 h of life, causing diarrhea characterized by yellow liquid feces (14). The second virus isolated was a coronavirus-like agent that was reported to infect calves between 5 d and 6 wk of age (15). Shortly thereafter, the reo-like (now known as rotavirus) and coronavirus-like viruses, as well as an adenovirus, were identified in dairy calves from Quebec and Ontario (16). Subsequently, other viruses were identified in feces of calves with gastroenteritis, such as calicivirus (17,18), torovirus (BT oV) (19), astrovirus (BA sV) (17), nebovirus (BN oV) (17), and enterovirus (BE nV) (20). Some of these viruses can be identified in feces from clinically healthy calves (Table 1), which makes assessing the clinical relevance of these viruses very difficult and the role of some of these viruses in NCD still remains undetermined.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Healthy calves</th>
<th>Diarrheic calves</th>
<th>Unique Agent</th>
<th>Co-infection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>2% to 45%</td>
<td>7% to 80%</td>
<td>16% to 27%</td>
<td>29% to 31%</td>
<td>23, 24, 26, 91, 92</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>1% to 8.2%</td>
<td>3% to 79%</td>
<td>1.4% to 4.2%</td>
<td>8% to 13%</td>
<td>23, 44, 91, 92</td>
</tr>
<tr>
<td>Torovirus</td>
<td>6% to 12%</td>
<td>14% to 28%</td>
<td>28%</td>
<td>7.6%</td>
<td>44, 57, 58</td>
</tr>
<tr>
<td>Nonovirus</td>
<td>10%</td>
<td>1.6% to 76%</td>
<td>4%</td>
<td>20%</td>
<td>64, 65, 66, 68</td>
</tr>
<tr>
<td>Nebovirus</td>
<td>0% to 1.6%</td>
<td>7% to 21%</td>
<td>ND</td>
<td>ND</td>
<td>26, 65, 67, 70</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>ND</td>
<td>46%</td>
<td>8% to 13%</td>
<td>24% to 87%</td>
<td>68, 73</td>
</tr>
<tr>
<td>Kobuvirus</td>
<td>4.8% to 24%</td>
<td>5.3% to 37%</td>
<td>ND</td>
<td>ND</td>
<td>75, 80</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>32%</td>
<td>5%</td>
<td>ND</td>
<td>ND</td>
<td>26</td>
</tr>
</tbody>
</table>

ND — not determined.

| Table 1. Reported detection rates of 8 viruses in healthy and diarrheic calves, and rates of detection of each virus as a sole potential pathogen or along with other infectious agents in diarrheic calves. |

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</tbody>
</table>

ND — not determined.

Viruses known to cause diarrhea in calves

**Bovine rotavirus**

Rotaviruses are non-enveloped RNA viruses that have 3 important antigenic specificities: group, subgroup, and serotype. Group A rotaviruses are major pathogens in calves, with Group B playing a minor role. Group A rotaviruses consist of 11 segments of double-stranded RNA, encoding 6 structural viral proteins (VP1 to VP4, VP6 and VP7) and 6 non-structural proteins (NSP 1 to NSP 6) (21).

**Bovine rotavirus** (BRoV) typically causes diarrhea in calves < 3 wk of age (22). Clinical signs are non-specific as is characteristic of NCD. Typically, pale yellow, non-bloody, profuse diarrhea is observed, often containing large amounts of mucus. Diarrhea usually lasts between 4 to 8 d. Fever can be present and the calves are usually dull and reluctant to drink. Limited studies have reported prevalence rates of 7% to 80% for shedding of BRoV by diarrheic calves (23,24). Two case-control studies from Brazil and the USA detected BRoV in feces of 11% and 30% of diarrheic calves, respectively, compared to 0% in healthy calves (25,26). However, other studies have demonstrated that BRoV can be detected in both healthy and diarrheic calves, including reports of BRoV in 2% to 12% of non-diarrheic and 7% to 30% of diarrheic fecal samples from dairy calves in Europe (27–29) and Central America (23). One study from France also reported BRoV in 49% of diarrheic and 45% of healthy beef calves (28). A recent study from Brazil also determined that BRoV was detected at significantly higher (P < 0.0001) frequency in the feces of dairy calves with diarrhea compared with the feces of non-diarrheic calves (30). Differences among studies include the age of the calves sampled, geographic location, management practices, experimental design, and assays to detect BRoV [e.g., polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and chromatographic lateral flow immunoassay]. Additionally, most of these studies were cross-sectional in design and the health status of the control (healthy) group was not followed up to determine if calves that were shedding BRoV developed diarrhea after the time of sampling. Overall, these results make it difficult to determine the clinical relevance of BRoV as a primary pathogen or a potential co-infection agent. Similarly, determination of the impact of BRoV is challenging since its role in disease is unclear. Mortality rates from 5% to
Bovine coronavirus
Coronaviruses are single stranded RNA viruses that can infect a wide range of hosts. Animal coronaviruses are divided into 3 antigenic groups: Group 1 has no hemagglutinin-esterase (HE), Group 2 has HE and includes BCoV (32), and Group 3 contains avian viruses including infectious bronchitis virus. Bovine coronavirus (BCoV) has been associated with gastrointestinal and respiratory diseases in cattle including diarrhea in neonatal calves (33), winter dysentery (34), and respiratory tract illness (35). In dairy and beef calves, BCoV can cause enteritis with naturally infected calves showing clinical signs of disease between 5 and 30 d of life. As with some other potential causes of NCD, BCoV can commonly be found in both healthy and diarrheic calves, complicating the assessment of its role as a primary pathogen (29). Some studies involving a limited number of calves (n < 100) have identified numerical, but not statistical, associations between the detection of BCoV in fecal or nasal samples and clinical signs (36–38). In contrast, 1 case-control study involving 380 calves found a statistical association between BCoV and diarrhea in dairy calves in Costa Rica (23). Discrepancies among studies can be explained, at least in part, by differences in the sample sizes in those investigations. The prevalence of BCoV was investigated in dairy farms from Ontario at 3 periods: 1982, 1990 to 1991, and 1995 to 1997 (39–41). The overall prevalence ranged from of 5% to 17%. In one study, the prevalence rates of BCoV in healthy and diarrheic calves were 13% (15/118) and 2.3% (1/43), respectively (40), but the remaining 2 studies failed to differentiate the prevalence of BCoV in both groups. Higher prevalence rates of BCoV in diarrheic calves have been reported recently compared to those identified 2 to 3 decades ago (42). Further, new strains of BCoV have been described worldwide during the last decade (43,44). At present, the prevalence of BCoV in healthy and diarrheic calves from dairy farms in Canada, the association of BCoV with diarrhea and whether new strains are circulating among dairy farms are unknown.

Clinical signs begin approximately 2 d after exposure and continue for 3 to 6 d. Typically, coronavirus infection causes profuse watery diarrhea, and feces can contain blood clots. Calves become moderately depressed, the suckling reflex is weak, and dehydration can develop rapidly. Decreased food intake, fluids, and electrolyte loss can result in dehydration, metabolic acidosis, and hypoglycemia. The diagnosis of BCoV enteritis can be achieved using viral culture, antigen-capture ELISA, hemagglutination assay using mouse erythrocytes, and PCR (45). Recently, a pancoronavirus reverse transcription (RT) PCR assay (PanCoV-RT) was described to identify human CoV from samples of humans with respiratory diseases (46). The utility of PanCoV to detect BCoV in samples of animals with clinical diseases has not been described.

As with other viral causes, treatment is supportive in nature. Clinically recovered calves may continue to shed low levels of virus for weeks (15,47).

Prevention of diarrhea caused by rotavirus and coronavirus
The basic tenets of preventing viral gastroenteritis is enhancing host immunity and reducing the load of viral agents in the environment. Infection control practices dealing with reducing exposure are beyond the scope of this review. The importance of good colostrum management, leading to an adequate passive transfer in the prevention of calf diarrhea is without debate (2,48). Most cows are seropositive to BRoV due to field exposure; however, antibody titers of milk decline to non-protective levels after parturition in unvaccinated cattle (49). It is unclear if vaccinating cows late in gestation improves calf antibody titers or whether the practice improves resistance of calves to disease. Colostral and milk antibodies against BRoV and BCoV can be enhanced via parenteral vaccination of the cows during the dry period (passive immunization). The success of the passive immunity against enteric viral infection depends on the continuous presence of a protective level of specific antibody in the gut lumen (50,51). BRoV and BCoV normally cause diarrhea between 5 and 14 d of age, a time that has been associated with a major decline in specific antibody concentration, as ingestion of high colostral antibody is replaced by ingestion of milk, which has much lower antibody concentration (52,53). Therefore, optimal protection would be from vaccines that can be given to cattle during pregnancy and that increase both colostrum and milk antibodies for a period of at least 3 wk. The efficacy of parenteral vaccines for prevention of diarrhea caused by BRoV and BCoV is unclear. In 1985, a field trial evaluating the efficacy of a vaccine consisting of modified live BRoV and BCoV with a F5-positive E. coli bacterin failed to detect differences in rates of diarrhea and associated mortality in calves from vaccinated and unvaccinated cows on dairy farms in Ontario (54). Importantly, colostral antibodies to BRoV and BCoV were similar in both groups, suggesting that the vaccine resulted in limited impact on passive transfer of immunity. Minimal increases of antibodies in milk or serum of calves from cows vaccinated with inactivated BCoV antigen were identified in some studies (55,56). Yet, several other studies have reported that pregnant cows vaccinated against BRoV and BCoV had increased titers of antibodies in colostrum and milk (39,51,53). A later study evaluated the concentration and persistence of antibodies in colostrum and milk against BRoV, BCoV and E. coli F5 antigens after cows were vaccinated 1 mo before expected calving date with a single dose of a vaccine containing an inactivated BRoV (serotype G6-P5), inactivated BCoV (originally isolated from a calf with diarrhea) and purified cell-free E. coli F5. This study demonstrated a 4-fold increase, for at least 28 days, in antibodies against BRoV and BCoV in colostrum and milk of vaccinated cows compared to the control group (53). However, this study failed to demonstrate vaccine efficacy through an animal challenge model. These results suggest that newer vaccines could be effective in the prevention and control of viral diarrhea in calves; however, randomized field trials are required to prove their efficacy.
Several studies have reported discrepancies between the genotypes of rotavirus (25) and coronavirus (43) in the commercial vaccine and those of the strains circulating in both beef and dairy herds. Differences between the vaccine and field strains suggest that the vaccines may not be protective against circulating strains (43). To provide optimal immunity, vaccine antigens should be as similar as possible to the circulating strains. Therefore, future studies should focus on epidemiological surveillance in order to avoid potential causes of vaccination failure.

Other viruses that might cause enteritis
Numerous animal viruses can be found in diarrheic calves. As sequence-based methods become more affordable and easy to use, it is almost certain that many more new viruses will be identified. However, identification of a virus in a diarrheic calf is typically much easier than determining what role, if any, it has in disease. The presence of viruses in both healthy and diarrheic calves does not rule out the potential for them to cause disease, but it complicates determination of their pathogenicity. Some viruses that may play a role in NCD, either as primary pathogens or co-infection agents, are discussed below.

Bovine torovirus
Torovirus is a genus of enveloped RNA viruses of the Coronavirus family. Toroviruses are similar in appearance to the crown-like coronaviruses but often have a donut-shaped structure within the particle. Toroviruses have been identified in humans, horses, cattle, and swine with gastroenteritis worldwide, but their role in disease etiology is still unclear. Similarly, bovine torovirus (B'ToV) has been identified in feces of diarrheic and healthy calves (57). One study identified B'ToV in 43/118 (36%) diarrheic and 5/43 (12%) healthy calves on Ontario farms, both as the sole detected pathogen (28% of diarrheic calves) and along with other pathogens (7.6%) (41). Another study failed to detect any association of B'ToV with calf diarrhea (58). Despite the passage of time since the first identification of B'ToV, its role in disease remains poorly characterized. Mixed results have been reported with attempted experimental infection, as some experimental infections have failed to produce clinical signs or histopathological lesions (59).

Natural infection usually occurs in calves between 2 and 5 d of age, but calves up to 4 mo appear to be susceptible (19,60,61). Diarrheic calves < 1 mo of age that are shedding B'ToV appear to be the major source of the virus (60,61). After ingestion or nasal exposure, the virus infects the epithelium of the distal half of the jejunum, the ileum, and colon. Viral replication is cytoplasmic and entrance into the enterocytes is achieved by attachment of the viral S protein to host cell receptors, which mediates endocytosis. Microscopic lesions consist of necrosis of the crypt and villous enterocytes and atrophy of the villi (62,63). Similar to coronavirus, lesions in the intestine caused by torovirus infection are expected to result in malabsorptive and hypersecretory diarrhea (48). Clinical signs observed in naturally occurring outbreaks include a yellow to white semisolid or profuse watery diarrhea (19). If the calf survives, it can be fully protected from infection but can intermittently shed BoTV (58,63). Specific preventive measures are not available.

Bovine norovirus
Noroviruses are non-enveloped RNA viruses that are members of the family Caliciviridae. On the basis of phylogenetic relationships inferred from the VP1 sequences, noroviruses have been divided into 6 genogroups (GI to GVI), with bovine noroviruses (BNoV) classified as GIII (64). The pathogenesis of BNoV is poorly understood; however, extrapolation from other species, especially humans, suggests that BNoV can be transmitted via the fecal/oral route, through contaminated food or water (65). The prevalence of BNoV in cattle has not been well established. Results from limited studies have reported ranges of 1.6% and 72% in Canadian dairy calves and USA veal calves, respectively (64,65), and up to 10% in healthy calves in Europe (66,67). One study identified BNoV as the sole detected pathogen in 4% of samples from diarrheic calves and along with other pathogens in 20% (68); however, this study only evaluated the presence of viral agents (BRoV, BAstV, BNoV, BCoV, B'ToV, and BVDV) and failed to investigate the presence of bacterial and parasitic agents. Therefore, conclusions regarding the true role of BNoV as a primary pathogen or co-infection were limited.

Gnotobiotic calves infected with the GIII BNoV strain exhibited anorexia and diarrhea associated with necrosis of the intestinal epithelium and villous atrophy (69). However, evidence that BNoV is a significant (or even rare) cause of diarrhea in calves in the field is limited. The potentially high prevalence of BNoV in healthy calves and lack of a significant difference in shedding between healthy and diarrheic calves (26,70) suggest this virus may be of limited clinical relevance, at least as a primary pathogen. Whether BNoV can be pathogenic in some situations, either as the sole infectious agent or a co-infecting agent, is as yet unknown. Therefore, a definitive causal relationship between BNoV and calf diarrhea remains to be determined.

Bovine nebovirus
Similar to norovirus, nebovirus is a non-enveloped member of the family Caliciviridae. Outbreaks of bovine nebovirus (BNeBV) associated gastroenteritis were initially reported in diarrheic calves in England (17) and Germany (18), but BNeBV has been detected in cattle worldwide (66,67). In Italy, BNeBV was detected in feces of diarrheic calves but not in healthy animals (67). In France, the United Kingdom and Korea, the prevalence of BNeBV in diarrheic calves ranged from 7% to 9% (67,70,71). In North America, BNeBV has been identified in 21% (43/199) of fecal samples of diarrheic calves and 1.6% (4/245) of samples from healthy calves. BNeBV was commonly detected in feces also positive for BCoV, Cryptosporidium parvum or B'ToV (26). Experimental infection of gnotobiotic calves with BNeBV causes lesions in the jejunum similar to those described for BNoV, and these lesions have been associated with malabsorption (72). The mechanism of diarrhea due to BNeBV remains poorly understood but malabsorptive and hypersecretory diarrhea can be expected (72). Described clinical signs in gnotobiotic calves infected with BNeBV included depression, anorexia, and diarrhea (72). However, despite the
potential over-representation of BNebV in diarrheic calves and experimental reproduction of disease in gnotobiotic calves, the role of BNebV in diarrhea in the field remains unclear.

**Bovine astrovirus**
The family Astroviridae includes 2 genera, Mamastroviruses and Avastroviruses that infect mammals and birds worldwide, respectively. Bovine astrovirus (BAstV) was initially isolated from a diarrheic calf in England in 1978 (17). However, exposure of gnotobiotic calves to this virus failed to produce clinical signs and, therefore, that strain of BAstV was considered to be non-pathogenic (17). In 1984, a similar BAstV was isolated from a diarrheic calf from USA that was also positive for BRoV (62). Experimental infection of gnotobiotic calves with the BAstV-USA strain caused infection and cytopathology of M-cells of the dome epithelium covering the Payer’s patches of the calf ileum but did not cause clinical signs. Interestingly, when BAstV-USA strain was mixed with BRoV or BToV, gnotobiotic calves developed severe diarrhea and more extensive BAstV infection (62). Study of BAstV in field situations has been limited. A recent study demonstrated a high prevalence (46%) of BAstV in diarrheic calves, with co-infection with other viruses, including BEnV, BCoV, BRoV and BVDV identified in 88% of those animals (73). Another study identified BAstV as the sole detected pathogen in 8% of the fecal samples and along with other viral agents in 24% (68).

One study from Scotland found that BAstV was common in calves [present in 74% (85/115) of samples] but uncommon in adult cattle [present in 15% (3/20) of samples]. However, no association was found between the presence of BAstV and calf diarrhea or the presence of a specific AstV lineage and calf diarrhea (74). The lack of comparative data with a healthy control group limits what can be concluded from that study. As with various other viruses, it is unclear whether BAstV is a relevant primary pathogen, a potential cause of disease with co-infections, or a clinically irrelevant virus.

**Bovine kobuvirus (aichivirus B)**
Kobuvirus, a genus of non-enveloped RNA viruses from the family Picornaviridae, contains 2 officially recognized species, Aichivirus and bovine kobuvirus (BkoV, now referred to as Aichivirus B) and 1 candidate species, porcine kobuvirus (75). Aichivirus was first isolated from a person with acute enteritis in Japan (76), although its role in disease in humans remains unclear. Initially, BkoV was only identified in bovine serum and feces from clinically healthy cattle (77), then, in 2008, it was isolated from feces of cattle with diarrhea (78). It has been suggested that BkoV can play a role in the pathogenesis of enteritis in calves; however, the role of BkoV infection in NCD still needs to be clarified because of limited data and the presence of this virus in clinically normal animals (75). While this virus has been isolated from diarrheic calves (78,79), studies comparing diarrheic and healthy calves are limited. Two recent studies compared the prevalence of BKV in healthy and diarrheic calves from Italy (80) and Korea (75). In the Italian study, the prevalence of BKV was similar in diarrheic (5.3%; n = 38) and non-diarrheic (4.8%; n = 104) calves, whereas in the Korean study, BKV was found in 37% (32/86) of diarrheic and 24% (5/21) of non-diarrheic samples. Both studies failed to investigate the presence of other etiologic agents causing diarrhea in calves and therefore it is not possible to attribute a causal association between BKV and NCD.

**Bovine enterovirus**
Bovine enterovirus (BEnV) belongs to the genus Enterovirus in the family Picornaviridae, a group of non-enveloped RNA viruses that includes numerous human and animal pathogens. The enterovirus genus consists of 12 species; 9 enteroviruses (A to J) and 3 rhinoviruses (81). The BEnVs are now classified into 2 subgroups E (1 to 4) and F (1 to 6) (81). Since 1959, BEnV has been isolated from cattle suffering from respiratory, gastrointestinal and reproductive diseases (82,83). However, respiratory and gastrointestinal disease could not be reproduced experimentally using viral isolates from affected calves. The pathogenesis and virulence of BEnV in cattle are largely unknown. One study described the pathogenesis associated with acute infection of BEnV in calves experimentally inoculated with the BEnV but found no clinical signs following acute infection (84). More importantly, the control group (unexposed group) used in this study was inadvertently infected with the inoculated BEnV, which largely limited the conclusions of the study.

Recently, BEnV was isolated from feces of diarrheic cattle from dairy herds in China (85). BEnV was detected in 25% of healthy and diarrheic calves, but the authors failed to report the prevalence in healthy and diarrheic calves separately. Therefore, conclusions regarding a potential association with disease cannot be made. Another recent study identified BEnV in calves with severe diarrhea from dairy herds in Egypt (86). Although the investigated diarrheic calves were negative for BCoV, BRoV, and BVDV on cell culture, other bacterial and parasitic causes of diarrhea were not investigated. There was also no corresponding study of healthy calves, so association between the presence of BEnV and diarrhea could not be investigated.

A case-control study conducted to assess the prevalence of 11 infectious agents in fecal samples from calves from Midwest USA revealed that prevalence of BEnV in healthy calves was significantly higher (32%) than in diarrheic calves (5%) (26). Difficulties in reproducing clinical signs following experimental infection, and the fact that BEnV appears to be more prevalent in healthy than diarrheic calves suggest that BEnV plays little to no role in neonatal calf diarrhea.

**Co-infection and calf diarrhea**
As testing becomes more comprehensive, identification of co-infection with known or potential pathogens becomes more common. Co-infection with multiple pathogens has been identified among children with diarrhea and has been associated with more severe diarrhea than infection with a single pathogen. For instance, one case-control study in children from China reported multiple pathogens in 185 (40%) diarrheic feces and in 69 (15%) controls (7). High rates of co-infection have also been reported in diarrheic foals (87), with co-infections being more frequent in the diarrheic foals (15 mono-infections versus 22 co-infections) than in the healthy group (12 versus 4,
respectively, $P = 0.0002$). Metagenomic studies have demonstrated that healthy piglets excrete enteric pathogenic viruses, but at lower concentrations when compared with diarrheic piglets. Interestingly, piglets that shed 6 or more distinct viruses were more likely to suffer from diarrhea (88). One study that evaluated the etiological agent in fecal samples of diarrheic piglets submitted to the Animal Health Laboratory of the University of Guelph identified rotavirus in 28 out of 237 samples. RoV was identified as the single etiological agent in 18 cases and associated with other pathogens (co-infection) in 10 cases (89). Furthermore, dogs with diarrhea can also simultaneously excrete several enteric viruses including rotavirus, coronavirus, parvovirus, norovirus, astrovirus, distemper virus, and paramyxovirus (90).

Studies investigating the interaction between viral micro-organisms and other microorganisms of the gastrointestinal tract of the calf are scarce; however, several studies investigating calf diarrhea have demonstrated high rates of co-infection (24,26,34,91,92). One study evaluated the prevalence of 5 known pathogens causing diarrhea and found rates of co-infection of 15% in diarrheic calves (33), whereas another study determined a rate of co-infection of 71% when fecal samples were tested for 4 known pathogens (24). A recent study testing for 11 pathogens associated with NCD documented a rate of co-infection of 55% in fecal samples from diarrheic calves. Notably, in this study the rate of co-infection in healthy calves was only 3% (26). One study in Ontario (Canada) farms, evaluating the presence of viruses (BToV, BCoV, BRoV, BVDV and small round-structured viruses) in feces of dairy calves reported a rate of co-infection of 14% (17/118) in diarrheic calves, whereas co-infection was not detected in clinically normal calves (41). This study failed to evaluate co-infection with bacterial or parasitic agents.

The pathogens associated with co-infection in diarrheic calves vary among studies. One study reported the most common combination of pathogens in diarrheic calves was *C. parvum* and BRoV (19%) followed by BRoV and *E. coli* K-99 (91). Similar results were reported in calves suffering from diarrhea in Australia in which the combination of *C. parvum* and BRoV accounted for 25% of the co-infections (24). Remarkably, a recent investigation determined that the most common co-infection in diarrheic calves from Unites States were viral pathogens with *C. parvum* (28%), viral and bacterial co-infection (7.5%), and viral, bacterial pathogens and *C. parvum* co-infection (1.5%). Furthermore, the presence of more than one pathogen increased the odds of diarrhea occurring in 2 studies (26,29). These studies suggest that co-infections with a large number of pathogens rather a single entity may be responsible for the diarrhea in a subset of neonates, likely by overwhelming the gut mechanisms of defense against pathogens.

In conclusion, viral gastroenteritis remains as an important cause of morbidity and mortality in neonatal calves. A large number of viruses in the gastrointestinal tract of calves are yet to be identified. Description of novel viruses will occur in the near future as next-generation sequencing technologies have facilitated virus discovery. Therefore, future clinical research should focus on determining the clinical relevance of the novel viruses, the role of co-infection in calf gastroenteritis, and the efficacy of vaccines in prevention and control of neonatal calf diarrhea. (C5)

**References**


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Incidence of gastric dilatation-volvulus following a splenectomy in 238 dogs

Lynn C. Maki, Kristina N. Males, Madeline J. Byrnes, Anthony A. El-Saad, George S. Coronado

Abstract — There is contradicting information in the veterinary literature regarding canine splenectomy and the increased risk for subsequent gastric dilatation-volvulus. The main purpose of this study was to determine the rate of occurrence of gastric dilatation-volvulus following splenectomy in medium to large breed dogs compared with a control group undergoing other abdominal procedures. Follow-up was performed by reviewing the medical records and conducting phone interviews. Weight, gender, and presence of a hemoabdomen at the time of surgery were not significantly associated with occurrence of gastric dilatation-volvulus, while increasing age was. Ten of 238 (4%) dogs in the splenectomy group and 3/209 (1.4%) dogs in the control group subsequently developed gastric dilatation-volvulus, which was not significantly different \( (P = 0.08) \). While the findings approach significance and support a need for future investigation, the current recommendation for gastropexy at time of splenic removal should be made on a case by case basis and while considering previously documented risk factors.

Résumé — Incidence de la dilatation gastrique-volvulus après une splénectomie chez 238 chiens. Il existe des renseignements contradictoires dans la littérature vétérinaire concernant la splénectomie canine et le risque accru pour la dilatation gastrique-volvulus subséquente. Le but principal de cette étude consistait à déterminer le taux d’occurrence de la dilatation gastrique-volvulus après la splénectomie chez des chiens de race moyenne ou grande comparativement à un groupe témoin subissant d’autres interventions abdominales. Le suivi a été réalisé en examinant les dossiers médicaux et en réalisant des entrevues par téléphone. Le poids, le sexe et la présence d’un hémoabdomen au moment de la chirurgie n’étaient pas significativement associés à l’occurrence de la dilatation gastrique-volvulus, tandis que l’âge avancé l’était. Dix des 238 (4 %) chiens dans le groupe de splénectomie et 3/209 (1,4 %) des chiens dans le groupe témoin ont subséquemment développé la dilatation gastrique-volvulus, ce qui n’était pas significativement différent \( (P = 0.08) \). Bien que les résultats soient près du seuil significatif et supportent le besoin d’études supplémentaires, la recommandation actuelle pour la gastropexie au moment de l’enlèvement splénique devrait être faite au cas par cas et en tenant compte des facteurs de risque documentés antérieurement.

Introduction

In veterinary medicine, total splenectomies have been performed for treatment of benign or malignant neoplasia, severe trauma, certain immune-mediated diseases, infiltrative disease, and splenic torsion (1). Reported complications following splenectomy include cardiac arrhythmias, damage to the left pancreatic lobe, hemorrhage, disseminated intravascular coagulation, sepsis/infection, and gastric dilatation-volvulus (GDV) (1–4). Of these complications, GDV would theoretically be one of the most preventable by performing a prophylactic gastropexy at the time of splenectomy (5,6). Prevention of GDV would be advantageous, as this disease can result in significant morbidity and mortality for the patient, as well as increased financial and emotional burden to the owner.

Gastric dilatation and volvulus occurs when the pylorus is malpositioned and there is severe gastric distension with gas, food, and/or fluid, resulting in secondary life-threatening complications (7–9). Genetics, breed, food texture, ingredients or size, feeding protocol, thoracic wall dimensions, emotional state, increased hepatogastric ligament length, age, foreign body, time of year, and previous splenectomy have all being proposed as contributing factors to GDV (10–19). Treatment is immediate medical and surgical intervention to stabilize the patient, return the stomach to its normal position, remove devitalized or necrotic tissue and secure the gastric antrum to the right side of the body wall to prevent recurrence (7,9,20,21).
Despite conflicting evidence in the literature regarding the association of splenectomy and subsequent GDV, it has been recommended to perform a gastropexy at the time of splenectomy regardless of breed, age, and underlying disease (1,2,21). Negative consequences following a gastropexy occur at a relatively low frequency and include pneumothorax, accidental penetration into the gastric lumen resulting in suture contamination, increased surgical and anesthetic time, increased financial costs, and possible changes in gastric motility (9,21,22).

The purpose of this study was to determine if the incidence of GDV following splenectomy in medium to large breed dogs differs significantly in comparison to a control group of medium to large breed dogs undergoing an exploratory laparotomy for procedures unrelated to splenic disease. We also aimed to define the time from surgery to occurrence of GDV in affected dogs, and determine if there was an association of age, weight, gender, or presence of a hemoabdomen at time of surgery with occurrence of GDV. Our hypothesis was that there would not be a significant difference in the incidence of GDV between the 2 groups.

Materials and methods
The medical records database at Ocean State Veterinary Specialists was searched for dogs weighing more than 20 kg which had undergone surgery with a vessel sealing or stapling device from 2008 to 2015. Dogs were excluded if a splenectomy was not performed, the dog had history of a GDV, the spleen was not submitted for histopathology, if the dog did not survive the 10 to 14 d until removal of staples, if the medical record was incomplete following surgery, or if the owner or primary care veterinarian could not be reached for follow-up. Dogs were also excluded if a prophylactic gastropexy was performed at any time before, during, or after the splenectomy. Dogs undergoing other procedures at the time of splenectomy were included.

For the control group, the database was searched for dogs that weighed more than 20 kg at adult weight that had undergone an emergency laparotomy for reasons other than treatment for splenic disease, dystocia, pyometra, or GDV from 2008 to 2015. Dogs were excluded if a splenectomy and/or prophylactic gastropexy were performed at any time before, during, or after the emergency laparotomy. Dogs which had abdominal adhesions noted at the time of the initial surgery, had a gastric feeding tube placed, had an abdominal mass larger than 5 cm, and/or had undergone more than 1 surgery for foreign body removal at any time were also excluded. Lastly, dogs were excluded if they did not survive 10 to 14 d until removal of the staples, their medical record was incomplete following surgery, or if the owner or primary care veterinarian could not be reached for follow-up.

For the splenectomy group, medical records were reviewed and data were collected regarding signalment (breed, age, weight, and neuter status at time of surgery), the reason for surgery, presence of a hemoabdomen, type of surgical procedures performed, size of splenic mass, histopathologic diagnosis, co-morbidities and administration of postoperative chemotherapy. For the control group, medical records were reviewed and data were collected regarding signalment (breed, age, weight, and neuter status at time of surgery), the reason for surgery, type of surgical procedures performed, and histopathology or stone analysis when appropriate. For both groups, if the dog was spayed or neutered at the time of surgery, then the intact status was changed to reflect this.

Case follow-up was performed by reviewing medical records, and conducting either an e-mail or telephone interview with the owner and/or the primary care veterinarian. Owners and veterinarians were asked if the dog had any complications following surgery, if the dog had any additional abdominal surgeries, and more specifically if the dog ever displayed clinical signs and/or had been diagnosed with a GDV. The date of occurrence and outcome were noted when indicated. If the dog had not displayed any signs related to a GDV it was confirmed that the dog had never undergone a prophylactic gastropexy. If the animal was no longer alive the owners and veterinarians were asked the date and cause of death. If the cause of death was suspected, but not confirmed, to be a GDV based on the clinical signs then it was considered a GDV.

Statistical analysis
Dogs were divided into control (n = 209) and splenectomy (n = 238) groups. Within those groups dogs were further subdivided into GDV (yes/no). Data were tested for normality (Shapiro-Wilk test and normal probability plot); data not normally distributed were analyzed by means of Wilcoxon rank-sum test. Comparisons between age, gender/neuter status, histopathology results, presence of hemoabdomens at the time of original surgery, survival/follow-up times, and occurrence of GDV were evaluated using the Chi-square test or Wilcoxon rank-sum test. Size of the splenic mass, administration of chemotherapy agents and co-morbidities were not evaluated due to inconsistency in the medical record. Breed was not evaluated for association because there were inadequate numbers of some breeds for proper analysis. Survival times were reported using Kaplan-Meier curve. Data were reported either as mean ± standard deviation or median (25th and 75th quartiles), as appropriate. Data were analyzed using computer software (SAS 9.3 software; SAS, Cary, North Carolina, USA) and differences were considered significant when P < 0.05.

Results
Overall 238 dogs met the inclusion criteria and were included in the splenectomy group, while 209 dogs met the inclusion criteria and were included in the control group. The splenectomy group included 32 breeds: mixed breeds (n = 58), Labrador retriever (n = 49), golden retriever (n = 34), German shepherd (n = 11), basset hound (n = 11), Staffordshire bull terrier (n = 9), rottweiler (n = 8), Weimaraner (n = 7), English bulldog (n = 7), boxer (n = 6), springer spaniel (n = 5), Siberian husky (n = 3), standard poodle (n = 3); 2 each of Airedale, Alaskan malamute, beagle, Bernese mountain dog, Australian cattle dog, English springer, Portuguese water dog, vizsla; and 1 each of American bulldog, American eskimo, border collie, bullmastiff, bearded collie, Doberman pincher, flat-coated retriever, greyhound, Irish setter, Rhodesian ridgeback, and St. Bernard. The control group included 35 breeds: Labrador retriever (n = 54), mixed breeds (n = 36), golden retriever (n = 34), English bulldog (n = 14),
Table 1. Age, gender distribution, and weight for dogs in the splenectomy and control groups. Age of dogs in the control group was a non-normal value and is reported as median value (25th; 75th quartiles). All other values were normal values and reported as mean +/- standard deviation. P-values represent intergroup difference between dogs with and without GDV using Wilson rank-sum test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (y) ± SD</th>
<th>Gender</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td>10.1 +/- 2.0</td>
<td>F = 2; M = 18</td>
<td>32.4 +/- 7.6</td>
</tr>
<tr>
<td>No GDV (n = 228)</td>
<td>10.1 +/- 2.0</td>
<td>F = 2; M = 18</td>
<td>32.4 +/- 7.6</td>
</tr>
<tr>
<td>GDV (n = 10)</td>
<td>11.0 +/- 1.8</td>
<td>F = 0; M = 0</td>
<td>32.4 +/- 7.0</td>
</tr>
</tbody>
</table>

P-value 0.14

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (y) ± SD</th>
<th>Gender</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 209)</td>
<td>6.0 (2.0; 8.4)</td>
<td>F = 4; M = 14</td>
<td>30.6 +/- 7.1</td>
</tr>
<tr>
<td>No GDV (n = 206)</td>
<td>6 (2.0; 8.4)</td>
<td>F = 4; M = 14</td>
<td>30.6 +/- 7.2</td>
</tr>
<tr>
<td>GDV (n = 3)</td>
<td>5.4 (4.3; 11.3)</td>
<td>F = 0; M = 0</td>
<td>30.9 +/- 5.1</td>
</tr>
</tbody>
</table>

P-value 0.51

SD — standard deviation of the mean; F — female; M — male; S — female spayed; N — male neutered.

Staffordshire bull terrier (n = 11), boxer (n = 8), Siberian husky (n = 5), Bernese mountain dog (n = 4), Dalmatian (n = 4), Airedale (n = 3), American bulldog (n = 3), Doberman pinscher (n = 3), Plott hound (n = 2), vizsla (n = 2); and 1 each of Alaskan malamute, basset hound, beagle, Australian cattle dog, border collie, bullmastiff, Cane corso mastiff, English springer, flat-coated retriever, German wirehaired pointer, German shepherd, standard poodle, Portuguese water dog, samoyed, greyhound, Irish setter, mastiff, redbone coonhound, Rhodesian ridgeback, Rottweiler, and springer spaniel.

There were 110 females (2 intact), 118 males (8 intact) in the splenectomy group. The control group consisted of 90 females (5 intact), and 119 males (16 intact). There was no significant difference in gender distribution between groups (P = 0.47).

The mean weight at time of surgery was 32.4 kg (range: 21.2 to 56.3 kg) for the splenectomy group and 30.6 kg (range: 12.4 to 55.5 kg) for the control group (Table 1). The mean age at time of surgery was 10.1 y (range: 4.8 to 15.0 y) for the splenectomy group and 5.6 y (range: 4 mo to 14.7 y) for the control group (Table 1). There was a significant difference for both weight (P = 0.01) and age (P < 0.01) between the 2 groups.

The reason for splenectomy was a splenic mass or nodule (n = 227, 95.3%), splenomegaly (n = 8, 3.4%) and an abnormal splenic appearance on diagnostic imaging (n = 3, 1.3%). No dogs underwent surgery for splenic torsion. Histopathology results revealed a benign underlying disease process for 130 (54.6%) dogs and a malignant neoplastic process for 108 dogs (45.4%). Of the dogs with a benign process, histopathology identified nodular hyperplasia (n = 94, 72.3%), congestion and extramedullary hematopoiesis (n = 14, 10.7%), hematoma (n = 12, 9.2%), unknown benign process (n = 3, 2.3%), diffuse hyperplasia (n = 2, 1.5%), and splenic necrosis (n = 2, 1.5%). Hemangiomia, ironation, and myelolipoma were reported in 1 dog each with a benign process. Of the 108 dogs with a malignant neoplastic process, histopathology results included hemangiosarcoma (n = 87, 80.5%), fibrohistiocytic nodule (n = 6, 5.6%), spindle cell sarcoma (n = 4, 3.7%), lymphoma (n = 3, 2.8%), and sarcoma (n = 3, 2.8%). Leiomyosarcoma, liposarcoma, mast cell tumor, neuroendocrine and tubular adenocarcinoma were reported in 1 dog each with a malignant process. The reason for surgery in the control group was gastrointestinal disease (n = 166, 79.4%), urinary disease (n = 25, 12%), reproductive disease (n = 8, 3.8%), hepatobiliary disease (n = 6, 2.9%), and endocrine disease (n = 4, 1.9%).

Of the 238 dogs undergoing a splenectomy 119 (50%) had a hemoabdomen at the time of surgery and 119 (50%) dogs did not. Of the 119 dogs with a hemoabdomen, 72 (60%) had a diagnosis of malignant neoplasia on histopathology and this association was significant (P < 0.01). Of the 119 dogs that did not have a hemoabdomen, 36 (30%) dogs had malignant neoplasia and 83 (70%) had a benign process.

Overall, 10 dogs (4%) in the splenectomy group and 3 dogs (1.3%) in the control group were considered to have had a GDV following surgery (Tables 2 and 3). Results of a Chi-square test showed there was no significant difference in incidence of GDV between the 2 groups (P = 0.08). Of the splenectomy group, 8 dogs (3.3%) had a confirmed GDV and 2 dogs (0.8%) died with a distended abdomen, for which GDV could not be ruled out (Table 2). Of the control group 2 dogs (1%) had confirmed GDV and 1 dog (0.05%) died with a distended abdomen, for which GDV could not be ruled out (Table 3). Two dogs in the splenectomy group had 1 episode each of gastric dilatation without volvulus confirmed on radiographs, and did not go on to develop GDV.

Overall median time from surgery to subsequent GDV for all dogs was 439 d (range: 15 to 1663 d). Median time from surgery to subsequent GDV was 124 d (range: 15 to 1273 d) in the splenectomy group and 1029 d (range: 570 to 1663 d) in the control group. This difference was significant (P = 0.05).

When evaluating both groups together the mean age and weight for all the dogs that developed a subsequent GDV was 10 y (range: 4.3 to 13.2 y) and 32 kg (range: 22.4 to 46.6 kg), while the mean age and weight for the dogs which did not develop subsequent GDV was 8.0 y (range: 4 mo to 15 y) and 21.6 kg (range: 12.4 to 56.3 kg). The difference in age was significant, and found to be associated with GDV (P = 0.02), while
Table 2. Detailed information for dogs that underwent splenectomy and had a subsequent GDV.

<table>
<thead>
<tr>
<th>Splenic weight (kg)</th>
<th>Time to GDV (d)</th>
<th>Histopathology</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Breed</th>
<th>Weight (kg)</th>
<th>Hemabdomen</th>
<th>Cause of death</th>
<th>Follow-up time (d)</th>
<th>Status</th>
<th>Survival time of death</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>30</td>
<td>Nodular hyperplasia (B)</td>
<td>2</td>
<td>F</td>
<td>Dobe</td>
<td>2.4</td>
<td>No</td>
<td>Yes</td>
<td>28</td>
<td>A</td>
<td>30</td>
<td>GDV</td>
</tr>
<tr>
<td>1.3</td>
<td>30</td>
<td>Nodular hyperplasia (N)</td>
<td>3</td>
<td>F</td>
<td>Doberman pinscher</td>
<td>2.0</td>
<td>Yes</td>
<td>8</td>
<td>D</td>
<td>15</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>180</td>
<td>Hemangiosarcoma (N)</td>
<td>20</td>
<td>F</td>
<td>Mixed breed</td>
<td>3.0</td>
<td>No</td>
<td>Yes</td>
<td>377</td>
<td>D</td>
<td>180</td>
<td>GDV</td>
</tr>
<tr>
<td>1.6</td>
<td>156</td>
<td>Fibrohistiocytic stromal tumor (N)</td>
<td>20</td>
<td>F</td>
<td>German shepherd</td>
<td>3.2</td>
<td>No</td>
<td>Yes</td>
<td>136</td>
<td>D</td>
<td>156</td>
<td>GDV</td>
</tr>
<tr>
<td>1.1</td>
<td>94</td>
<td>Hemangiosarcoma (B)</td>
<td>20</td>
<td>F</td>
<td>Mixed breed</td>
<td>0.3</td>
<td>Yes</td>
<td>Yes</td>
<td>36</td>
<td>D</td>
<td>94</td>
<td>GDV</td>
</tr>
<tr>
<td>0.9</td>
<td>54</td>
<td>Nodular hyperplasia (B)</td>
<td>20</td>
<td>F</td>
<td>Labrador retriever</td>
<td>3.0</td>
<td>Yes</td>
<td>Yes</td>
<td>118</td>
<td>D</td>
<td>54</td>
<td>GDV</td>
</tr>
<tr>
<td>1.6</td>
<td>1273</td>
<td>Fibrohistiocytic stromal tumor (N)</td>
<td>20</td>
<td>F</td>
<td>Labrador retriever</td>
<td>1.2</td>
<td>Yes</td>
<td>Yes</td>
<td>1273</td>
<td>D</td>
<td>1273</td>
<td>GDV</td>
</tr>
</tbody>
</table>

F — female; M — male; S — spayed; N — neutered; (B) — benign; (N) — neoplastic; D — Deceased; A — Alive; GDV — gastric dilation and volvulus.

Possible GDV.

Discussion

It has been theorized that splenic disease may cause stretching of the perigastric ligament from expansion secondary to a growing mass and/or hemoperitoneum, which may permit increased gastric mobility (4,7,23,24). Removal of the spleen could result in increased intra-abdominal dead space, allowing the stomach to twist on its longitudinal axis (4,7,23). In the present study, the rate of GDV occurrence was 4% (10/238) for the splenectomy group and 1.4% (3/209) for the control group, which appeared clinically relevant but was not statistically different. We also did not find an association between presence of a hemoabdomen at time of surgery and GDV, which is consistent with previous findings (25). If stretching of the ligament and creation of dead space are underlying contributing factors, then we would expect GDV to also occur at a higher incidence following treatment for dystocias, pyometras, and large liver mass resections. While this is not commonly noted or discussed in the literature, dogs undergoing abdominal surgeries for these procedures were excluded from both groups to avoid confounding results.
We considered dogs that died with a distended abdomen from an unknown cause to have had a GDV to avoid potentially underreporting the overall incidence of GDV. In total, 2 dogs from the splenectomy group (dogs 3, 5) and 1 dog from the control group (dog 13) fell into this category. It is possible that the distension was from another cause, such as a hemoabdomen. If these cases were not a GDV then overall incidence would have been 8/238 for the splenectomy group and 2/209 for the control group, which was still not significantly different ($P = 0.09$).

There are 2 previous studies that also did not find an association between splenectomy and GDV (25,26), while 1 study included 37 dogs with a follow-up time of 1 y (27). Of the 2 studies that found no association between splenectomy and GDV, 1 study included 37 dogs with a follow-up time of 1 y (26), while the other evaluated 172 dogs, of which 88/168 (52%) with histopathology performed were diagnosed with neoplasia and had a median survival time of 83 d (25). The limitation of both is that there may not have been adequate time to allow for subsequent GDV, making it harder to effectively evaluate the risk of GDV following a splenectomy.

The most recent study of 151 dogs by Sartor et al (27) did find a significant association between GDV and splenectomy; they noted that dogs with a GDV were 5.3 times more likely to have had a previous splenectomy compared with a control group. Unlike the 2 previously mentioned studies, in Sartor et al (27) the control group was age, weight, and breed matched, but included dogs that did not undergo a laparotomy. It is also harder to draw a definitive conclusion concerning the association between GDV and splenectomy because all dogs undergoing splenectomy were not evaluated, as association was found by looking back at the incidence of prior splenectomy in dogs known to have a GDV. Lastly, 2 previous case reports described GDV following splenectomy (3,4). They reported occurrence of GDV between 2 d and 17 mo after surgery in a mixed breed, Great Dane and German shepherd. Given that 2 of the 3 dogs were at-risk breeds, it is difficult know if a GDV would have occurred in these dogs regardless of splenectomy, or if removal of the spleen increased the risk for subsequent GDV.

There was no significant difference in gender distribution between our groups, but there was significant difference in both mean age and weight of dogs at the time of surgery. We suspect the difference in age was due to the inclusion criteria for each group, as most dogs in our splenectomy group were undergoing surgery for suspected splenic neoplasia, while in our control group most dogs were undergoing surgery for a gastrointestinal foreign body removal. The difference in weight was because juvenile dogs < 20 kg at time of surgery were included in the control group if they would be at least 20 kg as adults.

The overall significance of differences in both age and weight, and how they would affect accuracy when comparing GDV rates is unknown. Grange et al (25) found that age and weight were not associated with GDV occurrence. However, our results showed that while weight was not significantly associated with subsequent GDV, age was significantly associated with GDV when evaluating both groups together. Age was not significantly associated when each group was evaluated separately. Since the groups differed significantly in their age distribution, the authors felt the association of age and GDV occurrence would be most accurate when evaluating the total population of dogs. Furthermore, previous studies have also documented an association with both age and weight (10,16–18,28,29) and occurrence of GDV. It is plausible that GDV may be linked to a degenerative or age related process. However, since our splenectomy group was significantly older and may have been placed at an increased risk for GDV with removal of the spleen, it is possible that age is merely a correlation, and not causation. Also, our inclusion criteria for the splenectomy group selected for dogs that had not developed a GDV at a younger age. Including younger dogs in the control group allowed for both a longer follow-up time and for comparison between the splenectomy group and dogs undergoing surgery for other reasons. In regard to weight, deBattisti et al (28) found an increased occurrence of GDV with a lower body weight, while Bredal (29) found an increased occurrence of GDV with a higher body weight. However, just as in our study, body condition score was not noted. Future studies are needed to further define the risk of age, weight, and body condition score in relation to GDV.

Breed has been shown to be associated with GDV occurrence (18,30–32). In our study, we elected to not analyze breed, as many breed categories had too few numbers. The largest breed categories in both study and control groups were mixed-breed dogs, Golden retrievers, and Labrador retrievers. It is interesting to note that our splenectomy group contained a larger number of German shepherds, Weimaraners, and basset hound dogs, which are considered at risk breeds for GDV (30–32) and could influence GDV occurrence compared with the control group. However, the dogs that subsequently developed GDV in the splenectomy group were primarily mixed-breed or Labrador retrievers (Table 2).

The median time from surgery to subsequent GDV for dogs in the splenectomy group was significantly shorter than for the control group. This difference is the result of the control group having a significantly younger population with both longer survival and follow-up times, which would then allow more time for development of GDV. We also observed that although the median time from surgery to occurrence of GDV was

### Table 3. Detailed information for dogs in the control group that had a subsequent GDV.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Gender</th>
<th>Weight (kg)</th>
<th>Age (y)</th>
<th>Surgery/Diagnosis</th>
<th>Time to GDV (d)</th>
<th>Status</th>
<th>Survival time (d)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>English springer</td>
<td>MN</td>
<td>25</td>
<td>11.3</td>
<td>Gastrotomy/Linear FB</td>
<td>570</td>
<td>D</td>
<td>1350</td>
<td>Paralysis</td>
</tr>
<tr>
<td>12</td>
<td>Boxer</td>
<td>MN</td>
<td>33.7</td>
<td>5.4</td>
<td>Enterotomy/Jejunal FB</td>
<td>1663</td>
<td>D</td>
<td>1663</td>
<td>GDV</td>
</tr>
<tr>
<td>13</td>
<td>Dalmatian</td>
<td>MN</td>
<td>33.9</td>
<td>4.3</td>
<td>Cystotomy/CaOx stones</td>
<td>1029</td>
<td>D</td>
<td>1029</td>
<td>Suspect GDV</td>
</tr>
</tbody>
</table>

F — female; M — male; S — spayed; N — neutered; FB — foreign body; D — Deceased; GDV — gastric dilation and volvulus; CaOx — calcium oxalate bladder stones.
longer than the survival time for dogs with splenic neoplasia, a prophylactic gastropexy should still be considered in those dogs which belong to an at-risk breed because GDV was documented to occur as quickly as 15 d after splenectomy (Dog 5). This is consistent with other studies that reported intervals of 2 d and 12 d between splenectomy and subsequent GDV (4.25).

The limitations of this study are mainly related to its retrospective nature. Most importantly we relied on the medical records to be both accurate and complete. If owners were unsure if a gastropexy had been performed prior to surgery, the surgery report was then referenced. It is possible that presence of an intact gastropexy site may have been omitted from a surgery report. This would have falsely decreased our overall occurrence of GDV. We were also limited in the details we could obtain from the medical record, and therefore were unable to control for other GDV risk factors, such as anatomy, environment, and diet. Our control group was not weight and age matched, which are factors that may influence both the rate and timing of occurrence of GDV. Lastly, follow-up was performed by contacting owners, which sometimes required them to recall details from up to 7 years earlier. It is possible that some owners may have incorrectly recalled relevant details regarding history of gastropexy, cause of death, and time of death, all of which could have confounded our findings.

In conclusion, we did not find gender, weight, or the presence of a hemoabdomen to be significantly associated with occurrence of GDV. Age was significantly associated with subsequent GDV when both groups were evaluated together, but was not significant when evaluated separately. Presence of hemoabdomen at time of surgery and splenic neoplasia were associated with a shortened median survival time. The difference in occurrence of GDV between dogs that underwent a splenectomy and dogs that underwent exploratory laparotomy for other reasons appeared clinically relevant, but was not statistically significant. Given the limitations of our study, a larger prospective study would be helpful in clarifying these issues. Current recommendation for gastropexy at time of removal of the spleen should be made on a case-by-case basis while taking into consideration previously documented risk factors.

Acknowledgment

The authors thank Dr. Joe Hauptman for his contribution to statistical analysis.

References

Comparison of surgical time and complication rate of subcutaneous and skin closure using barbed suture or traditional knotted suture in dogs

Laura K. Nutt, Megan L. Wilson, Sherisse Sakals

Abstract — This prospective study evaluated the handling, intraoperative and postoperative complication rates of a barbed knotless suture for closure of subcutaneous tissue and skin in 17 client-owned dogs (group A) following a tibial plateau leveling osteotomy procedure. Clinical characteristics, surgical time, and complication rates were compared to a control group of 17 client-owned dogs (group B) with subcutaneous tissue and skin closure using traditional suture material. Signalement was not significantly different between groups and did not have an effect on complication rates. Surgical times were not significantly different for subcutaneous tissue or skin closure between the 2 groups. There were significantly more intraoperative complications in the barbed suture group (A: 4/17; B: 0/17; \(P = 0.033\)) but no difference in minor or major postoperative complication rates (minor A: 2/16; B: 1/14; \(P = 0.626\), major A: 2/16; B: 0/14; \(P = 0.171\)).

Résumené — Comparaison du temps opératoire et du taux de complication de la fermeture sous-cutanée et de la peau en utilisant des points de suture barbelés ou des points de suture noués traditionnels chez les chiens. Cette étude prospective a évalué les taux de complication liés à la manipulation, peropératoire et postopératoire des points de suture barbelés sans nœuds pour la fermeture du tissu sous-cutané et de la peau chez 17 chiens appartenant à des clients (groupe A) après une intervention d’ostéotomie de nivellement du plateau tibial. Les caractéristiques cliniques, le temps opératoire et les taux de complication ont été comparés à un groupe témoin de 17 chiens appartenant à des clients (groupe B) avec du matériel de suture traditionnel pour la fermeture du tissus sous-cutanés et de la peau. Le signalement n’était pas significativement différent entre les groupes et n’a pas eu d’effet sur les taux de complication. Le temps opératoire n’était pas significativement différent pour la fermeture du tissu sous-cutané ou de la peau entre les deux groupes. Il y avait significativement plus de complications peropératoires dans le groupe à points de suture barbelés (A : 4/17; B : 0/17; \(P = 0,033\)) mais aucune différence pour les taux de complications postopératoires mineures ou majeures (mineure A : 2/16; B : 1/14; \(P = 0,626\), majeure A : 2/16; B : 0/14; \(P = 0,171\)).

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Introduction

Barbed suture has been used in human surgery for many years with good results. Uses have included urogenital reconstruction, retroperitoneoscopic partial nephrectomy, arthroscopy, tenorrhaphy, laparoscopic myomectomy, laparoscopic hysterectomy, laparoscopic gastric bypass, and cosmetic surgery such as esthetic breast surgery and wound and incision closure (1–10). Studies in humans using knotless barbed suture for closure of surgical incisions resulted in a decrease in scarring in the long term, with less pruritus and fewer adverse skin tissue reactions during healing and shorter surgical times (6,11,12). Its use in veterinary medicine is still in its infancy, with many of the studies involving swine models for human application (13). It has been reported experimentally for gastrotomy, jejunal and colonic enterotomy, tendon laceration repair, diaphragmatic herniorrhaphy, incisional gastropexy, urinary tract reconstruction including bladder neck anastomosis, and laparoscopic gastropexy, gastrotomy, enterotomy, cystostomy, and pyeloplasty (14–24). There have been few studies on client-owned patients. Laparoscopic prophylactic gastropexies on client-owned dogs using self-anchoring barbed sutures appear to be the most common area of clinical study, showing long-term success (25,26). A single case report of the use of barbed suture for laparoscopic closure of internal inguinal rings in a horse was performed without recurrence or complication (27). It has been reported that strength of closure and complications with knotless barbed suture are comparable to traditional

Small Animal Clinical Sciences, Western College of Veterinary Medicine, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4.

Address all correspondence to Dr. Laura K. Nutt; e-mail: laura.nutt@usask.ca

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monofilament knotted suture, while surgery time can be significantly reduced (3,16–24). The use of knotless barbed suture in clinical cases in veterinary medicine has been minimal, and, to the authors’ knowledge, the use of barbed suture has not been evaluated in clinical cases for subcutaneous and intradermal closures in veterinary medicine.

The barbed knotless suture used for the study was Stratafix Spiral PGA-PCL (Ethicon, Guaynabo, Puerto Rico), which has small bidirectional barbs cut into the suture material in a spiral fashion to allow the suture to engage the tissue without the risk of backing out or loosening under moderate tension (2). This suture is formed with a copolymer of glycolide and e-caprolactone, is a sterile synthetic absorbable monofilament which maintains tension for 1 to 2 wk, and absorbs in 90 to 120 d. A surgical needle is present on each end of the suture, allowing the surgeon to perform a 2-layer closure with 1 thread. Current label indications for use are in soft tissues in which absorbable suture is deemed appropriate. The barbed nature of the suture allows for closure without requiring knots, eliminating the weakest point of the closure and the amount of suture needed (8). Barbed suture also allows for consistent tension throughout the continuous closure (2,4,8). The lack of knots also has been reported to improve efficiency, allowing for shorter anesthesia times, while increasing the ease of suturing incisions with limited access (1,2,4). Barbed suture combines the security and strength of interrupted suture patterns with the efficacy of a continuous suture pattern (2).

The purpose of our study was to compare the clinical use, intraoperative and postoperative complication rate of a knotless suture and a traditional knotted suture in the closure of subcutaneous and dermal tissues layers following a tibial plateau leveling osteotomy (TPLO). Our hypotheses were that there would be no difference in intraoperative or postoperative complication rates between the 2 groups and that closure times using the knotless barbed suture would be faster than for the control group.

Materials and methods

Between March and November 2014, 17 client-owned dogs scheduled for TPLO were entered into the study as the barbed suture group (Group A) at the Veterinary Medical Centre (VMC) at the Western College of Veterinary Medicine, University of Saskatchewan. Owner’s consent was obtained before enrolling the patient in the study. The protocol for this study was approved by the institutional Animal Care Committee. Seventeen client-owned dogs scheduled for TPLO between December 2015 to May 2016 were entered as the control group (Group B). All animals were deemed healthy prior to surgery. Preoperative analyses of blood and urine were performed based on recommendations by anesthesiologists and surgeon preference. Stifle arthroscopy followed by TPLO was performed based on recommendations by anesthesiologists and surgeon preference. Stifle arthroscopy followed by TPLO was performed based on recommendations by anesthesiologists and surgeon preference. Stifle arthroscopy followed by TPLO was performed based on recommendations by anesthesiologists and surgeon preference. Stifle arthroscopy followed by TPLO was performed based on recommendations by anesthesiologists and surgeon preference.

In Group A, the subcutaneous layer and intradermal layer were closed with 1 package of 3-0 tensile strength, 2-0 size polydioxanone suture (PDSII; Ethicon) in a simple continuous pattern. The skin was closed with the same suture in a continuous intradermal pattern. Square knots were used for the beginning and end of each suture line and all knots were buried.

Any intraoperative complications including suture breakage, suture knotting, incomplete or poor apposition of the incision edges and, in Group A, the suture material not locking were noted. All subcutaneous and intradermal closures were performed by a single surgical resident.

All patients were discharged within 1 to 2 d after surgery with an Elizabethan collar and instructions to prevent the patient from licking or chewing at the incision. Antibiotics were not routinely prescribed for the postoperative period. Tramadol hydrochloride (Ultram; Janssen, Toronto, Ontario) and a non-steroidal anti-inflammatory drug were sent home for analgesia. Clients were instructed to not apply any ointments or creams to the incision, and to monitor the incision at least twice daily for any evidence of superficial incisional infection, as outlined by the Centers for Disease Control (CDC), including purulent discharge, pain/tenderness, localized swelling, erythema, heat, or deliberate opening of the incision (28).

Patients were rechecked at approximately 2 wk after surgery and any incisional complications were recorded, as well as any treatment required. If they were not brought in to the VMC for this recheck, the clients were contacted by telephone and/or e-mail at least 2 wk after surgery to inquire about healing of the incision if they were rechecked with the referring veterinarian. If a superficial incisional infection was reported by the owner in the dogs that did not return to the VMC, the referring veterinarian was contacted to qualify what complications were observed and the treatment performed. Complications were divided into
minor, which resolved without any surgical intervention, and major, which required further surgical intervention, including any form of subsequent closure of the incision.

**Statistical analysis**

A prospective cohort study comparing the 2 groups of dogs was performed. Data were analyzed using a statistical software program (Stata SE 12; Statacorp LP, College Station, Texas, USA). Shapiro-Wilk testing was performed to assess the normality of the distribution of the data for age, weight, length of time to close subcutaneous tissues and skin, incision length, and length of follow-up. Mean, median, and standard deviation were calculated. If there was normal distribution, a 2-sample t-test was performed. If the data were not normally distributed, a Wilcoxon rank-sum test was performed. To assess for any learning curve and increase in speed of closure with the knotless suture, the length of time to close subcutaneous tissues and intradermal tissues in Group A as well as intraoperative and postoperative complication rates of the first 5 cases and the last 5 cases were analyzed. A Fisher’s exact test was performed on variables with non-numeric variables including breed, gender, and neuter status, on which leg the surgery was performed, the occurrence of intraoperative complications, and the occurrence of minor and major postoperative complications. Any patient that was discharged with antibiotics was excluded from statistical analysis of postoperative complications. A P-value < 0.05 was considered significant. Post-hoc power analysis calculations were performed on subcutaneous and intradermal tissue closures, intraoperative and postoperative complication rates.

**Results**

**Patient parameters**

A total of 34 client-owned dogs were admitted into the study, 17 in each group. Mixed breed dogs were the most common breed in the study (n = 14), followed by Labrador retrievers (n = 5), and golden retrievers (n = 3). The remaining dogs were other medium to extra-large breeds, including Akita, basset hound, bulldog, dogue de Bordeaux, Alaskan malamute, rottweiler, Newfoundland, American pitbull, Maremma sheepdog, and Tibetan mastiff. There was no significant difference between the 2 groups for breed (P = 0.544), age [mean A: 5.65 y, standard deviation (SD) ±/− 3.02; B: 4.76 y, SD ±/− 2.17, P = 0.3349], body weight (mean A: 39.15 kg, SD ±/− 9.79; B: 38.51 kg, SD ±/− 9.11, P = 0.8362), gender (P = 0.522), or surgery side (P = 0.303).

**Surgery**

There was no significant difference in incision size between the 2 groups (mean A: 9.34 cm, SD ±/− 1.64; B: 9.65 cm, SD ±/− 1.13, P = 0.2938). There was no significant difference between the 2 groups in closure times for subcutaneous tissue (mean A: 2.75 min, SD ±/− 0.97; B: 2.23 min, SD ±/− 0.40, P = 0.0871) and skin (mean A: 3.41 min, SD ±/− 0.66; B: 3.69 min SD ±/− 0.48, P = 0.1673). The first 5 cases and last 5 cases of Group A showed no significant difference in the surgical time to close the subcutaneous tissues (mean first 5: 2.70 min, SD ±/− 0.69; last 5: 2.81 min, SD ±/− 0.51, P = 0.7886) and the skin (mean first 5: 3.0 min, SD ±/− 0.67; last 5: 3.4 min, SD ±/− 0.49, P = 0.3278). Post-hoc power analysis of surgical times to close the subcutaneous and skin tissues were 53.1% and 29.4%, respectively.

**Intraoperative complication**

There were significantly more intraoperative incisional complications in Group A than in Group B (A: 4/17; B: 0/17, P = 0.033). All complications in Group A were associated with the suture not locking into the tissues at the midway point where the bars change direction. There was no significant difference in intraoperative complications between the first and last 5 cases of Group A (1/5 versus 0/5, P = 0.292). Post-hoc power analysis for intraoperative complication was 57.1%.

**Follow-up**

There was no significant difference in mean follow-up time between the 2 groups (A: 169.2 d; B: 79.9 d, P = 0.6324). Patients that were lost to follow-up or were discharged with postoperative antibiotics were excluded from follow-up analysis. There was no significant difference in the number of dogs that were rechecked at the VMC between the 2 groups (A: 8/16; B: 10/14, P = 0.232).

**Postoperative complications**

One dog from Group A and 3 from Group B were excluded from postoperative complications due to either being lost to follow-up (1 dog) or were discharged with antimicrobial therapy (3 dogs). There was no significant difference in minor postoperative complications between Group A and Group B (A: 2/16; B: 1/14, P = 0.626) or in major postoperative complications (A: 2/16; B: 0/14, P = 0.171). There was no significant difference in postoperative complication rates in the dogs that were rechecked at the VMC (3/15) compared to those rechecked at the referring veterinarian (2/15) (P = 0.624). There was a significant difference in the number of postoperative complications between the first and last 5 cases of Group A (first 5, 0/5; last 5, 3/5; P = 0.038). Post-hoc power analysis for postoperative complication was 24.9%.

Of the 4 postoperative complications in Group A, all were suspected to have a superficial incisional infection based on the previously described parameters (pain, erythema, swelling with or without discharge, and dehiscence) and were treated with broad-spectrum antibiotics. One dog had incisional infection confirmed with culture of *Staphylococcus pseudintermedius*. All the patients treated with broad-spectrum antibiotics had complete resolution of the clinical signs and healing of the incision. The 2 patients that were treated at the VMC were treated with cephalexin (Apo-Cephalex; Apotex, Weston, Ontario), 20 mg/kg body weight (BW), PO, q8h for 7 d. Of the 2 patients which were not rechecked at the VMC, 1 was treated with cephalexin (25 mg/kg BW, PO, q12h for 21 d) and the other was treated with amoxicillin and clavulanic acid (Clavaseptin; Véroquinol, Lavaltrie, Quebec), 15 mg/kg BW, PO, q12h for 7 d. Of these 4 with suspected superficial infections, 2 were classed as major as there was partial incision dehiscence treated with the additional intervention of closure of the dehiscence with skin staples. All
patients had resolution of clinical signs with complete healing of the incision.

The minor postoperative complication in Group B involved 1 dog that developed a suspected seroma that resolved with massage, warm compresses, and passive range of motion.

Discussion

Many studies have shown that the use of knotless suture will significantly decrease surgical time (3,4,6,9–12,23,24). Improved surgical times have been reported in canine models using barbed suture for jejunal and colonic enterotomy, gastrotomy, and prophylactic laparoscopic incisional gastropexy; however, there have been several studies with no significant difference or increased surgical times when comparing barbed to traditional suture (17,25). Surgical times were increased for laparoscopic cystopexy using knotless barbed suture in a cadaveric canine model compared with traditional open cystopexy using traditional suture, suggesting that there may be a learning curve present; however, this also could be attributed to the laparoscopic versus open aspect of the study (15). A study comparing barbed suture and conventional suture for laparoscopic pyloroplasty and bladder wall anastomoses in pigs revealed no significant difference in duration of surgery (21). It is possible that the learning curve of using the novel barbed suture material contributed to our study not obtaining a significant difference between the 2 groups. The surgeon practiced closing several incisions on cadavers prior to this study; however, the barbed suture was not routinely used other than in this study. There may have been an improvement in surgical time if the surgeon had used the suture for a longer period before the study to become more comfortable with the handling of the knotless suture. When comparing the first 5 closure times to the last 5 closure times in Group A, there was no significant improvement in surgical time for either subcutaneous or skin closure and intraoperative complication rate, which could indicate that there is a more prolonged learning curve than was allowed in this study. Interestingly, there was a significantly higher postoperative complication rate in the last 5 closures, which could also support the need for a longer period of practice with barbed suture.

In vivo studies demonstrate that the barbed suture material used herein retains approximately 62% of its original strength 7 d after implantation and approximately 27% of original tensile strength at 14 d. In in vivo studies, complete absorption of the barbed suture occurred between 90 and 120 d. Similarly, the traditional suture’s breaking strength is 50% to 60% at 7 d, and 20% to 30% at 14 d with complete absorption reported at 90 to 120 d. A study of breaking strength of barbed 2-0 polypropylene suture compared to conventional polypropylene suture of different sizes revealed that the barbed 2-0 suture was at least as strong as 3-0 traditional suture, which should be a consideration when selecting size of barbed suture (29). The packaging of the barbed suture gives both the actual size of the suture and the equivalent tensile strength on the package to minimize confusion during selection of suture. We selected a barbed suture with the tensile strength of 3-0, which should be equivalent strength to the suture used in the control group. Therefore, it is unlikely that a strength difference existed between the 2 suture materials or contributed to the postoperative complication rates in our study. A 2010 study showed that geometric design of barbed monofilament suture, including cut angles and depths of barbs, affected tensile and tissue anchoring properties in different tissue types, suggesting that tissue-specific barbed suture could be beneficial (29). Histological analysis has shown that tissue reaction to the barbed suture is increased compared to traditional suture of the same material in intradermal wound closure in dogs; however, this did not cause a significant difference in short-term complication rates (30). Several studies on the use of barbed knotless suture in humans have shown lower complication rates compared to traditional suture, including less scarring, pruritus, adverse skin reactions, and wound complications. This was not observed herein, as our results showed no significant difference in minor or major postoperative complications between the 2 groups (6,12).

Another consideration with the use of barbed suture is the placement and type of end-pass anchor configuration technique, which is the additional bites at the end of the continuous suture line through the tissues to end the closure line. In the current study, we took 2 bites backwards in the opposite direction at the end of each suture line as our end-pass anchor technique. The package insert recommends “an additional backstitch or bite of tissue lateral to the end of the incision” to lock the suture. A study comparing 3 end-pass configurations, 1 of which was the configuration used in our study, as well as without an end-pass configuration for intradermal closures in canine cadavers, showed that maximum load, displacement, and stiffness of all end-pass configurations were higher than the no-end-pass configuration with slippage of the suture in all samples without an end-pass configuration. This suggests that while the type of end-pass anchor technique is likely not significant, one should be used (31). We therefore consider it unlikely that the end-pass anchor configuration contributed to the dehiscence that occurred in our study.

Tibial plateau leveling osteotomy procedures have a reported infection rate of 2.5% to 15.8% (32–35). The CDC defines superficial incisional infection as occurring within 30 d after surgery affecting the skin or subcutaneous tissues and involving 1 of the following: purulent discharge, a positive bacterial culture, pain/tenderness, localized swelling, erythema, heat or deliberate opening of the incision (28). All but one of the suspected superficial incisional infections in the barbed suture group were diagnosed by the clinical signs described by the CDC rather than bacterial culture, and may have resolved on their own even if broad-spectrum antibiotic therapy had not been initiated. Although our suspected infection rate was 20% (4/16), which is higher than the expected rate of infection for TPLO, none of the suspected infected patients required implant removal, which may indicate that the suspected infection sites were merely inflamed or partially dehisced. Nevertheless, these cases still constitute a postoperative complication. A recent study showed that postoperative antimicrobial administration was protective against surgical site infections in patients undergoing TPLO procedures (35). One patient in Group A and 2 in Group B were discharged with antibiotics due to pyoderma; none of these patients developed postoperative complications.
Any patients that were discharged with antibiotics were excluded from postoperative complication analysis.

The handling of barbed suture is different than the handling of traditional suture and was subjectively noted to be more difficult by our surgeons, with multiple intraoperative complications which included failure of the suture material lock in the tissue and poor apposition of the incision which required the placement of skin sutures in 1 patient. The subjective opinion of the suture handling would likely improve with more exposure to the barbed suture material, as it has been reported to be associated with increased surgeon satisfaction (12).

Limitations to this study include limits to the power caused by the small number of patients in both the experimental and control groups. Post hoc power analysis for surgical times of subcutaneous tissue and intradermal closures, intraoperative complication rate and postoperative complication rate ranged from 24.9% to 57.1%, putting this study at risk for type II errors. The surgeon was not able to be blinded to the suture being used, allowing for bias. The postoperative incisional recheck for approximately half of the patients was performed by the referring veterinarian due to distance from the VMC. Since there was no significant difference in postoperative complication rate between those patients rechecked at the VMC and those rechecked with the referring veterinarian, and classification of complication was based on objective measures including tenderness, erythema, swelling and discharge described by the CDC, it is unlikely the site of recheck affected our results.

Based on our results, our first hypothesis was partially supported, as there were significantly more intraoperative complications in the barbed suture group, with no significant difference in postoperative complications between the 2 groups. We expected that closure times using the barbed suture would be less, but this did not occur. We expect our results in this aspect varies from much of the human literature because our study reflects the early clinical exposure to and usage of the barbed suture. Further study using knotless suture for subcutaneous and skin closure in veterinary patients for both orthopedic and soft tissue procedures is recommended. It is possible that with increased familiarity with the use of the barbed suture, both the surgical times and intraoperative complication rates would decrease.

Acknowledgment

The authors thank Dr. Germaine Hung for her assistance with the surgical procedures in this study.

References

NovaVive Presents Data Demonstrating Reduction in Antibiotic Use with MCWF in Young Calves

NovaVive Inc., an animal health immunobiology company, today presented a Research Report at the 2017 American College of Veterinary Internal Medicine (ACVIM) Forum in National Harbor, Maryland. The NovaVive research report, presented by Dr. Aleksandar Masic, Vice-President of Research and Development, summarized the results of a study conducted in a large Canadian veal operation with NovaVive’s Mycobacterium Cell Wall Fraction (MCWF) technology (Amplimune™).

High levels of mortality in dairy calves represent a significant health issue and a major source of economic losses. A survey from the U.S. Department of Agriculture’s National Animal Health Monitoring System (NAHMS) showed that 9.1% of female heifer calves and 7.3% of male dairy calves die during the pre-calving or growing period. Forty-two percent of the deaths of male dairy calves occurred in the first 21 days following arrival on veal farms. “Antimicrobials (antibiotics) are traditionally used to reduce mortality on veal and dairy heifer raising operations,” said Dr. Masic. “The majority of producers introduce antimicrobials to calves immediately on arrival at the farm to prevent disease and again within the first week following arrival. As a result of the abundant use of antimicrobials in the veal industry, high levels of antimicrobial resistant bacteria, compared to conventional cattle, have been detected.” With antimicrobial resistance becoming of greater concern from both an animal and public health point of view, it is imperative to determine strategies to reduce antimicrobial resistance and use without comprising animal welfare.

In the study presented at the ACVIM Forum, a total of 699 calves age 1–10 days old were randomly assigned into two experimental groups (N = 354 MCWF/Treatment; N = 345 Control) and housed in four barns (Delimax, Quebec). Standard farm procedures and metaphylactic therapies (electrolytes and antibiotics) were administered to all animals upon arrival. One dose (1 mL) of MCWF was administered to the Treatment group on the day of arrival and a second dose was administered 7 days later. Calves were monitored daily for adverse events. Data were collected for various clinical conditions in all calves for the duration of the study (154 days). Data for additional antibiotic usage, supplemental treatments (duration of use and costs) and weight of animals were also collected and analyzed.

The study demonstrated:

<table>
<thead>
<tr>
<th></th>
<th>MCWF/Treatment Group</th>
<th>Control Group</th>
<th>% Reduction in Treatment vs. Control Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of calves requiring treatment</td>
<td>42</td>
<td>62</td>
<td>32.3%</td>
</tr>
<tr>
<td>Incidence of clinical conditions requiring treatment</td>
<td>41</td>
<td>123</td>
<td>66.7%</td>
</tr>
<tr>
<td>Volume of antimicrobials and supportive therapies</td>
<td>298.5 mL</td>
<td>1090 mL</td>
<td>72.6%</td>
</tr>
<tr>
<td>Total number of treatment days</td>
<td>65</td>
<td>191</td>
<td>66%</td>
</tr>
<tr>
<td>Average number of treatment days per calf</td>
<td>1.6</td>
<td>3.8</td>
<td>57.9%</td>
</tr>
</tbody>
</table>

In addition to the above results, there was a 57% lower mortality rate in the MCWF/Treatment Group than in the Control Group.

“Based on preliminary analysis of the data, MCWF (Amplimune™) can be used as an aid or an antimicrobial alternative to not only reduce illness and death in calves, but also significantly reduce the number and volume of antimicrobials used in veal production,” concluded Dr. Masic. Further studies are underway to demonstrate MCWF efficacy as a standalone metaphylactic treatment in both dairy and beef calf operations.

Contact: NovaVive Inc., Belleville, Ontario; phone: (613) 391-3837; website: www.NovaVive.ca
Short-term effects of dietary supplementation with amino acids in dogs with proteinuric chronic kidney disease

Andrea Zatelli, Paola D'Ippolito, Xavier Roura, Eric Zini

Abstract — This retrospective study investigated the impact of amino acid supplementation on body weight, serum albumin, creatinine and urea concentrations, and urine protein-to-creatinine (UPC) ratio in proteinuric dogs with chronic kidney disease (CKD). Forty-six client-owned azotemic dogs with spontaneous proteinuric CKD already on a renal diet and in therapy with enalapril were included. After approximately 1 month of treatment (baseline), 29 dogs received oral amino acid supplementation daily (group A) and 17 dogs did not (group B). The parameters under investigation were determined at baseline and after 4 to 8 weeks in both groups. Compared to baseline, body weight and serum albumin increased ($P < 0.01$, $P < 0.05$, respectively) at follow-up in group A, but did not change in group B. Serum creatinine concentration did not change in both groups; urea concentration ($P < 0.05$) and UPC ratio ($P < 0.01$) decreased in group B, but not in group A. Supplementation with amino acids increased body weight and serum albumin concentration in these dogs but it might have prevented a decrease in proteinuria and urea concentration.

Résumé — Effets à court terme de la supplémentation alimentaire avec des acides aminés chez les chiens atteints de la maladie rénale chronique protéinurique. Cette étude rétrospective a étudié l’impact de la supplémentation avec des acides aminés sur le poids corporel, l’albumine sérique, les concentrations de créatinine et d’urée et le rapport protéines/créatinine urinaire (UPC) chez les chiens albuminuriques atteints de maladie rénale chronique (MRC). Quarante-six chiens azotémiques, appartenant à des clients, atteints de MRC albuminurique spontanée consommant déjà une diète rénale et un traitement d’enalapril ont été inclus. Environ 1 mois après le traitement (données de référence), 29 chiens ont reçu une supplémentation quotidienne aux acides aminés (groupe A) et 17 ne l’ont pas reçu (groupe B). Les paramètres à l’étude étaient déterminés aux données de référence et après 4 à 8 semaines dans les deux groupes. Comparativement aux données de référence, le poids corporel et l’albumine sérique ont augmenté ($P < 0.01$, $P < 0.05$, respectivement) au suivi dans le groupe A, mais n’ont pas changé dans le groupe B. La concentration de créatinine sérique n’a pas changé dans les deux groupes; la concentration d’urée ($P < 0.05$) et le rapport d’UPC ($P < 0.01$) ont baissé dans le groupe B, mais non dans le groupe A. La supplémentation avec des acides aminés a augmenté le poids corporel et la concentration d’albumine sérique chez ces chiens mais elle peut avoir empêché une baisse de la concentration de protéinurie et d’urée.

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Introduction

In dogs, proteinuria is often associated with chronic kidney disease (CKD) and studies in this species led to the hypothesis that proteinuria may promote the progression of renal damage, as it does in humans (1–8). In endemic areas for certain vector-borne diseases, such as leishmaniosis, the prevalence of dogs with proteinuria, azotemia, or both, has been reported to be up to 50% (8,9). Among dogs at risk for developing proteinuric nephropathy, other than those living or having lived in endemic areas, there are also breeds that are genetically predisposed to proteinuric CKD (3,4,6,8). Early identification and treatment of proteinuria appears to be crucial in dogs, as its management slows the progression of renal disease, risk of uremic crisis, and renal-related death (1,3,4). Together with treatment of the underlying disease, the major cornerstones of therapy in proteinuric dogs with CKD are angiotensin converting enzyme inhibitors (ACEI), dietary intervention, and omega-3 fatty acids which slow the progression of renal disease, minimize clinical signs of uremia and, at least for the diet, maintain an optimal body weight (BW) and body condition score (1–3,6,9–14). However, in some dogs, anti-proteinuric therapy may not reduce proteinuria despite concurrent administration of diets lower in protein compared to maintenance diets (2–4,8,11,13,14). Furthermore, in proteinuric dogs renal diets may not adequately meet protein requirements, thus possibly leading to low BW, hypoalbuminemia, and malnutrition. One study on a model of spontaneous proteinuric nephropathy in dogs showed that a low protein content diet (14% dry matter), similar to the renal diets commonly recommended in dogs with kidney disease, caused a significant reduction in BW and plasma albumin concentration that were noticeable at 4 wk of administration (8).

In humans with CKD, nutritional status is helpful to identify patients with increased risk of morbidity and mortality: a significant association was observed between decreased baseline BW and subsequent risk of hospital admission (15–18). For these reasons, oral supplementation with amino acids (AA) or intradialytic AA administration has been proposed in malnourished humans with CKD (15–19).

In dogs with CKD and severe proteinuria, either low BW or hypoalbuminemia is frequent and can be associated with increased morbidity and risk of mortality (20). Indeed, albumin hypercatabolism and its down-regulated synthesis can contribute to glomerular disease-associated hypoalbuminemia, possibly leading to marked hypoalbuminemia in dogs with CKD with subnephrotic range proteinuria, thus worsening the prognosis (21). Based on this premise, it seems plausible that the amount of protein needed should be individually tailored in dogs, depending on the stage of CKD and the extent of proteinuria (8). As in humans, also in dogs, AA supplementation may represent the easiest means to correct an insufficient daily intake of proteins. Thus, the aim of this retrospective case-control study was to investigate the impact of an oral AA supplementation during a short period of time on BW, serum concentrations of albumin, creatinine, and urea, and on the urine protein-to-creatinine (UPC) ratio in proteinuric dogs with CKD treated with enalapril and fed a commercial renal diet (RD).

Materials and methods

Animals and inclusion criteria

Medical records of proteinuric dogs in IRIS stages ≥ 2 (14) admitted in 2007 and 2008 at one of the authors’ institutions (AZ, PDI) were reviewed. All the data available on clinical history, physical examination, BW, complete blood (cell) count (CBC), serum biochemical profile, urinalysis, UPC ratio, indirect blood pressure, abdominal ultrasonographic findings, ongoing treatments, and follow-up examinations were collected. Dogs without stable renal function were excluded; stable renal function was defined by serum creatinine concentration that did not increase or decrease by ≥ 20% within 1 mo of initial determination (10). Dogs were considered to be proteinuric if the UPC ratio was above 0.5 (IRIS stage P) (14) in 2 urine samples collected at 1-month interval; dogs that did not fulfill this criterion were excluded. Furthermore, to be included in the study, dogs had to receive enalapril (Enacard; Merial Italia spa, Milano, Italy) at 0.5 mg/kg BW, q12h, and a commercial renal diet (Hill’s Prescription Diet Canine k/d; Hill’s Pet Nutrition, Topeka, Kansas, USA or Royal Canin Renal Canine; Royal Canin SA, Aimargues, France); the amount of diet was according to the recommendation of the companies and corrections were not made if dogs received or did not receive AA supplementation.

Information was collected from each record, to identify dogs that received or did not receive oral AA supplementation [IT IS pet; ACME srl, CAVIARGO (RE), Italy; formulation shown in Table 1]. Among dogs on AA supplementation, only those taking the daily amount (X mg) of AA arbitrarily calculated using the following formula, BW (kg) × UPC ratio × 20 = X (22), were included. One tablet provided approximately 675 mg of AA. Dogs that had received oral or intravenous AA supplementation within 1 mo from the time of admission were excluded. Finally, dogs were excluded if the diagnostic workup identified inflammation or infection of the genitourinary tract (based on ultrasonography and urinalysis), a pre-renal cause of proteinuria (based on serum biochemistry), and if cardiac disease, neoplasia, or endocrinopathies were diagnosed or suspected. All dogs had been tested for leishmaniosis, ehrlichiosis, and babesiosis, and were not included if an active form of infection was identified or suspected.

Additional treatments and follow-up

As a standard of care at the authors’ institution, dogs classified as “severely hypertensive” (systolic arterial pressure ≥ 180 mmHg) according to the IRIS staging system (14) were treated with oral amlopidine (Norvasc; Pfizer Italia srl, Latina, Italy), 0.1 to 0.5 mg/kg BW, q24h, in order to reduce systolic arterial pressure to < 160 mmHg (substage “normotensive” or “borderline hypertensive”). In addition, dogs with severe hypoalbuminemia received oral acetylsalicylic acid at 2.0 mg/kg BW, q24h, to prevent thrombosis. Based on the reference range of serum albumin (28 to 38 g/L), dogs were considered hypoalbuminemic if the albumin concentration was ≤ 27 g/L; severe hypoalbuminemia was arbitrarily defined as a value < 20 g/L.

As stated, dogs with proteinuric CKD were reassessed after 1 mo to check if the renal disease was stable; all throughout the
Table 1. Composition of the amino acid supplement per 100 grams.

<table>
<thead>
<tr>
<th>Amino acid type</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched-chain aliphatic amino acids</td>
<td></td>
</tr>
<tr>
<td>isoleucine, leucine, valine</td>
<td>26</td>
</tr>
<tr>
<td>Aliphatic amino acids</td>
<td></td>
</tr>
<tr>
<td>threonine, arginine, lysine</td>
<td>24</td>
</tr>
<tr>
<td>Sulfur-containing amino acids</td>
<td></td>
</tr>
<tr>
<td>cysteine, methionine</td>
<td>7</td>
</tr>
<tr>
<td>Aromatic amino acids</td>
<td></td>
</tr>
<tr>
<td>tyrosine, phenylalanine</td>
<td>11</td>
</tr>
<tr>
<td>Heterocyclic amino acids</td>
<td></td>
</tr>
<tr>
<td>tryptophan, histidine</td>
<td>6</td>
</tr>
<tr>
<td>Carrier</td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>10</td>
</tr>
<tr>
<td>sucrose</td>
<td>10</td>
</tr>
<tr>
<td>pregelatinized rice</td>
<td>6</td>
</tr>
</tbody>
</table>

manuscript this time-point will be called baseline. After baseline, dogs were re-evaluated between 4 and 8 wk.

Blood sampling and assay
During each examination, blood samples were collected in dogs fasted overnight, and serum was obtained within 30 min, stored at 4°C and analyzed within 24 h. Results from CBC and serum biochemical analysis, including albumin, total protein, glucose, bilirubin, cholesterol, amylase, alanine transferase, alkaline phosphatase, urea nitrogen, creatinine, sodium, potassium, chloride, and phosphate, were obtained by the same methods (BC-2800Vet, MINDRAY, Mindray Co., Shenzhen, China; Cobas Mira, Roche Diagnostic AG, Basel, Switzerland) in all samples.

Urine collection and urinalysis
An ultrasound-guided cystocentesis was performed in all dogs using a 5-mL syringe connected to a 23-G needle. All urine samples were placed in 10 mL, sterile, evacuated collection tubes, and analyzed by the same operator. Urine samples were examined within 60 min from collection if samples were stored at room temperature (≈20°C), or within 4 h if stored at 4°C to 8°C. Urine sediment was obtained by centrifugation (10 min at 900 × g) of 5 mL of urine, followed by removal of 4.5 mL of supernatant, and resuspension of the remaining 0.5 mL of sediment. A sample of 12 μL of the resuspended urine sediment was microscopically assessed. The supernatant was transferred into separate tubes and stored at ≈20°C to determine the UPC ratio within 7 d. Red blood cells and white blood cells were expressed as mean number of cells/10 high power fields (hpf, 40 × magnification). Urine sediment with bacteriuria, and/or > 5 red blood cells or white blood cells/hpf, was considered indicative of active inflammation and excluded from the UPC ratio evaluation (23).

UPC ratio
To calculate the UPC ratio, protein concentration was measured with pyrogallol red, and creatinine was measured using the Jaffé method on undiluted urine supernatant that was thawed before analysis. Analytes were measured in an automated spectrophotometer (Cobas Mira, Roche Diagnostic AG) in each case.

Table 2. Age, body weight (BW), serum concentration of creatinine, urea and albumin, and urine protein to creatinine (UPC) ratio at baseline in dogs receiving amino acid (AA) supplementation (group A) and in dogs not receiving AA supplementation (group B).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (mean ± SD)</th>
<th>Group B (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6 ± 3</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>30 ± 14</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>256 ± 115</td>
<td>407 ± 248</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>24 ± 11</td>
<td>25 ± 16</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>24 ± 7</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>UPC ratio</td>
<td>4.1 ± 4.5</td>
<td>4.3 ± 4.6</td>
</tr>
</tbody>
</table>

SD — standard deviation.

Statistical analysis
For data evaluation, BW, serum albumin, creatinine and urea concentrations, and the UPC ratio were retrieved from baseline and after 4 to 8 wk in all dogs. Dogs that received the AA supplementation were included in group A, those that did not receive the AA supplementation belonged to group B. To verify whether population characteristics were similar in the 2 groups, baseline age, BW, serum albumin, creatinine and urea concentrations, and UPC ratio were compared with unpaired t-test. Gender distribution, and frequency and severity of hypoalbuminemia were compared between groups with Chi-squared test or Fisher’s exact test.

To study the effect of AA supplementation, BW, serum albumin, creatinine and urea concentrations, and UPC ratio at baseline and after 4 to 8 wk were compared between the 2 groups with paired t-test. Because severe hypoalbuminemia may be associated with morbidity and mortality in dogs (3,20,21), the effect of AA supplementation was also explored in the subset of cases with serum albumin concentration < 20 g/L by paired comparisons between baseline and 4 to 8 wk for the discussed parameters. Normality of all data sets was investigated with Kolmogorov-Smirnov test and non-normally distributed variables were log-transformed to achieve Gaussian distribution before using parametric tests. Results are reported as mean ± standard deviation or as percentages. A P < 0.05 was considered statistically significant. Statistical analysis was performed with commercial software (GraphPad Prism version 4.0; GraphPad Software, La Jolla, California, USA).

Results
Baseline
Forty-six proteinuric CKD dogs in IRIS stages 2, 3, or 4 were included; 29 of them received AA supplementation (group A), while 17 did not (group B).

Age and BW of both groups are reported in Table 2. In group A, 20 (69%) dogs were males (17 intact and 3 castrated) and 9 (31%) were females (8 intact and 1 spayed). Regarding dog breeds, 7 were boxer, 2 of each were German shepherd, dogue de Bordeaux, epagneul Breton or Italian pointer, and 1 of each was cocker spaniel, dachshund, Dalmatian, doberman, dogo Argentino, German pointer, golden retriever, Jack
Russell terrier, pit bull and rottweiler; the remaining 4 dogs were cross-breed. In group B, 11 (65%) dogs were intact males and 6 (35%) were females (5 intact and 1 spayed). Regarding dog breeds, 3 were boxer, 2 were Great Dane, and 1 of each was American Staffordshire, Dalmatian, dogo Argentino, dogue de Bordeaux, English setter, German shepherd, Irish wolfhound, Labrador and Pomeranian; the remaining 2 dogs were cross-breed. Age, gender distribution and BW did not significantly differ between groups.

Serum concentration of creatinine, urea and albumin, as well as UPC ratios are reported in Table 2. In group A, 18 (62%) dogs were in IRIS stage 2, 9 (31%) were in IRIS stage 3, and 2 (7%) were in IRIS stage 4. In group B, 7 (41%) dogs were in IRIS stage 2, 5 (29%) were in IRIS stage 3, and 5 (29%) were in IRIS stage 4. Serum concentration of creatinine was significantly lower in dogs in group A ($P < 0.05$), whereas albumin and urea, and the UPC ratio did not significantly differ between groups. In group A, 20 (69%) dogs had low albumin concentration, 11 of which showed severe hypoalbuminemia; in group B, 11 (65%) dogs had low albumin concentration, 4 of which showed severe hypoalbuminemia. The frequency of dogs with hypoalbuminemia or severe hypoalbuminemia was not significantly different between groups A and B.

**Follow-up at weeks 4 to 8**

In group A at follow-up, BW increased in 16 (55%) dogs (range: 0.5 to 4 kg), remained unchanged in 11 (38%), and decreased in 2 (7%); by arbitrarily considering BW as stable if it increased or decreased by $\pm 2.5\%$, 14 (48%) of the 29 dogs had stable BW. The mean BW of dogs (32 $\pm$ 15 kg) significantly increased by 6.2% ($P < 0.01$) compared to baseline. Body weight was available for 10 out of 17 dogs of group B and was increased in 1 dog, equal in 5, and decreased in 4; BW was stable in 5 of the 10 dogs. The mean value did not differ from baseline (26 $\pm$ 12 kg; $P > 0.05$) (Figure 1).

In group A, serum albumin concentration increased in 19 (65%) dogs, was equal in 2 (7%), and decreased in 8 (28%); by arbitrarily considering albumin concentration as stable if it increased or decreased by $\pm 5\%$, 8 (27%) of the 29 dogs had stable albumin concentration. The mean albumin concentration (26 $\pm$ 7.0 g/L) significantly increased by 2.0 g/L ($P < 0.05$), compared to baseline. None of the 19 dogs with higher than baseline albumin had concentrations above the reference range. In group B, albumin concentration increased in 8 (47%) dogs, was equal in 2 (12%), and decreased in 7 (41%); albumin was stable in 4 (23%) of the 17 dogs. The mean value (24 $\pm$ 8.0 g/L) did not differ from baseline ($P > 0.05$) (Figure 2).

Serum concentration of creatinine in group A increased in 6 (21%) dogs and decreased in the remaining 23 (79%); by considering creatinine as stable if it increased or decreased by $\pm 20\%$, 10 (45%) of the 29 dogs had stable creatinine. In group B, creatinine concentration increased in 4 (23%) dogs, was equal in 2 (12%), and decreased in 11 (65%); creatinine was stable in 6 (35%) of the 17 dogs. In both groups, mean serum concentration of creatinine measured at 4 to 8 wk did not statistically differ from baseline (group A: 177 $\pm$ 177 $\mu$mol/L; group B: 301 $\pm$ 292 $\mu$mol/L; $P > 0.05$).

In group A, serum concentration of urea increased in 9 (31%) dogs, was equal in 1 (3%), and decreased in 19 (65%); by arbitrarily considering urea as stable if it increased or decreased by $\pm 20\%$, 13 (45%) of the 29 dogs had stable urea concentration. The mean urea concentration did not statistically differ from baseline (18.9 $\pm$ 27.1 mmol/L; $P > 0.05$). In group B, urea concentration increased in 5 (29%) dogs, was equal in 2 (12%) and decreased in 10 (59%); urea was stable in 6 (35%) of the
17 dogs. The mean urea concentration significantly decreased by 5.7 mmol/L (18.9 ± 12.5 mmol/L; \( P < 0.05 \)) (Figure 3).

In group A, the UPC ratio increased in 9 (31%) dogs and decreased in 20 (69%); by arbitrarily considering UPC ratio as stable if it increased or decreased by ≤ 20%, 7 (24%) of the 29 dogs had stable UPC ratio. The mean UPC ratio did not differ from baseline (3.9 ± 4.9; \( P > 0.05 \)). In group B, the UPC ratio increased in 2 (12%) dogs, was equal in 1 (6%), and decreased in 14 (82%) of the 17 dogs. The mean UPC ratio significantly decreased by 1.9 (2.4 ± 3.5; \( P < 0.01 \)) (Figure 4).

The time at which the examination was performed within the 4- to 8-week interval did not differ between groups (5.5 ± 1.0 wk, both groups).

Dogs with hypoalbuminemia

In group A, serum albumin concentration at 4 to 8 wk was increased compared to baseline in all 11 dogs with severe hypoalbuminemia (serum albumin < 20 g/L). The mean albumin concentration significantly increased by 7.0 g/L (\( P < 0.001 \)). None of these dogs had detectable subcutaneous edema or ascites, based on physical examination or abdominal ultrasonography, respectively. No significant differences were observed for BW, serum creatinine and urea concentrations, or the UPC ratio. At 4 to 8 wk, the 9 dogs with hypoalbuminemia between 20 and 27 g/L had no significant change compared to baseline in BW, serum albumin, creatinine and urea concentrations, or the UPC ratio.

In group B, the 4 dogs with severe hypoalbuminemia had albumin concentration that was decreased in 2 of them and was equal and increased in one of each at 4 to 8 wk compared to baseline. Due to the limited number of cases (4 dogs), statistical analyses were not performed for BW, serum albumin, creatinine and urea concentrations, or the UPC ratio. The 7 dogs with hypoalbuminemia (serum albumin between 20 and 27 g/L) had no significant change in BW, serum albumin, creatinine and urea concentrations, or the UPC ratio at 4 to 8 wk compared to baseline.

**Discussion**

A significant increase in serum albumin concentration compared to baseline was evident in proteinuric dogs with CKD showing severe hypoalbuminemia (serum albumin < 20 g/L) when receiving AA supplementation. Along with a beneficial effect on serum albumin level, supplementation with AA also increased dogs’ BW, albeit mildly. The effect on BW was evident in the whole group of proteinuric dogs with CKD but not in those with severe hypoalbuminemia, possibly due to the worse nitrogen balance of the latter cases. On the other hand, even though supplementation with AA increased BW and serum albumin concentration in proteinuric dogs with CKD, it prevented the decrease in proteinuria and lowering of urea.

Indeed, at follow-up, proteinuria and urea significantly decreased in dogs that did not receive AA while they did not differ in dogs supplemented with AA. It is therefore possible that in these dogs the reduced efficacy of enalapril and the commercial renal diet on either proteinuria or azotemia was a direct consequence of the positive nitrogen balance and increased protein synthesis induced by the AA supplementation. In fact, diets lower in protein compared to maintenance diets offer a chance to reduce the overall renal trafficking of protein, and if serum protein can be lowered then there is less risk of protein overload across the glomerular barrier, thus leading to less tubular protein reabsorption and inflammation (3). The amount of protein in the diet has a well-known effect on the magnitude of proteinuria, and dogs fed a diet lower in protein compared to maintenance.
diets have reduced proteinuria, which can in turn improve serum albumin concentration despite the reduction of albumin synthesis that can occur in dogs with CKD (2,3,8). Too strict restriction of protein intake can lead to loss of BW and decreased plasma albumin concentration; therefore, in proteinuric dogs with CKD the protein amount administered daily with food should be tailored to the degree of proteinuria. In these patients, dietary therapy should minimize proteinuria and control plasma albumin concentration while not compromising the nutritional status (8,24). The correct amount of protein might differ depending on the dog's stage of renal disease and extent of proteinuria (8). The commercial renal diets currently available for dogs have lower protein compared to maintenance diets, and it is possible they do not meet the minimum requirements in the case of severe proteinuria (thus leading to hypoalbuminemia and loss of BW). Meanwhile, the degree of proteinuria is strictly associated with survival and CKD progression in dogs (1,3,11). In light of these findings, it is the authors' opinion that supplementation with AA should be carried out with caution in proteinuric dogs with CKD, but it might be considered as an adjunctive therapy in severely hypoalbuminemic dogs in which the anti-proteinuric treatment has failed to control proteinuria and maintain plasma albumin concentration within normal limits.

This study has some limitations including its retrospective nature and consequent lack of blinding. It is therefore possible that some of the effects would have been different if cases were randomly allocated to receive or not receive the AA supplementation and the 2 groups were more homogeneous. Indeed, serum concentration of creatinine at baseline was significantly higher in dogs that did not receive the AA supplementation. Then, it cannot be excluded that administering AA supplementation to dogs with higher creatinine concentration is associated with detrimental effects on renal function. In addition, follow-up time of all dogs included in the study was short. A longer follow-up period might have allowed detection of additional differences between the groups.

Additionally, even though owners were instructed to feed their dogs with just 1 of the 2 renal diets available, sometimes they switched to the other. However, the effect of this potential bias was probably minor because both commercial renal diets were expected to be randomly provided to dogs. Furthermore, studies comparing the effect of different diets in dogs with CKD have not been published, but it is likely that the 2 commercial renal diets used for the present study provided similar beneficial effects. The IRIS simply suggests the use of a renal diet, without offering specific guidance on a particular brand on the market (14).

Another limitation is represented by the fact that from medical records it was possible to retrieve BW but not the body or the muscle condition score of the dogs; the latter might have provided more information regarding the potential beneficial effect of AA supplementation. Furthermore, the increase of BW in dogs receiving supplementation of AA at follow-up might have been biased by the concurrent presence of subcutaneous edema or abdominal effusion; however, none of the dogs with severe hypoalbuminemia in the present group developed any of the above. With regard to the same group of dogs, it is worth noting that at follow-up BW increased on average by only 6.2%, thus the beneficial effect of AA supplementation would be questionable. However, by considering the 16 dogs that had an increase of BW, the increase was from 0.5 to 4 kg, possibly suggesting a more relevant gain.

The re-evaluation at 4 to 8 wk may be considered a rather large interval, which might have affected the results. Although this hypothesis is conceivable, the potential bias was evenly distributed in the 2 groups, likely limiting the source of error.

Another factor that might have affected the study results is that the AA provided with the supplementation were predominantly essential AA. Even though it has been demonstrated that humans with CKD have a decrease in circulating essential AA relative to non-essential AA (25,26), there are no data available on the AA blood profile of dogs with renal disease, particularly in those affected by spontaneous proteinuric CKD. Determining the AA profile of these dogs might prove useful in identifying the specific AAs that are needed to correct their imbalance. Finally, even though BW and serum albumin concentrations have been historically considered as insensitive and late indicators of malnutrition, in a previous study these values were considered clinically useful in assessing the adequacy of the nutritional status in dogs with renal proteinuria (8). Our results support the notion that serum albumin concentration represents a helpful indicator to plan dietary modification in proteinuric dogs affected by spontaneous CKD.

In conclusion, proteinuric dogs with CKD treated with enalapril and fed commercial renal diets that received supplementation with AA had improved BW and serum albumin concentration, while maintaining stable serum creatinine. However, administration of AA appeared to prevent the reduction of proteinuria and lowering of urea. In light of these findings, the authors propose the use of AA supplementation in proteinuric dogs with severe hypoalbuminemia that are not adequately controlled with standard treatments consisting of renal diets and ACEI. Relying on serum albumin was useful to identify the benefits of dietary changes in proteinuric dogs with CKD. Further clinical trials are expected to be valuable in order to evaluate the impact of different AA formulations on BW, hypoalbuminemia, and survival time of dogs affected by CKD and severe proteinuria.

Acknowledgment

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A retrospective study of owner-requested testing as surveillance for equine infectious anemia in Canada (2009–2012)

Sara N. Higgins, Krista J. Howden, Carolyn R. James, Tasha Epp, Katharina L. Lohmann

Abstract — This retrospective study was undertaken to estimate i) the surveillance coverage for equine infectious anemia (EIA) based on owner-requested testing, and ii) the incidence of case detection from this surveillance activity to inform a review of Canada’s national disease control strategy. Based on sample submissions by accredited veterinarians to laboratories CFIA-approved for EIA testing between 2009 and 2012, the estimated national surveillance coverage was 14% for all years, and 72 cases of EIA were detected. The annual national incidence of EIA detection ranged from 0.03 to 0.08 cases/1000 horses. On average, a greater proportion of the horse population was tested in eastern Canada (32%) than in western Canada (6%, \( P < 0.0001 \)). The cumulative incidence of EIA detection was higher in western Canada (0.25 cases/1000 horses) than in eastern Canada (0.02 cases/1000 horses, \( P < 0.0001 \)). This study identified regional differences in owner-requested EIA testing and case detection resulting from this testing activity.

Résumé — Étude rétrospective des tests demandés par les propriétaires comme surveillance pour l’anémie infectieuse équine au Canada (2009–2012). Cette étude rétrospective a été entreprise afin d’estimer i) la couverture de surveillance pour l’anémie infectieuse équine (AIE) basée sur les tests demandés par les propriétaires et ii) l’incidence de détection des cas à partir de cette activité de surveillance pour documenter un examen de la stratégie nationale de contrôle des maladies du Canada. L’estimation de la couverture nationale de surveillance, basée sur les soumissions d’échantillons par les vétérinaires autorisés aux laboratoires approuvés par l’ACIA pour l’AIE entre 2009 et 2012, était de 14 % pour toutes les années et 72 cas d’AIE ont été détectés. L’incidence nationale annuelle de la détection de l’AIE variait de 0,03 à 0,08 cas/1000 chevaux. En moyenne, une proportion supérieure de la population équine de l’Est du Canada (32 %) subissait des tests par rapport à l’Ouest canadien (6 %, \( P < 0,0001 \)). L’incidence cumulative de la détection de l’AIE était supérieure dans l’Ouest canadien (0,25 cas/1000 chevaux) par rapport à l’Est du Canada (0,02 cas/1000 chevaux, \( P < 0,0001 \)). Cette étude a identifié des différences régionales pour les tests de l’AIE demandés par les propriétaires et la détection des cas découlant de cette activité d’épreuve diagnostique.

(Traduit par Isabelle Vallières)

Introduction

Equine infectious anemia (EIA) is a persistent and incurable viral disease to which all equines (i.e., horses, donkeys, mules, and zebras) are susceptible (1,2). Equine infectious anemia virus (EIAV) is a lentivirus of the family retroviridae, which are enveloped RNA viruses (1). The natural mode of transmission of EIAV is the mechanical transfer of infectious blood on mouthparts of biting insects, most commonly tabanids (horse flies, deer flies, and stable flies), during interrupted feeding on horses (here and subsequently used to refer to all equines) (1,2). Iatrogenic (e.g., blood-contaminated needles and equipment) and \textit{in utero} transmission have also been reported (1,3). Clinical disease is associated with high levels of viremia (4) and has been characterized by acute or chronic recurring fever, lethargy, thrombocytopenia, anemia, edema, weight loss, or sudden death (2). However, no combination of clinical signs is pathognomonic for EIA and many infected horses exhibit mild or inapparent disease. Subsequent to primary infection...
with EIAV, surviving horses are considered “carriers” with the potential to remain infectious (1,2,5). The subclinical EIAV carrier poses significant challenges for disease control, and spread of EIA among herds occurs subsequent to movement of horses and the introduction of a persistently infected horse into a disease-free population. This issue highlights the importance of surveillance to identify subclinically infected horses that serve as the only known reservoir of the virus.

Equine infectious anemia became a reportable disease in Canada in 1971 when a serological test to detect infected animals became commercially available (6,7). The national disease control program for EIA was subsequently introduced by Agriculture and Agri-Food Canada in 1972, and has been modified several times since then. The most notable change occurred in 1994 when the federal government significantly reduced its role in the EIA program as a result of a larger government deficit reduction initiative. In 1997, the Canadian Food Inspection Agency (CFIA) was established as the authority responsible for delivery of the national EIA disease control program under legislation governed by the Health of Animals Act and Regulations (8). In 1998, the newly formed CFIA responded to an industry request to restate its involvement in the control of EIA and a new program was created. As EIA is listed by the World Organisation for Animal Health (OIE), Canada follows the standards for the safe international trade and movement of equines as identified in the OIE Terrestrial Animal Health Code (9). The current goal of CFIA’s EIA disease control program is to reduce the frequency of infection with EIAV in the domestic, owned horse population in Canada. This is in contrast to a disease eradication program in which the objective is to eliminate the disease or the organism causing the disease. The current program combines testing initiatives with disease intervention strategies, including the requirement for the humane euthanasia or permanent quarantine (with strict vector control) of all confirmed cases of EIA to prevent further disease transmission. Additional disease control interventions implemented by the CFIA in response to a case of EIA being detected may include movement restrictions (e.g., temporary or permanent quarantine) and an epidemiological and diagnostic investigation to identify and test horses which may have been exposed to EIAV in the 30 d prior to sampling of the index case. Horses maintained under quarantine must be separated from uninfected horses by at least 200 m to prevent mechanical transmission of EIAV by tabanids (10). Owners of infected horses which are ordered destroyed by the CFIA and are humanely euthanized are eligible to receive compensation up to a maximum of $2000, as specified in the Compensation for Destroyed Animals Regulations (11). At present, no licensed vaccine or approved treatment for EIA is available in Canada.

Testing for EIA in pleasure, breeding, and performance horses in Canada is voluntary and driven primarily by owner-initiated requests for EIA testing to their private veterinarian. Only licensed veterinarians who are accredited under the CFIA’s Accredited Veterinary Program can submit samples for EIA testing in Canada. Samples may be submitted for testing to meet industry requirements for entry at commingling events (e.g., shows, sales, races), as part of biosecurity protocols at boarding stables, or to meet conditions of export to the US or Mexico. Testing for EIA as a result of the Accredited Veterinary Program is a form of enhanced passive surveillance which is defined as “observer-initiated provision of animal-health data with active investigator involvement” (12). The CFIA collects $2 for each sample submitted by an accredited veterinarian, and this partial cost recovery program supports the intervention strategies applied by the CFIA when a case of EIA is detected, meeting the criteria to consider it an “enhanced” program. Additional surveillance data used to assess Canada’s EIA status are collected during testing of samples collected by CFIA veterinarians and inspectors designated under the Health of Animals Act during disease investigations and for the purpose of export to countries other than the US and Mexico. Samples collected for these purposes are submitted directly to the CFIA National Reference Laboratory in Saint-Hyacinthe, Quebec. Information generated from the description and analysis of all available surveillance data for EIA in Canada serves to inform program evaluation and explore potential risk factors associated with observed cases.

A review of Canada’s current EIA disease control program, including stakeholder consultations on future options for EIA surveillance and disease policy, was initiated by the CFIA in 2014. To inform this program review, it was necessary to improve knowledge and increase understanding of the EIA situation in Canada. To address this need, a collaborative research study was undertaken between the CFIA and the Western College of Veterinary Medicine. The objectives of this study were to estimate i) the surveillance coverage for EIA in Canada based on owner-requested testing, and ii) the incidence of case detection from this surveillance activity.

Materials and methods

The time period for this study was January 1, 2009 to December 31, 2012. The target population included all domestic owned horses in Canada for each year of the study period. The sample population was all horses from which a sample was submitted for EIA testing by a private veterinarian accredited by the CFIA for this function.

Private or provincial laboratories in Canada must be approved by the CFIA to perform EIA testing, and use a competitive enzyme-linked immunosorbent assay (IDEXX EIA cELISA kit;
Table 1. Owner requested surveillance testing for equine infectious anemia (EIA) from 2009 to 2012. The annual surveillance coverage estimated in each province, in each region (east, west), and in Canada was calculated as the number of tested samples divided by the estimated number of horses, assuming horses were only tested once per year. Only test submissions by accredited veterinarians were considered. The average surveillance coverage differed significantly between eastern and western Canada ($P < 0.0001$). The number of horses is based on data from the Statistics Canada 2011 Census of Agriculture (15) and the Yukon Bureau of Statistics 2003 Survey of Yukon's Horse Owners and Horse Boarding Operators (16).

<table>
<thead>
<tr>
<th>Province</th>
<th>Estimated number of horses (% of the Canadian population)</th>
<th>Number of samples tested (% annual surveillance coverage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td>Yukon territory</td>
<td>1748 (0.4)</td>
<td>269 (15.4)</td>
</tr>
<tr>
<td>British Columbia</td>
<td>45 791 (11.6)</td>
<td>3481 (7.6)</td>
</tr>
<tr>
<td>Alberta</td>
<td>139 410 (35.4)</td>
<td>9940 (7.1)</td>
</tr>
<tr>
<td>Saskatchewan</td>
<td>54 093 (13.7)</td>
<td>1232 (2.3)</td>
</tr>
<tr>
<td>Manitoba</td>
<td>33 752 (8.6)</td>
<td>1891 (5.6)</td>
</tr>
<tr>
<td>Ontario</td>
<td>86 642 (22)</td>
<td>24 868 (28.7)</td>
</tr>
<tr>
<td>Quebec</td>
<td>25 190 (6.4)</td>
<td>11 189 (44.4)</td>
</tr>
<tr>
<td>Atlantic$^a$</td>
<td>7462 (1.9)</td>
<td>1957 (26.2)</td>
</tr>
<tr>
<td>East$^b$</td>
<td>119 294 (30.3)</td>
<td>38 014 (31.9)</td>
</tr>
<tr>
<td>West$^c$</td>
<td>274 794 (69.7)</td>
<td>16 813 (6.1)</td>
</tr>
<tr>
<td>Canada</td>
<td>394 088</td>
<td>54 827 (13.9)</td>
</tr>
</tbody>
</table>

$^a$ Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland and Labrador.<br>$^b$ Ontario, Quebec, Atlantic provinces.<br>$^c$ Yukon territory, British Columbia, Alberta, Saskatchewan, Manitoba.

IDEXX Laboratories, Westbrook, Maine, USA), with a relative sensitivity and specificity compared to the reference agar gel immunodiffusion (AGID) test of 99.7% (97.8% to 100%) and 99.7% (98% to 100%), respectively (13). For this study, sample level data were provided to the CFIA by all approved laboratories ($n = 14$) and included the total number of samples tested for EIA with results authorized by month and year, and the location (by province) of the veterinary practice associated with the accredited veterinarian submitting the sample. Based on the data provided by the approved laboratories, it was not possible to identify repeat testing on individual horses that tested negative, during a calendar year or over the course of the entire study period. Thus, for the analysis, it was assumed that horses were not tested repeatedly within a given year; however, no such assumption was made for between years.

Samples reported as non-negative on the cELISA were forwarded to the CFIA National Reference Laboratory and confirmatory testing undertaken in series using the cELISA followed by the AGID test, i.e., “Coggins” test. The AGID test is the diagnostic gold standard for EIA and is the official test required by the OIE (14,15). In order to meet the case definition of a confirmed case as per the CFIA’s EIA Disease Control Manual of Procedures, both tests must be reported as positive. The objective of this diagnostic testing approach is to maximize sensitivity during serological screening (i.e., minimize the probability of false negative test results) while maximizing specificity during confirmatory testing (i.e., minimize the probability of false positive test results). For samples reported as non-negative at an approved laboratory and forwarded to the National Reference Laboratory for confirmatory testing, sample and animal level data were queried from CFIA’s Laboratory Sample Tracking System (LSTS) or collected manually from the case files and provided by the CFIA. Data included the reason for testing as indicated on the EIA sample submission form (race, show, or sale, export to the US or Mexico, owner request when exposure is suspected, other), the sampling location as indicated by the owner’s postal code, and descriptive data on each horse. For horses that tested EIA positive, therefore, animal level data allowed for the identification of any repeated positive tests of the same horse, thus ensuring that each case of EIA reported during the study period represented a unique horse.

National and provincial level horse demographic data were queried from Statistics Canada using the most recent national census data (16). Territorial data were not available from Statistics Canada; therefore, the estimated equine population for the Yukon was sourced from the Yukon Bureau of Statistics 2003 Survey of Yukon’s Horse Owners and Horse Boarding Operators (17). No equivalent source of information was available for the Northwest Territories or Nunavut and these were not included in the study. Provincial/territorial data were aggregated by region and categorized as either “east” or “west” according to the province of origin of the submitting accredited veterinarian. “East” included Ontario (ON), Quebec (QC), and the Atlantic provinces (Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland and Labrador) and “west” included the Yukon (YT), British Columbia (BC), Alberta (AB), Saskatchewan (SK) and Manitoba (MB). Using commercial geographical information system software (ArcGIS version 10.2.1; Environmental Systems Research Institute, Redlands, California, USA), cases of EIA were mapped to the centroid of the postal code region identified by 6- or, in a few instances, 3-digit postal codes.

Surveillance coverage (%) was calculated for each year of the study by dividing the total number of samples submitted and tested for EIA at an approved laboratory in Canada by the estimated horse population at the national, regional, and provincial/territorial level. The annual incidence of case detection was calculated by dividing the total number of new EIA cases detected...
Results

During the study period, 220 078 blood samples from horses in Canada were submitted by accredited veterinarians to a CFIA-approved private or provincial laboratory for EIA testing. The number of sample submissions by month is presented in Figure 1. Approximately half of all samples (50.2%) were submitted in the spring and early summer months (March through June). The month with the lowest total proportion of samples submitted was December (4.1%). The national surveillance coverage for EIA in Canada was 14% for each year of the study (Table 1). At the subnational level, surveillance coverage ranged from 2% in Saskatchewan (2011) to 51.4% in Quebec (2011). The annual surveillance coverage remained relatively consistent for each province/region over the study period but varied markedly among provinces/regions. The average regional surveillance coverage in eastern Canada (32%) was significantly greater than in western Canada (6%, \(P < 0.001\); Table 1).

During the 4-year study period, 72 cases of EIA were detected in Canada as a result of owner-requested testing (Table 2). The annual national incidence of case detection ranged from 0.04 cases/1000 horses in 2010 to 0.08 cases/1000 horses in 2012, with a median of 0.04 cases/1000 horses over the study period. The reasons for testing identified at the time of initial sample collection were owner request where exposure to EIA was suspected \((n = 47)\), race \((n = 5)\), export to the US or Mexico \((n = 8)\), and “other” \((n = 12)\). Cases originated from 4 provinces and 1 territory, and most (97%) were identified in the western region (Table 2; Figure 2). The annual regional incidence of case detection was consistently higher in western Canada \((0.04 to 0.11 cases/1000 horses)\) than in the east \((0 to 0.02 cases/1000 horses)\), and the cumulative incidence over the study period differed significantly between the regions \((P < 0.0001; \text{Table 2})\).

On a provincial/territorial level, the highest incidence of case detection was in the Yukon, while no cases were identified in Manitoba, Ontario, or the Atlantic provinces during the study period (Table 2).

Discussion

Canada’s current EIA control program is a partnership between the CFIA and the equine industry. The equine industry encourages testing in the domestic population, while the CFIA manages the response to confirmed cases of EIA. Data generated from owner-requested testing of blood samples submitted by private veterinarians under the Accredited Veterinarian Program provide surveillance information which is essential when evaluating the overall effectiveness of the national EIA control plan. In 2014, the CFIA began engaging stakeholders with the objective of collaborating with industry and provincial/territorial animal health regulators to re-design the national EIA control program. This study serves to inform decision-making during the program review by identifying an objective measure for the adequacy of owner-requested testing to detect EIA cases and provide surveillance coverage. The impact of surveillance coverage on incidence of case detection is explored in time and space. The coverage of owner-requested surveillance for EIA at the national level remained consistent throughout the study period with an estimated 14% of horses being tested for EIA annually. Average surveillance coverage for the 4 y of study was significantly higher in eastern Canada than in the west. This regional variation could reflect differences in industry-driven EIA test requirements for equine events. It is also possible that historical EIA outbreaks...
in the eastern region served to increase awareness of EIA with subsequent intensified disease surveillance efforts that are still noticeable. Whatever the reason for this east/west discrepancy, it indicates that owner-requested testing is not uniform and non-random.

Increased requirements for EIA testing at equine events and commingling sites should serve to increase surveillance coverage and improve case detection if EIA is endemic in a population. As carrier animals are detected and removed in a population, the prevalence at the population level and the incidence proportion are expected to decrease, provided that the rate of new cases does not exceed the rate of case detection. However, the impact of increasing surveillance coverage is dependent on the risk of EIA in the tested horses. If a large proportion of the tested population is horses of lower risk (e.g., previously tested negative), a relative increase in surveillance coverage may not correlate with an increased case detection nor decrease in disease prevalence over time, so values must be interpreted with caution. Correspondingly, the higher surveillance coverage for EIA observed in eastern Canada may reflect an increase in efficiency of surveillance to detect cases of EIA, but this cannot be assumed as not all tested horses are likely to have an equal risk of being infected. It has been suggested that most horses tested for EIA represent a low-risk population, and that an increased focus on previously untested populations will be necessary to improve the efficiency and cost effectiveness of EIA control programs (18).

Given the current lack of mandatory unique horse identification for equines in Canada and inconsistent collection of descriptive animal level data by laboratories approved by the CFIA to test for EIA, it was not possible to identify duplicate testing on most horses either annually or for the entire study period. Thus, for descriptive reporting and analysis of EIA surveillance coverage, we assumed independence between samples for each year of the study (i.e., no repeat sampling of horses within a calendar year). As at least some horses are likely tested more than once per year, our figures may overestimate testing coverage within the horse population. Frequency of testing is expected to depend on the purpose for testing; for example, export to the US or Mexico from Canada requires that a negative EIA test result is obtained no more than 180 days before the date of travel, while on-farm biosecurity programs and entry requirements for horse events are not uniform.

Another factor affecting our estimate is that the estimated surveillance coverage reported for the provincial/territorial, regional, and national levels was based on demographic data

Figure 2. Cases of equine infectious anemia in Canada (2009–2012).
reported for the 2011 Census of Agriculture (16). These data only capture horses on census farms, and in 2010, a Canadian Horse Industry Profile Study commissioned by Equine Canada (19) reported an estimated 963 500 horses in Canada, i.e., more than double those reported from the census. Correspondingly, if surveillance coverage was estimated based on this alternative source of demographic data, the value would be lower (5.7% of horses tested for EIA per year on average).

During the study period, EIA cases originated from 4 provinces and 1 territory (YT, BC, AB, SK, QC), and most EIA cases (70/72) were identified in the western region. It should be emphasized that EIA cases reported here represent only those detected through owner-requested testing, i.e., they do not include additional cases that were detected as part of outbreak investigations that occurred in response to the detection of these index cases. In addition, although we could ensure that all reported EIA cases represented individual horses, we did not have information about any testing activity before detection of their positive EIA status. Therefore, we could not determine whether detected cases represented new infections or, possibly, chronic virus carriers that had never previously been tested. Our data, therefore, estimate the incidence of case detection through owner-requested testing but not the true incidence of infection with EIAV in Canadian horses.

The annual incidence of case detection was consistently higher in western Canada than in the east, and the cumulative incidence differed significantly between the regions. As we do not know whether owner-requested testing affected the same risk groups in the east and west during the time frame of the study, and because of the difference in surveillance coverage, we cannot be certain there truly is a regional difference in incidence of EIA. With this caution, however, potential reasons for an increased incidence of EIA in western Canada include increased probability of disease transmission associated with environmental, spatial, or human-related factors (e.g., weather, vector abundance, and vectorial capacity, potential for exposure to feral or other untested populations, use of community pastures) (20,21).

Interestingly, the number and locations of EIA cases were inversely related to surveillance coverage at the regional level. While this study does not provide evidence of a causal relationship between surveillance coverage and disease incidence, it appears plausible that increased surveillance in a population may lead to a decreased incidence over time. When the intensity of surveillance is increased initially, there is often a corresponding increase in the number of cases detected simply as a result of increased population level sensitivity, although this effect may be less pronounced in situations in which the same animal is repeatedly tested on an annual basis. As indicated, increased surveillance in response to higher numbers of EIA cases in eastern Canada may have reduced the incidence of disease in this region over time. In this context, it is noteworthy that a 6-year retrospective study between 1976 and 1981 reported a higher cumulative incidence of EIA in eastern Canada (25.88 cases/1000 horses) compared with the west (8.78 cases/1000 horses) (22). While it is tempting to speculate that EIA incidence in western Canada could be reduced through intensified surveillance efforts, or surveillance directed at particular groups of horses, we cannot conclude this from our data given the discussed limitations. As we did not assess regional climatic conditions as part of the study, their potential effect on vector populations and differences in EIA incidence between regions or years is also unknown. The variable incidence of case detection in the Yukon despite relatively consistent and, comparatively, high surveillance coverage over the years of the study may serve as an example of our study’s limitations. While it is possible that the overall incidence of EIA truly differed in the Yukon horse population over time, it is also possible that owner-requested surveillance affected different groups within the entire population in each year of the study, and that disease incidence differs between these groups. Further investigation and description of the populations undergoing owner-requested testing in Canada are therefore warranted to assess its value as a surveillance tool.

This study was limited by the fact that data extraction from EIA submission forms was not consistent among private laboratories approved for EIA testing, and that data were entered into each laboratory’s data management system rather than one central, searchable database. The reasons for testing were available only for EIA cases and, therefore, not for most of the testing results used in this study. We could not evaluate the contribution of different activities, such as showing, sale, or export, to regional or seasonal differences in testing activity. The seasonal pattern of test submissions likely reflected increased activity within the equine industry during the summer season, and may suggest that tested horses were largely those used for performance; however, further work is necessary. Ideally, an active population-based surveillance system for EIA would be designed and implemented. Owner-requested testing could be a component of this system. A mandatory national unique identification for horses and consistent collection and maintenance of sample and animal level data by laboratories testing for EIA would facilitate future research on EIA surveillance as well as improve traceability during outbreaks affecting the equine industry.

The objective of the national EIA disease control program, to reduce the frequency of infection with EIAV in the domestic owned horse population, appears to have been met based on the declining number of cases detected in Canada since the inception of the program. However, it is unclear whether the current combination of voluntary industry-driven testing and CFIA-managed response when cases are detected is the most effective and efficient national strategy for this disease. The use of diagnostic testing data based on voluntary testing to estimate incidence is not ideal and cannot be considered an accurate measure of disease frequency in the Canadian horse population. The number of new cases detected is likely informative of incidence risk sometime in the past rather than current incidence. Therefore, in the current form, this method cannot be expected to reliably detect recent changes in incidence, which would be very important in monitoring a national epidemic. Complete surveillance coverage for EIA in Canada is necessary to identify animal or spatial-level risk factors which may be used to target sampling and improve the efficiency and cost-effectiveness of a future surveillance program.

In conclusion, the average annual surveillance coverage based on owner-requested testing for EIA in Canada was 14% of the
population nationwide and higher in eastern Canada, while significantly more index cases were detected in western Canada during the time of the study. Findings from this study will serve to inform ongoing national EIA policy reviews and add to the discussion regarding the implementation of additional testing requirements for horses in areas where surveillance coverage is lower, and incidence of disease detection is higher. However, to fully assess the national EIA disease control program, joint considerations of surveillance and intervention will also be necessary, including assessment of the economic values of surveillance.

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The authors thank Maria Funk, Hayley Kosolofski, and Thuy Nguyen for their assistance with data cleaning, and Dr. Julie Paré for reviewing the manuscript. Laboratories providing data associated with the accredited veterinarian program including the Atlantic Veterinary College, Biovet, Palliser/ Biocheck Labs, Idexx Markham, Idexx Delta, Animal Health Centre, Faculty of Medicine, Animal Health Lab, University of Guelph, Prairie Diagnostic Services, True North Veterinary Diagnostics, Veterinary Diagnostic laboratory, MAPAQ, International Bio Institute, and Meyer Service and Supply Ltd.

References

Effects of probiotic VSL#3 on glomerular filtration rate in dogs affected by chronic kidney disease: A pilot study

Ilaria Lippi, Francesca Perondi, Gianila Ceccherini, Veronica Marchetti, Grazia Guidi

Abstract — The aim of this study was to evaluate the effects of probiotic VSL#3 on glomerular filtration rate (GFR) in dogs affected by chronic kidney disease (CKD). The treatment group (n = 30) received prescription renal diet and probiotic VSL#3 (112 to 225 × 10^9 lyophilized bacteria per 10 kg body weight, PO, q24h for 2 months); the control group (n = 30) received prescription renal diet and standard therapy. All dogs underwent GFR measurement at the beginning of the study (T0) and were re-evaluated by GFR measurement after 2 months (T1). The GFR was significantly higher (P = 0.0001) in the treatment group compared to the control group at T1. In the treatment group, the GFR was significantly higher (P = 0.0008) at T1 compared to T0. In the control group, the GFR was significantly lower (P = 0.001) at T1 compared to T0. VSL#3 supplementation seemed to be efficient in reducing deterioration of GFR over time in dogs affected by CKD.

Introduction

Uremic retention solutes are generated along the gastrointestinal tract and mostly cleared by the kidneys. Their accumulation in serum is negatively correlated with the level of renal function and glomerular sclerosis (1,2). According to recent studies in human medicine (3), the gastrointestinal tract seems to be involved in the pathophysiology of uremic syndrome and contributes to its clinical signs. The ability of probiotics to modulate the intestinal microbiota and to reduce the progression of chronic kidney disease (CKD) has been investigated in *in vitro* and *in vivo* studies in animals and humans (4).

VSL#3 is a high-dose, multi-strain probiotic product containing viable lyophilized bacteria consisting of 4 strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp. *bulgaricus*), 3 strains of *Bifidobacterium* (*B. longum*, *B. breve*, and *B. infantis*), and 1 strain of *Streptococcus salivarius* subsp. *thermophilus*. The VSL#3 strains have shown efficacy in humans for the prevention, treatment, and maintenance of remission of pouchitis and ulcerative colitis (5); it also seems to accelerate healing of gastric ulcers (6) and reduce portal pressure in patients with cirrhosis (7). Recently, VSL#3 has also been used in dogs with idiopathic inflammatory bowel disease (IBD) with promising results (5).

The aim of this study was to investigate the effects of the administration of VSL#3 on GFR in dogs affected by spontaneous CKD.
Materials and methods

Sixty client-owned dogs affected by CKD were recruited for this study. Sample size was calculated based on a power analysis with an alpha of 0.05 and power of 0.80. There were no restrictions on breed or gender of the dogs. Dogs in International Renal Interest Society (IRIS) stages 2 and 3 were persistently azotemic, had ultrasound findings consistent with CKD (decreased cortico-medullary distinction) and glomerular filtration rate (GFR) < 60 mL/min/m²; dogs in IRIS stage 1 had ultrasound findings consistent with CKD (decreased cortico-medullary distinction) and GFR < 60 mL/min/m² (8). All patients were classified according to the plasma concentration of creatinine based on IRIS guidelines. Stage 1 (IRIS) included non-azotemic dogs (creatinine < 123.8 μmol/L), with ultrasound findings consistent with CKD, inadequate urinary concentrating ability (USG < 1.030), and GFR < 60 mL/min/m². Patients were considered proteinuric if they were found repeatedly with a urine protein: creatinine (UPC) ratio ≥ 0.5 in 3 or more specimens, obtained ≥ 2 wk apart. IRIS 1 dogs, which met the inclusion criteria for CKD IRIS stage 1, but with a USG > 1.030, were considered eligible for the study if they had protein-losing nephropathy. For ethical reasons, animals were excluded from the study if they were in IRIS stage 4 (creatinine > 442 μmol/L). Dogs with evidence of acute kidney injury (AKI) or other significant systemic or organ-related disease, such as, neoplastic, cardiovascular, liver, or gastrointestinal disease, assessed by clinical and ultrasound evaluation and serum biochemistry were not included in the study. Dogs with CKD with evidence of positive urine culture were excluded from the study. After the full workup 24 dogs in IRIS stage 1, 16 dogs in IRIS stage 2, and 20 dogs in IRIS stage 3 were considered eligible for the study.

Dogs with persistent proteinuria (n = 32) were treated with benazepril (Fortekor; Novartis Animal Health, Varese, Italy), 0.25 to 0.5 mg/kg body weight (BW), PO, once to twice daily. Dogs with vomiting and/or poor appetite (n = 9) were treated with maropitant (Cerenia; Pfizer Italia, Latina, Italy), 1 mg/kg BW, PO, once daily and ranitidine (Zantadine; CEVA Salute Animale, Agrate Brianza, Italy), 2 mg/kg BW, PO, twice daily. Dogs showing hypo-proliferative anemia (n = 4) with hematocrit (HCT) < 20% were treated with darbepoetin-alpha (Aranesp; AMGEN, Milano, Italy), 0.5 to 1 μg/kg BW, SC, once weekly. Dogs (n = 20) with a history of hypertension [blood pressure (BP) > 160 mmHg] were maintained on a combination of benazepril and amlodipine (Amodip; CEVA Salute Animale), 0.25 to 0.5 mg/kg BW, PO, once daily. Dogs with serum phosphate > 1.6 mmol/L (n = 10) were treated with aluminium hydroxide, 50 to 100 mg/kg BW, PO, daily. Dogs in IRIS stages 2 and 3 with clinical signs (vomiting, poor appetite) and/or proteinuria, anemia, hypertension, and hyperphosphatemia were started on appropriate treatment weeks to months prior to T0. These drugs were continued during the study period.

On the day of enrolment (T0) 12 of the 24 dogs with IRIS stage 1, 8 of the 16 dogs with IRIS stage 2, and 10 of 20 the dogs with IRIS stage 3 were randomized into 2 groups (control group and treatment group) using a computer-generated randomization list. The control group (CG) consisted of 30 dogs (IRIS stage 1, n = 12; IRIS stage 2, n = 8; IRIS stage 3, n = 10). The treatment group (TG) consisted of 30 dogs (IRIS stage 1, n = 12; IRIS stage 2, n = 8; IRIS stage 3, n = 10). Dogs in the TG received VSL#3 at the dose of 112 to 225 × 10⁶ lyophilized bacteria per 10 kg BW, PO, q24h for 60 d (5), in addition to the ongoing therapy. After randomization, patients of both groups were submitted to GFR evaluation through the plasma clearance of iohexol (8), evaluation of serum creatinine, urea, phosphate, complete urinalysis and UPC, urine culture, and blood pressure monitoring (PetMAP-Ramsey Medical, Tampa, Florida, USA). For blood pressure a mean of 5 consecutive measurements was considered. Hydration status of patients was assessed before sampling of blood and evaluation of GFR, in order to be sure they were not dehydrated. None of the dogs was dehydrated at the time sampling of blood and determination of GFR. Data were recorded as T0. For both groups, GFR, serum creatinine, urea, phosphate, complete urinalysis and UPC, blood pressure, and urine culture were reassessed at T1. This study was conducted in a single-blinded manner. To keep the investigator blinded to the study, a dispenser was used to supply VSL#3, according to a predetermined randomization code. Each owner was instructed not to mention VSL#3 at the time of the recheck. The study was approved by the University of Pisa animal care committee.

Statistical analysis was conducted with commercial software (GraphPad Prism-Software; La Jolla, California, USA). Data were tested for normality with D’Agostino and Pearson test. Data were non-normally distributed and were presented as median (range). Differences among groups were assessed using a Wilcoxon signed-rank test. A level of P ≤ 0.05 was considered significant for all tests.

Results

At baseline (T0) among the 30 dogs in the CG, 14 were proteinuric (UPC > 0.5) and 4 were borderline proteinuric (UPC > 0.2 and < 0.5); 1 dog was severely hypertensive (BP > 180 mmHg) and 3 dogs were moderately hypertensive (BP > 160 mmHg and < 180 mmHg). Among the 30 dogs in the TG, 18 were proteinuric (UPC > 0.5) and 3 were borderline proteinuric (UPC > 0.2 and < 0.5); 4 dogs were moderately hypertensive (BP > 160 mmHg and < 180 mmHg). At T0 there were no significant differences in age, weight, GFR, serum creatinine, urea, phosphate, blood pressure, UPC, and USG between dogs of the CG and TG (Table 1). A combination of benazepril and amlodipine was used to control blood pressure in 11/30 dogs in the CG and in 9/30 dogs in the TG. In dogs of the CG, GFR was lower (P = 0.0002), and creatinine and USG were higher (P = 0.001 and P = 0.04 respectively) at T1 compared with T0. There was no significant difference in urea, phosphate, blood pressure, and UPC between T0 and T1. At T1, 24/30 dogs of the CG were proteinuric and 2/30 were borderline proteinuric; while 15/30 dogs of the TG were proteinuric and 3/30 were borderline proteinuric. There was no significant difference in the number of proteinuric and non-proteinuric dogs between the CG and the TG (P = 0.18). When only proteinuric dogs (n = 14) of the CG were considered, no significant difference in UPC was found between T0 and T1.
In dogs of the TG, GFR, and USG were higher ($P = 0.001$ and $P = 0.0001$, respectively), and UPC was lower ($P = 0.006$) at T1 compared with T0. When only proteinuric dogs ($n = 18$) of the TG were considered, a significant reduction ($P = 0.006$) in UPC was found at T1. At T1, 4/30 dogs of the CG were moderately hypertensive; while 2/30 dogs of the TG were moderately hypertensive and 1 dog was severely hypertensive. No significant difference was observed in creatinine, urea, phosphate, and blood pressure between T0 and T1. Values of GFR, creatinine, urea, phosphate, blood pressure, UPC, and USG for the CG and the TG at T0 and T1 are reported in Table 2.

### Discussion

In this study we determined that GFR, measured through the plasma clearance of iohexol, is increased in dogs treated with VSL#3, compared with dogs treated with standard therapy (Figure 1). Our findings are in agreement with previous results (9), in which the group of patients on probiotic plus prebiotic supplementation showed a significantly reduced decline of GFR over time, compared with the group of patients on a protein-restricted diet only (9). In our study, patients on prescription renal diet only showed a significant reduction in GFR over time. This finding may be due to an incomplete ability of prescription renal diet to block the production of uremic retention solutes. Koppé et al (4) postulated that the production of uremic retention solutes, mainly generated by protein degradation, cannot be completely blocked by a low-protein diet, and modelling intestinal microbiota can be considered as an additional beneficial intervention (4). The reason for using probiotics during CKD is to enhance the intestinal removal of uremic retention solutes. A food-grade, Gram-positive bacterium, in a probiotic formulation, was previously found to be beneficial to rodents (10), miniature pigs (11), and cats (12) with renal failure. Ranganathan et al (13) reported that probiotic dietary supplements facilitated the reduction of blood concentrations of uremic toxins, reduced the progression of renal impairment, and prolonged survival in rats with CKD. In 1 report (14) the use of probiotics (in particular Kibow biotics) in 2 uremic dogs showed favorable and encouraging results, while Polzin (15) did not find any significant difference between 32 CKD dogs treated with Azodyl (Vétoquinol, Paris, France) versus placebo. In human medicine, CKD has been associated with alterations of the gastrointestinal mucosa and disequilibrium in the intestinal flora. This condition is responsible for an increased transformation of amino acids into uremic retention solutes (16). Elevated serum concentrations of indoxyl-sulfate, p-cresyl sulfate, and trimethylamine n-oxide were negatively correlated with the level of kidney function and were predictors of CKD progression (1). These uremic toxins would be responsible for a worsening of renal function by different mechanisms. One study in experimental rats suggested that elevated serum concentration of uremic toxins may accelerate the onset of kidney tubular damage. In nephrectomized rats, GFR was significantly lower in rats treated with uremic toxins compared with controls. The reduction in GFR correlated with a higher glomerular sclerosis, which was promoted by the elevated levels of uremic toxins (17). In a previous study by Miyazaki et al (2), the administration of indoxyl-sulfate to uremic rats mediated the kidney expression of genes related to tubule-interstitial fibrosis and was associated with significant decline in renal function and worsening of

### Table 1. Signalement and baseline values (T0) of GFR, serum creatinine, urea, phosphate, blood pressure, UPC, and USG of dogs in the control group (CG) and the treatment group (TG).

<table>
<thead>
<tr>
<th></th>
<th>CG ($n = 30$)</th>
<th>TG ($n = 30$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.2 (1 to 12)</td>
<td>6.8 (1 to 13)</td>
<td>0.32</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>28.5 (7.4 to 72)</td>
<td>29.3 (8 to 72)</td>
<td>0.51</td>
</tr>
<tr>
<td>GFR (ml/min/m²)</td>
<td>37 (12 to 59)</td>
<td>40 (15 to 56)</td>
<td>0.23</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>159.1 (79.5 to 212.1)</td>
<td>123.7 (79.5 to 265.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>22.8 (9.6 to 56)</td>
<td>20.7 (6.4 to 39.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.3 (0.9 to 2.1)</td>
<td>1.3 (0.9 to 3.1)</td>
<td>0.78</td>
</tr>
<tr>
<td>BP (beats/min)</td>
<td>128 (115 to 189)</td>
<td>130 (115 to 176)</td>
<td>0.98</td>
</tr>
<tr>
<td>UPC</td>
<td>0.9 (0.08 to 7.5)</td>
<td>1.29 (0.13 to 4.94)</td>
<td>0.89</td>
</tr>
<tr>
<td>USG</td>
<td>1.016 (1.005 to 1.046)</td>
<td>1.015 (1.002 to 1.038)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Data were non-normally distributed and presented as median (range). *P ≤ 0.05* was considered significant. BP — blood pressure; GFR — glomerular filtration rate; UPC — urine protein to creatinine ratio; USG — urine specific gravity.

### Table 2. Values of GFR, serum creatinine, urea, phosphate, blood pressure, UPC, and USG of dogs in the control group (CG) and treatment group (TG) at T0 and T1.

<table>
<thead>
<tr>
<th></th>
<th>CG ($n = 30$)</th>
<th>T0</th>
<th>T1</th>
<th>$P$-value</th>
<th>CG ($n = 30$)</th>
<th>T0</th>
<th>T1</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min/m²)</td>
<td>37 (12 to 59)</td>
<td>30 (10 to 47)</td>
<td>0.0002</td>
<td>40 (15 to 56)</td>
<td>48 (11 to 75)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>150.2 (79.5 to 521.5)</td>
<td>159.1 (79.5 to 212.1)</td>
<td>0.001</td>
<td>123.7 (79.5 to 265.2)</td>
<td>123.7 (79.5 to 415.4)</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>22.8 (9.6 to 56)</td>
<td>22.1 (8.2 to 89.2)</td>
<td>0.28</td>
<td>20.7 (6.4 to 39.9)</td>
<td>18.2 (6.4 to 54.6)</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.3 (0.9 to 2.1)</td>
<td>1.7 (0.8 to 4.7)</td>
<td>0.27</td>
<td>1.3 (0.9 to 3.1)</td>
<td>1.2 (1 to 3.6)</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP (beats/min)</td>
<td>128 (115 to 189)</td>
<td>127 (115 to 170)</td>
<td>0.58</td>
<td>130 (115 to 176)</td>
<td>130 (115 to 185)</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPC</td>
<td>0.9 (0.08 to 7.5)</td>
<td>1.2 (0.1 to 9.3)</td>
<td>0.04</td>
<td>1.29 (0.13 to 4.94)</td>
<td>0.77 (0.09 to 3.76)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USG</td>
<td>1.016 (1.005 to 1.046)</td>
<td>1.012 (1.005 to 1.048)</td>
<td>0.04</td>
<td>1.015 (1.002 to 1.038)</td>
<td>1.018 (1.010 to 1.047)</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were non-normally distributed and presented as median (range). *P ≤ 0.05* was considered significant. BP — blood pressure; GFR — glomerular filtration rate; UPC — urine protein to creatinine ratio; USG — urine specific gravity.
glomerular function (2). Elevated levels of indoxyl sulfate were also associated with vascular stiffness, aortic calcifications and high cardio-vascular mortality in humans affected by CKD (18).

If we compare the serum values of creatinine and urea in the 2 groups of patients, we notice that, for the treatment group, there was no significant difference between T0 and T1, while for the control group there was a significant increase in serum creatinine at T1. The increase in serum creatinine in CG seems to reflect a worsening of renal function; in TG both creatinine and urea showed only a non-significant trend to reduction at T1, compared with T0, despite a significant improvement in GFR. This finding was not unexpected. Serum creatinine and urea are generally used as indirect markers of renal function, but they may be affected by extra-renal factors. We opted to measure GFR, as it is universally considered the gold standard test to assess overall renal function (19). It is also possible that the trend to reduction in creatinine and urea in the TG may be due to a direct degradation by VSL#3. VSL#3 contains, among others, Lactobacillus delbrueckii, which has been reported to hydrolyze urea in vitro (13). Therefore, reduction in serum levels of urea and creatinine in patients treated with probiotics should be evaluated carefully, as it may not reflect an actual improvement of kidney function (4).

The improvement of GFR in the TG was also accompanied by a significant increase in USG and reduction of UPC at T1. In dogs in the CG, USG was significantly reduced at T1, compared with T0, while UPC showed only a non-significant trend towards reduction. These findings may reflect an overall improvement of kidney function in patients treated with VSL#3. A recent study (20) reported that supplementation with Lactobacillus species in rats with CKD reduced systemic inflammation and proteinuria, playing a protective role in reducing the progression of CKD (20).

The present study has a few limitations. As we had no clear evidence of potential benefits of VSL#3 in controlling clinical signs of CKD and reducing the progression of the disease, we did not consider it ethical to enrol dogs with IRIS stage 4 and/or end-stage renal disease. As a consequence, we have no data regarding the effects of VSL#3 on GFR in these 2 populations. Because of the relatively low number of patients enrolled in the study, we opted to consider all CKD patients together. A larger study is recommended to compare the effects of VSL#3 on GFR in dogs at different stages of CKD, in order to determine whether the severity of CKD may or may not affect the efficacy of VSL#3. Another limitation of the present study is that during the study period 20/60 dogs were on a combination of benazepril and amlodipine to control blood pressure. Although no randomization for blood pressure was done prior to T0, the number of dogs on benazepril and amlodipine was almost equal in both CG (n = 11) and TG (n = 9). However, the authors cannot exclude that the concomitant use of benazepril and amlodipine in association with VSL#3 might contribute to improve UPC at T1 in this group of patients. It should also be noted that the slightly higher number of hypertensive dogs in CG might affect the progression of CKD and contribute to the worsening of GFR and UPC at T1.

In conclusion, the administration of VSL#3 at the dose of 112 to 225 × 10⁹ lyophilized bacteria per 10 kg BW, PO, q24h for 60 d seemed to affect significantly GFR, USG, and UPC in dogs with CKD. After 2 months of VSL#3 supplementation, treated dogs showed a significant improvement in GFR and USG and a significant reduction of UPC compared to control dogs. Our findings seem to support a potential role of VSL#3 in reducing the progression of CKD in dogs. Results from this pilot study should encourage a larger study.

References


Atypical hypocalcemia in 2 dairy cows, after having been fed discarded vegetable cooking oil

Allan J. Gunn, Angel Abuelo

Abstract — Two mid-lactation dairy cows were presented sternally recumbent 4 days after the herd had been fed discarded vegetable cooking oil ad libitum. In both affected animals hypocalcemia was confirmed by clinical chemistry and response to treatment. This atypical presentation of hypocalcemia associated with feeding discarded cooking oil is previously unreported.

Case description

Two Holstein/Friesian cows in a milking herd of approximately 60 cows in the Riverina region of New South Wales, Australia were noticed to be showing signs of an uncoordinated (“wobbly”) gait on the evening of April 7, 2016. The following morning, both cows were sternally recumbent in the paddock.

On April 4th the herd had been fed about 60 L of discarded used vegetable cooking oil ad libitum in a trough, of which approximately half (0.5 L/cow) was consumed by April 5th, and then removed from the cows. The oil was discarded “deep fryer” oil from local restaurants that was stored unsheltered in 20-L plastic containers that were marked as cottonseed oil, rice bran oil, and canola oil.

The weather had been hot (mean monthly maximum/minimum temperatures of 31.5/17.8°C; 32.6/16.6°C; and 30.6/16.4°C for January, February, and March, 2016, respectively) and dry (48; 33; and 23 mm rainfall in January, February, and March, 2016, respectively) for the previous 3 mo. The estimated pasture growth was < 10 kg/ha per day in January, up to 40 kg/ha per day in February and < 20 kg/ha per day in March; with at least 5 wk within that period deemed to have no forage production. The pastures were not irrigated.

The cows were being fed “oaten” hay and silage overnight ad libitum, and were fed silage in a lucerne/grass sward paddock during the day. The silage was harvested from a Lucerne paddock that had a substantial “barley grass” (Hordeum spp.) content. The cows had access to 2 tonnes of silage daily but did not appear to find it palatable, probably due to the barley grass. The farmer fed discarded vegetable oil to the herd in an attempt to increase the palatability of the silage, and inexpensively enhance the dietary energy availability to the cows. They were also fed 3.5 kg of a commercial concentrate pellet containing barley and canola with 16% crude protein and 1.4 g Ca/kg DM, at each twice daily milking. Water from a bore that was gravity fed to troughs in the paddocks via storage dams was available ad libitum, except during milking times. The average milk yield of the herd between April 4th and April 15th was 16.5 L/cow/d of 3.3% fat and 4.5% protein; which did not alter as a result of the feeding of oil.

Laboratory findings of blood samples from the recumbent cows indicated hypocalcemia, hypophosphatemia, and mild hypermagnesemia (Table 1). To the authors’ knowledge, this is the first reported finding of hypocalcemia in dairy cattle after having been fed a relatively large quantity of oil or fat.

On the morning of April 8, 2016, 2 cows (Cow A — 6 y old, 8 mo in lactation, and 6 mo in gestation; and cow B 7 y old, 3 mo in lactation, and diagnosed not pregnant 2 wk previously) that had shown signs of ataxia the previous evening were found recumbent in the overnight paddock. No abnormal signs were observed in the rest of the herd. Both cows were milked on the evening of April 7, 2016.

Both cows had similar clinical signs: they were in sternal recumbency, alert and responsive, and when approached they...
unsuccessfully attempted to stand. Heart rates were 80 beats/min with no abnormal sounds, rates, or rhythms; respiratory rates 20 breaths/min without obvious effort, and no cracks or wheezes were auscultated. No rumen activity was auscultated, and the gastrointestinal tract (GIT) was subjectively quiet; however, the cows were capable of defecating. Rectal temperatures were 37°C, slightly lower than expected (38°C to 39°C). Per rectum examinations of the cows revealed a gravid uterus in cow A, and were otherwise unremarkable, except that the feces were moist but firm, and had an obvious oil sheen and slippery feel. No abnormalities were detected on examination of the udder or the musculoskeletal system. No obvious cranial nerve or central nervous system abnormalities were detected, and subjectively the cows had a slightly “s-shaped” curvature of the neck. The clinical appearance of the animals was suggestive of hypocalcemia, or milk fever, despite them not being periparturient — the typical time for presentation of hypocalcemia (1,2).

Likely differential diagnoses included: metabolic derangements, of which hypocalcemia was considered most likely; traumatic reticulo-peritonitis; heart and/or liver aberrations; infectious causes such as Listeria spp. meningocencephalitis, salmonellosis, metritis or mastitis, botulism, tick paralysis, or other causes of endotoxemia; polioencephalomalacia; starvation (protein calorie malnutrition); pneumonia; water intoxication/other causes of endotoxemia; polioencephalomalacia, infectious causes such as Listeria spp. meningocencephalitis, salmonellosis, metritis or mastitis, botulism, tick paralysis, or other causes of endotoxemia; polioencephalomalacia; starvation (protein calorie malnutrition); pneumonia; water intoxication/other causes of endotoxemia; polioencephalomalacia, infections such as salmonellosis, metritis or mastitis, botulism, tick paralysis, or other causes of endotoxemia; polioencephalomalacia; starvation (protein calorie malnutrition); pneumonia; water intoxication; fractures and/or dislocations; and toxins such as lead or plant poisonings. Based on the clinical signs, and high suspicion of “dietary induced” hypocalcemia, a blood sample was taken from each cow for determination of calcium, magnesium, and phosphorus concentrations (Table 1). The laboratory results confirmed substantial hypocalcemia and mild to moderate hypophosphatemia in both cows. Five hundred milliliters of a 4-in-1 solution (Vet-cal 4in1; Malindi, Dandenong VIC 3175, Australia), 12.25 g Ca, 42.35 g Mg, 6.05 g P, and 91 g glucose were administered intravenously to each cow. Both cows became ambulatory within an hour of the IV infusion.

Cow A was “dried off” after this incident, and calved uneventfully on July 15, 2016. Cow B recommenced milking in the afternoon of April 9, 2016, as she no longer showed signs of ataxia. Three days later (April 12, 2016), however, she had diarrhea and decreased milk production. She continued to have diarrhea, was not re-examined, and died on April 21, 2016. Unfortunately, veterinary attention was not sought for this cow, nor was a postmortem examination carried out to assist in determining the cause of death.

**Discussion**

Vegetable oils are polyunsaturated fats that undergo hydrogenation in the rumen as a result of the action of rumen microflora. It is not known exactly what concentrations of various vegetable oils were in this feed source, nor how much was consumed by individual animals. Saponification is the formation of soap by long hydrocarbon fatty acid chains of oil or fat, chelating positively charged cations. Typically, this occurs with sodium or potassium, but it is possible for any cations to be chelated. As a result of this chelation, absorption of calcium and magnesium is reduced when the fat content of the diet is high (3). Some oils such as palm oil can contain as much as 8% calcium (3), which is only available once digested.

If the small intestine is “overloaded” with fat, and pancreatic enzyme secretion is not induced with the increase in dietary fat (4), it is probable that the dietary calcium, which is likely to have been saponified in the rumen, would remain unavailable to the cow in these circumstances. The inability to digest the fat would also explain the presence of undigested oil in the feces of these cows at the clinical examination.

Calcium metabolism and homeostasis is well-documented (5). Briefly, cattle blood calcium concentration is maintained between approximately 2.0 and 2.5 mM. Generally, this occurs by utilizing the skeletal calcium stores to maintain the plasma and extracellular fluid (ECF) calcium concentration. The primary mechanisms for calcium homeostasis involve detection of parathyroid artery calcium concentration adjacent to the parathyroid gland. Parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D3 increase plasma calcium concentration and thyroid calcitonin lowers plasma calcium concentration. Parathyroid hormone and 1,25 dihydroxyvitamin D3 enhance the absorption of soluble calcium from the gastrointestinal tract (primarily the duodenum and jejunum) by active and passive mechanisms. Parathyroid hormone and 1,25 dihydroxyvitamin D3 also enhance osteoclastic activity, via PTH receptors on osteoblasts which activate the osteoclasts in bones to facilitate calcium release into the extracellular fluid (ECF) pool, and increase renal reabsorption of calcium. Renal excretion and reabsorption of calcium and phosphorus are also regulated by these hormones. However, renal reabsorption of Ca is not sufficient to counteract large Ca deficits, due to the small amount of Ca (< 2 g/day) that is excreted in urine. The estimated amount of Ca needed to be absorbed just for maintenance in a cow is 10 to 12 g/day (1).

These homeostatic mechanisms require magnesium to be bound to the intra-cellular adenyl cyclase complex, and a non-alkalotic plasma pH to facilitate PTH-receptor binding (2). These tight homeostatic mechanisms to control blood Ca concentration require a 2- to 3-day period for their activation (6). Hence, cows are not equipped with mechanisms to facilitate a quick response to abrupt changes in calcemia, such as a sudden decrease in absorbed Ca, as it is hypothesized in this report.

Hypocalcemia usually occurs as a result of increased calcium demand in the dairy cow, and this is most often due to the calcium component in colostrum and milk (1). Most body calcium is in the skeleton, with approximately 2% of calcium in ECF. The plasma calcium concentration is normally 2.0 to 2.5 mM in adult cattle (1,3). Extracellular calcium is important for skeletal tissue formation, nervous impulse transmission, excitation of skeletal and cardiac muscle contraction, blood clotting, and is secreted in milk. Calcium is an integral component of various

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**Table 1.** Laboratory results illustrating the calcium, phosphate, and magnesium concentrations in mmol/L from blood samples from the recumbent cows A and B.

<table>
<thead>
<tr>
<th>Cow identity</th>
<th>Calcium</th>
<th>Phosphate</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.51</td>
<td>0.61</td>
<td>1.41</td>
</tr>
<tr>
<td>B</td>
<td>0.95</td>
<td>0.99</td>
<td>1.17</td>
</tr>
<tr>
<td>Reference range</td>
<td>2.4 to 3.1</td>
<td>1.1 to 2.8</td>
<td>0.7 to 1.1</td>
</tr>
</tbody>
</table>

**Discussion**

Vegetable oils are polyunsaturated fats that undergo hydrogenation in the rumen as a result of the action of rumen microflora.
enzymes and is an important second messenger from the exterior to the interior of cells. In ruminants, the primary presentation of hypocalcemia is a result of the effect on the neuromuscular motor end plate, and a failure of acetylcholine release resulting in paresis and recumbency. Other classical signs include muscle fasciculations, tachycardia with “muffled” heart sounds, and reduced thermoregulation, typically resulting in cool extremities (1,3).

Reduced GIT movement occurs in clinical hypocalcemia, but could also occur with peritonitis, e.g., due to traumatic reticulo-peritonitis, such as feeding silage contaminated with wire. Other factors that can induce hypocalcemia include metabolic alkalosis, hypomagnesemia, toxemia — especially endotoxemia from metritis and mastitis, and the decrease in osteoclastic activity as a result of estrogen (1). At the time of presentation, no clinical signs suggestive of endotoxia, meningoencephalitis, polioencephalomalacia, toxin ingestion (including phytoestrogens), fracture/dislocation or traumatic reticulo-peritonitis were detected. Effects of estrogen were unlikely to have been the cause of hypocalcemia, at least in the pregnant cow. There were no clinical signs suggestive of botulism: flaccid paralysis of the tongue, decreased jaw tone, flaccid tail, mydriasis, and bradycardia; poultry litter was not being fed to the cows.

Metabolic alkalosis induces conformational changes in the PTH receptor, impairing the physiological activity of PTH in increasing circulating Ca concentrations. Blood gas analysis was not performed in these cases, hence metabolic alkalosis could not definitively be excluded as a contributing factor to the hypocalcemia. However, the described scenario of cation chelation in the rumen will result in metabolic acidosis following the strong ion difference theory of acid-base physiology (7). The decreased availability of cations for absorption while anions remain available will result in an increase in proton ions in blood to maintain electroneutrality, with a consequent decrease in pH. Metabolic alkalosis, therefore, was not considered a relevant contributing factor to the observed hypocalcemia.

In the scenario reported here, there is substantial circumstantial evidence to suggest that there is a link between the consumption of discarded used vegetable cooking oil on April 4th to 5th, 2016, with the atypical onset of recumbency and the laboratory detection of hypocalcemia and hypophosphatemia on April 8th, 2016. It is unlikely that calcium demand resulted in these cows being hypocalcemic and recumbent, as they were in mid-/late-lactation. The sudden availability of a relatively large amount of oil in the diet and their clinical presentation (including oily feces) are highly suggestive of a dietary induced hypocalcemia in these particular animals.

In this scenario, unlike typical periparturient hypocalcemia and recumbency (milk fever), it is likely the hypocalcemia was due to a decrease in calcium availability, rather than as a result of an increase in demand. Speculative reasons for this reduction in calcium availability to the cows include: saponification of calcium, and other cations in the rumen (8); fat-induced decrease in voluntary food intake (3); fat-induced decreased efficiency of absorption of calcium (9); inability of the pancreatic lipases to be “induced” (4), thereby preventing the digestion of the fatty acids to release the saponified calcium; the adverse effect of dietary fat on the conversion of vitamin D to 1,25 dihydroxyvitamin D3 (1). Hypocalcemia was detected in the blood samples, and the animals responded, albeit slightly delayed, to calcium administration; enhancing the likelihood of hypocalcemia as the reason for recumbency in these cows.

It is interesting to speculate as to why cow B had diarrhea 4 d after being found recumbent, and ultimately died with diarrhea. There is a report of Salmonella Mbandaka being isolated from dairy cattle where the source was a contaminated vegetable fat supplement that was fed to the cows. None of the cattle were clinically affected in that report (10), but it illustrates the vagaries of feeding unusual rations to animals, which could lead to clinical aberrations.

To the authors’ knowledge, this is the only reported case of dietary oil or fat related hypocalcemia in dairy cows. It is likely that other unusual manifestations of disease will occur in Australasian, and other, dairy cattle as the economic margins on global milk production continue to pressure farmers into unconventional feeding practices.

Acknowledgments

We thank the farmer for his support and for reporting this case, and to Charles Sturt University librarian Lee-Anne McInerney for her assistance.

References

Inguinal herniation of a mineralized paraprostatic cyst in a dog

Kyle P. Vititoe, Federico Vilaplana Grosso, Stephanie Thomovsky, Chee Kin Lim, Hock Gan Heng

Abstract — A firm mass was noted in the right inguinal subcutaneous region of an 11-year-old intact male Labrador retriever dog presented for right pelvic limb weakness. Pelvic radiographs showed 2 large ovoid structures with circumferential thin eggshell-like mineralization in the right external inguinal region. The structures were confirmed sonographically, and on magnetic resonance imaging as a large folded herniated mineralized paraprostatic cyst through a defect in the right inguinal wall. To the author’s knowledge, this is the first published report of an inguinal herniated mineralized paraprostatic cyst.

Case description

An 11-year-old intact male Labrador retriever dog was presented to the neurology section at Purdue University with a 2-year history of right pelvic limb weakness and licking at the right paw of the same limb. The dog had a history of an acral lick granuloma on the right paw of this limb, as well as right radial and ulnar osteotomies, and surgical correction of a right patella luxation several years before presentation. Pelvic radiographs taken by the referring veterinarian revealed hip dysplasia and secondary degenerative joint disease, with the right coxofemoral joint

Department of Veterinary Clinical Sciences, Purdue University — College of Veterinary Medicine, 625 Harrison Street, West Lafayette, Indiana 47907, USA.
Address all correspondence to Dr. Kyle P. Vititoe; e-mail: kvititoe@purdue.edu
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being more affected than the left. At presentation to Purdue University, the dog had generalized muscle wasting (worse on the right side) and a large tubular to ovoid subcutaneous firm mass in the right inguinal region. Neurological examination showed paresis with a mild proprioceptive ataxia observed in the pelvic limbs. The dog was lame in all 4 limbs with the right pelvic limb lameness being most severe. Conscious proprioception was reduced in both pelvic limbs but normal in the thoracic limbs. Patella reflexes were absent bilaterally and withdrawal reflexes were reduced in both pelvic limbs. Thoracic limb reflexes were normal. There was absence of cutaneous trunci reflex caudal to the thoracolumbar junction. The lesion was localized to the T3-L3 and L4-S2 spinal cord bilaterally.

Radiographs of the abdomen, thorax, and lumbar spine were taken to rule out obvious neoplastic or infectious causes of paraparesis before pursuing magnetic resonance imaging (MRI) and for further investigation of the right inguinal mass. The thoracic radiographs were unremarkable.

On abdominal radiographs 2 well-defined and large structures with thin eggshell-like mineralization were present within the right external inguinal region, extending beyond the ventral margins of the abdominal wall. One of the structures measured 7.8 cm × 4.5 cm and was ovoid in shape, while the other structure measured 3.1 cm × 3.1 cm and was triangular in shape with moderate centrally stippled mineralization (Figures 1A, 1B). An enlarged, well-defined, smoothly margined prostate was present in the normal anatomic location. The differential diagnoses for the inguinal changes include: inguinal herniation of mineralized paraprostatic cysts, less likely dystrophic mineralization or mineralized masses (e.g., granuloma, chronic hematoma, or neoplasia). The prostatomegaly was likely attributable to benign prostatic hyperplasia.

An abdominal and inguinal ultrasound (Philips Ultrasound iU22 SonoCT system; Bothell, Washington, USA) examination was performed subsequently to further characterize the abdominal radiographic findings. The prostate was symmetrically

Figure 1. Lateral (A) and ventrodorsal (B) radiographs of the caudal abdomen. The mineralized structures are present ventral and ventrolateral to the abdominal wall within the right inguinal region. Note the smaller mineralized structure (arrowheads) with the thicker rim and stippled central mineralization and the larger mineralized structure (arrows) with the classic eggshell-like thin rim.

Figure 2. (A–E) Sonographic images of the prostate and herniated mineralized paraprostatic cyst within the right inguinal subcutaneous space. A – Transverse image of the enlarged prostate (P) and intraprostatic cyst (demarcated by 2 different calipers). B – Transverse image of the smaller lobe (SL) and hypoechoic stalk (white arrow) extending from the right ventral aspect of the prostate (P) into the right inguinal subcutaneous space. C – Transverse image of the paraprostatic cyst at the folding site (white arrow) creating a bilobed appearance and the parallel orientation of the 2 lobes (larger lobe – LL; smaller lobe – SL). D – Transverse image of the larger lobe (LL) with the thin hyperechoic rim (white arrow), likely consistent with the eggshell-like radiographic appearance. E – Transverse image of the smaller lobe (SL) with the thicker irregularly marginated hyperechoic rim (white arrow), likely consistent with the centrally stippled mineralized radiographic appearance. Note there is no body wall ventral to the cysts within images C–E, consistent with herniation into the right inguinal subcutaneous space.
enlarged and mildly diffusely hyperechoic with multifocal intraparenchymal round to oblong anechoic structures of variable sizes, consistent with cystic benign prostatic hyperplasia. A large round-to-tubular anechoic pedunculated cystic structure extended from the right ventrolateral aspect of the right prostatic lobe through a large right inguinal wall defect into the subcutaneous space. The proximal portion was smaller (~2.5 cm in diameter) and oriented cranially with a more irregularly marginated and thicker hyperechoic wall. This segment was folded on itself ~180° caudolaterally then increased in diameter to about 4 cm with a thinner smoother hyperechoic wall. The folding created a bilobed cyst with the proximal segment as the smaller lobe and the distal segment as the larger lobe. This cystic structure had a diffusely hyperechoic rim. The ultrasonographic findings were consistent with inguinal herniation of a bilobed mineralized paraprostatic cyst that extended from the prostate into the right inguinal subcutaneous space (Figures 2A to 2E).

Magnetic resonance imaging of the thoracic, lumbar, and sacral spine was performed using a 1.5 Tesla device (General Electric Signa, Milwaukee, Wisconsin, USA) to further investigate the T3-L3 and L4-S2 neurological deficits. The MRI showed no significant spinal cord compression, and medical management (including but not limited to pain medications, rest, and physical therapy) was advised for the pelvic limb weakness and mild neurological deficits. Coincidentally, the enlarged prostate and bilobed paraprostatic cyst were also visualized within the limited field-of-view. The prostate was diffusely symmetrically enlarged with multiple T1W hypointense and T2W hyperintense cystic structures located within the parenchyma. The paraprostatic cyst was homogenously T1W hypointense and T2W hyperintense to the pelvic musculature. The thin eggshell-like rim was poorly discernible on the T1W images but a hypointense rim on the T2W image was more conspicuous (especially surrounding the smaller lobe) (Figures 3A to 3C).

Castration and repair of the right inguinal hernia was recommended. The owner opted to forego any further treatment and the dog was subsequently discharged.

Discussion

This unique case describes the radiographic, ultrasonographic, and MRI features of an inguinal herniated mineralized paraprostatic cyst in a dog. Previously reported herniated organs and tissues through inguinal defects in dogs include: small intestine, colon, urinary bladder, omentum, fat, ovary, spleen, normal uterus, gravid uterus, mucometra/hydrometra, hysterocele, paraprostatic fat, and an ectopic fetus (6–8,13). Herniated paraprostatic cyst through the inguinal defect has not been reported. Paraprostatic cysts, along with the urinary bladder and prostate, have been reported in perineal hernias (2,4). Interestingly, inguinal and perineal hernias have been suggested to share a common pathogenesis in the intact adult male dog secondary to relaxin hormone within the prostatic cysts causing weakening of the pelvic ligaments and musculature (7,14). This report represents only 1 case of paraprostatic inguinal herniation, nevertheless this may indicate inguinal hernias can also be seen in dogs with paraprostatic cysts.

Radiographically, paraprostatic cysts vary in size and shape and may appear as a second bladder in the caudal abdomen (10). If not mineralized, discerning the paraprostatic cyst from the urinary bladder can be difficult. The inguinal herniation may have gone undiagnosed in this case if the paraprostatic cyst was not mineralized, because the cyst's fluid opacity and overlying soft tissues would have been superimposed. Also, the patient's main clinical signs were neurologic in origin and the inguinal mass was an incidental finding. Mineralization of the wall of paraprostatic cysts was previously thought to be uncommon, displaying a thin mineral opaque rim or eggshell appearance (4). More recent literature demonstrates mineralization to be more common and the radiographic appearance has been proven to be more variable (4). Mineralization patterns of the paraprostatic cysts such as random linear, amorphous or stellate, focal stippling, stalk-like and a woven web-like wall have been described (4). The paraprostatic cyst in the dog herein had a thin eggshell-like rim appearance within both lobes and additional stippled mineralization within the smaller lobe. Since
the radiographic appearance of the cyst in this dog resembled features previously described for paraprostatic cysts (4), the tentative diagnosis of inguinal herniation of mineralized paraprostatic cysts was made. The lack of clinical signs related to the paraprostatic cyst in this case is likely because the paraprostatic cyst was herniated, preventing compression of the colon or urinary bladder.

The sonographic appearance of the paraprostatic cyst was similar to that in previous studies; an anechoic structure with a thin echogenic wall (4,15). A stalk was present between the paraprostatic cyst and the prostate gland, similar to that previously described (4). However, in this case the stalk was visualized to extend from the ventrolateral aspect of the prostate into the right inguinal SQ space through a large right inguinal abdominal wall defect. Also, the cyst was folded on itself giving the appearance of 2 separate cysts on radiographs and a single cystic but bilobed sonographic appearance. The wall of the proximal lobe was also thicker and more irregular which may account for the stippled appearance. The larger lobe was thinner and more smoothly marginated which may be the reason for the thin egg-shell radiographic appearance. Distal acoustic shadowing was present within the far walls, but much more evident within the smaller proximal portion with the more stippled radiographic appearance. There were multifocal regions of reverberation artifact in both portions of the cyst, extending distally from the cyst wall in the near field. Both of these changes provided sonographic evidence of mineralization of the wall in this case similar to a previous study (4). Septations within the paraprostatic cyst have also been described (15). This characteristic was not identified in this patient; however, the folding or lobulated appearance could have represented a large septated region.

To date, there has been no MRI description of the signal intensity of paraprostatic cysts in dogs. In a previously published case of 2 castrated male dogs, paraprostatic cysts on MR imaging were noted but not described (3). Albeit, only the smaller lobe and the dorsal half of the larger lobe of the paraprostatic cyst of this dog were within the field-of-view, the signal intensity noted on T1W, and T2W sequences were as one would have predicted. The fluid within the paraprostatic cyst was T2W hyperintense, T1W and STIR hypointense to the musculature. The thin eggshell-like rim was also as expected — hypointense on T1W and T2W sequences.

To the authors’ knowledge, this is the first report of a herniated mineralized paraprostatic cyst in a dog with characterization on MR imaging. Inguinal herniation of paraprostatic cysts should be included on any differential diagnosis list in male dogs with inguinal swelling or masses. Based on this case, radiography and/or ultrasonography are adequate to confirm the diagnosis of paraprostatic cyst (mineralized or not) due to its unique (pathognomonic) appearance.

References
Severe upper airway obstruction following bilateral ventral bulla osteotomy in a cat

Chiara De Gennaro, Enzo Vettorato, Federico Corletto

Abstract — A cat that underwent bilateral ventral bulla osteotomy (VBO) for treatment of otitis media and otitis interna secondary to bilateral inflammatory polyps, developed upper airway obstruction (UAO) soon after tracheal extubation. The cat was re-intubated but the UAO did not resolve at the next extubation. Eventually, tracheostomy was performed. Upper airway obstruction is a potential postoperative complication of bilateral VBO in cats.

Case description

A 1.5-year-old, spayed female domestic shorthaired cat, weighing 3.1 kg, was referred to our neurological department for investigation of an acute onset of left-sided head tilt, ataxia, vomiting, and mild positional rotatory nystagmus. No history of recurrent otitis was reported and the upper airway was unremarkable at clinical examination. Magnetic resonance imaging (MRI) of the head showed that both bullae were filled with a relatively homogeneous material, which had a low signal in T1-weighted images and had a rim of strong mucosal contrast enhancement. More solid contrast enhancement was seen in the rostral compartments of both ears, in particular the left, possibly consistent with a polyp. The MRI changes were consistent with bilateral otitis media and otitis interna of the left ear only. Cerebrospinal fluid was collected at the end of the MRI and its analysis was unremarkable. Differential diagnosis included idiopathic, inflammatory, or infectious diseases and possible presence of bilateral inflammatory polyps. Recovery from anesthesia was uneventful. The cat was discharged with a 10-day course of clindamycin (Antirobe; Pfizer, Tadworth, Surrey, UK), 50 mg PO, q12h, and bilateral VBO was scheduled for 2 wk later.

The day before surgery the cat was admitted to the hospital. The cat was bright, alert, and in excellent body condition, the neurological signs were improved but a marked left head tilt was still present. Pre-anesthetic evaluation was unremarkable: heart rate (HR) was 150 beats/min (bpm), and the respiratory rate (RR) was 30 breaths/min (brpm). Abnormal upper respiratory noises were not detected. Hematology and biochemistry revealed only eosinophilia \(3.34 \times 10^9/L\) (reference interval (RI): 0.1 to \(0.79 \times 10^9/L\)) and mild hyperglobulinemia (54 g/L; RI: 28 to 51 g/L). Twenty minutes after administration of methadone (Comfortan; Vétoquinol, Great Slade, Buckingham, UK), 0.3 mg/kg body weight (BW), IM, general anaesthesia was induced with alfaxalone (Alfaxan; Dechra Veterinary Products, Shrewsbury, UK), 3.3 mg/kg BW, IV, and, after applying lidocaine spray directly on the larynx (with subjectively normal shape and function), tracheal intubation was performed with a 4.5-mm internal diameter cuffed endotracheal tube (ET).
General anesthesia was maintained with isoflurane (IsoFlo; Abbott, UK) vaporized in oxygen and delivered using a pediatric Mapleson D breathing system in the preparation room and a circle system during surgery. During surgery, the animal was positioned in dorsal recumbency on an electrical heating blanket (Hot Dog; Augustine Temperature management, Eden Prairie, Minnesota, USA), with the neck fixed in an extended position using tape. To facilitate extension, a swab pack was placed under the neck. The cat was allowed to breathe spontaneously. Lactated Ringer's solution (Aquapharm Animalcare, York, UK), 10 μg/kg BW, Ringer's lactated solution, 10 mL/kg BW, IV, and tetrastarch (Voluvén; B. Braun Medical, Sheffield, UK), 2 mL/kg BW, boluses were administered IV in an attempt to increase heart rate (110 bpm) and blood pressure (MAP 40 mmHg), respectively. At this point FE’Iso was 1.1%. Normotension was re-established after starting a constant rate infusion of dopamine (Dopamine Hydrochloride; Hospira, Warwickshire, UK) 4 μg/kg BW/min.

Ventral bulla osteotomy was performed as previously described (4). Material was found in both bullae; therefore, samples were taken for histological examination. Total duration of anesthesia was 90 min. Median HR was 150 bpm (range: 120 to 170 bpm), MAP was 60 mmHg (range: 30 to 90 mmHg), PE’CO₂ was 28 mmHg (range: 21 to 37 mmHg), temperature was 36.5°C (range: 36.2°C to 38.2°C), and SpO₂ % was maintained between 98% and 100%. Fentanyl (Fentanyl; Dechra Veterinary Products), 2 μg/kg BW, IV, was administered to control nociception in one occasion during the closure of the skin. At the end of the surgery, which was uneventful and lasted 60 min, the cat was positioned in lateral recumbency and isoflurane administration was discontinued. The cat was extubated 5 min later, when the pinna reflex reappeared. Immediately after extubation, an increase of inspiratory and expiratory efforts, dyspnea, and paradoxical breathing were noted; the upper airways were evaluated and a marked peri-laryngeal and peri-pharyngeal swelling was detected. Furthermore, the ventral cervical area appeared swollen. The cat became rapidly cyanotic and the cat was initially able to breathe without evident UAO, respiratory distress and dyspnea developed 5 min after extubation. Alfaxalone, 1 mg/kg BW, IV, was then administered and the upper airways were examined: marked peri-laryngeal and peri-pharyngeal swelling was still present; therefore, the cat was re-intubated and phenylephrine (Phenylephrine injections BP; Amdipharm, Basildon, UK), 0.01 mg/kg BW, was splashed on the larynx. After discussion with the soft tissue surgeon in charge of the case, it was decided to explore the ventral cervical area and recover the cat with a temporary tracheostomy. Anesthesia was maintained with isoflurane delivered in oxygen using a circle breathing system. No obvious bleeding or other abnormalities were detected during surgical exploration. After placement of a tracheostomy tube (3-mm external diameter) the cat was allowed to recover from general anesthesia. Recovery was uneventful and the cat was able to maintain SpO₂ > 95% while breathing 21% oxygen. Buprenorphine (Vetgesic; Sogeval, Hutton, York, UK), 20 μg/kg BW, IV, was administered and repeated every 6 h as postoperative analgesia. The quality of recovery was excellent and the cat was constantly monitored for any signs of UAO overnight. The following day the cat was comfortable and able to breathe even when the tracheostomy tube was intentionally occluded; alfaxalone, 0.5 mg/kg BW, IV, was administered to evaluate the upper airway: the peri-laryngeal and peri-pharyngeal swelling was markedly reduced as was the ventral cervical swelling, thus, the tracheostomy tube was removed and the tracheostomy site was sutured. At 2 wk postsurgical re-examination the cat was bright, alert, and in excellent condition; no upper airway respiratory noises were present; the head was slightly tilted but there was no residual ataxia. Both surgical wounds and the tracheostomy site were completely healed. The results of histological examination were consistent with bilateral ear polyps.

**Discussion**

Upper respiratory obstruction has been commonly reported in brachycephalic canine breeds — brachycephalic obstructive airway syndrome — or in large breed dogs following unilateral or bilateral laryngeal paralysis (5). In cats, the most common causes of UAO are laryngeal masses, inflammatory laryngeal diseases, laryngeal paralysis, laryngospasm, tracheal disruption, and severe laryngeal or pharyngeal swelling (1). Upper airway obstruction following surgical procedures at the level of the ear canal is not a commonly reported complication. In dogs, UAO can occur due to significant pharyngeal swelling if total ear canal ablation (TECA) and lateral bulla osteotomy (LBO) are performed bilaterally (6) mainly related to edema, inflammation, and hemorrhage caused by the surgical access or in the early postoperative period due to encircling head bandages that can further constrict the pharynx, enhancing the obstruction (2). In
cats, VBO is often performed to treat otitis media, inflammatory polyps, and neoplasia of the middle ear. Horner’s syndrome, facial paralysis, infections, otitis media, and vestibular syndrome are the most common postoperative complications associated with VBO in cats (7). To the authors’ knowledge, UAO after VBO or other surgical procedures at the level of the ear canal has only been mentioned in a retrospective study evaluating indications, complications, and outcome of tracheostomy in cats (3).

Swelling of the pharynx, larynx, and the surrounding soft tissue structures near the ear canal is likely to be the triggering event leading to UAO in the postoperative period (6). In cats, one of the most reported causes of UAO in the perioperative period is laryngospasm, which may occur after irritation of laryngeal tissue by secretions and/or blood, or by a direct stimulation during tracheal intubation or extubation, especially in presence of a light plane of anesthesia (8). Laryngospasm and laryngeal edema have been mainly associated with the use of xylocaine spray, due to the irritant effect of one of the excipients, rather than the drug itself (9,10). In this case lidocaine was used rather than xylocaine to desensitize the larynx and it was sprayed only during the first intubation; therefore, it seems unlikely that sprayed lidocaine could have triggered laryngeal edema or spasm. However, although intubation proceeded smoothly, we cannot completely rule out that the laryngeal stimulation during extubation could have triggered partial laryngospasm and therefore UAO. Upper airway obstruction developed immediately after extubation and, in our opinion, it was mainly caused by the presence of peri-laryngeal and peri-pharyngeal edema, and by direct external compression caused by the swelling of the ventral cervical area.

The severity of UAO clinical signs, which depends on the degree of functional obstruction and the underlying etiology, dictates the initial therapeutic approach. Clinical signs of UAO in cats can be mild like voice change, gagging, retching, cough, dysphagia, weight loss, and anorexia or severe dyspnea, paradoxical breathing, inspiratory and/or expiratory stridor (11). Depending on the severity, UAO impairs ventilation causing hypercapnia, hypoxemia, stress, and increasing oxygen requirement. This is accompanied by a compensatory increase of RR and effort; according to the Bernoulli effect, the increased velocity through an obstruction decreases upper airway pressure, worsening the airway diameter and efficiency of ventilation. Furthermore, the increase of respiratory effort and consequent muscular work increase body temperature, which might further increase RR. If not promptly resolved, UAO can lead to non-cardiogenic pulmonary edema, hypoxemia, collapse and death (12). Medical interventions in the case of UAO include sedation of the patient, administration of oxygen, active cooling, and short-acting glucocorticoids. Sedation reduces stress, oxygen consumption, RR, effort, and development of hyperthermia. Cold water, low environmental temperature, and the use of a fan might be helpful to reduce body temperature. Oxygen might be beneficial to increase arterial oxygen content and therefore oxygen delivery, especially if achieved in a non-stressful way (5). In severe UAO, or when medical treatment fails, intubation is strongly recommended to restore airway patency by by-passing the obstruction (11). In the present case, the cat showed marked respiratory distress and cyanosis not responding to flow-by-oxygen administration. The cat’s trachea was promptly re-intubated with the help of a stylet as peri-laryngeal swelling and secretions did not allow clear visualization of the larynx.

As previously recommended (5), a short-acting corticosteroid was administered to try to decrease inflammation and reduce soft tissue edema. Corticosteroids reduce the production of tissue transudate and cell edema in acute inflammation, inhibiting the release of inflammatory mediators and decreasing capillary permeability (11). In humans, high-dose corticosteroids were effective in improving dyspnea caused by neoplastic obstruction of the upper airways (13). Moreover, they were effective in reducing the incidence of post-extubation laryngeal edema (14). Nevertheless, dexamethasone was ineffective in reducing peri-laryngeal and soft tissue edema in the present case. The recommended dose of dexamethasone in dogs and cats ranges between 0.1 and 1 mg/kg BW (15); it is possible that the dose used here was insufficient. It is also possible that we did not allow enough time for dexamethasone to work. Although dexamethasone has rapid onset of action, in humans about 12 h were needed to relieve dyspnea caused by UAO after administration of dexamethasone (13). In this case, we maintained the cat anesthetized for only 40 min before re-attempting recovery. In humans, corticosteroids are commonly nebulized to treat UAO (16). The efficacy of nebulized corticosteroids (budesonide), intramuscular dexamethasone, and placebo were compared in children suffering from acute viral laryngotracheobronchitis (croup), causing UAO. Both nebulized budesonide and dexamethasone resulted in more rapid clinical improvement than placebo, with dexamethasone offering the greatest improvement (17). In our case, nebulized corticosteroids were not applicable due to the severity of obstruction requiring immediate re-intubation of the trachea under anesthesia.

A cold pack was also applied to the skin of the ventral neck to try to reduce the swelling and soft tissue edema. The use of local cryotherapy is based on the reduction of the skin and tissue temperature promoting vasoconstriction and subsequently a decrease of neuronal activity and arterial and capillary blood flow, thereby minimizing fluid leakage and edema (18). However, application of local hypothermia should be limited to multiple short sessions (5 to 15 min) to prevent reflex vasodilation and edema (19). In our case cryotherapy was applied for 30 min and although it was able to partially reduce the external soft tissue swelling it was not effective in decreasing the compression of the upper airways. It is possible that the application time was not adequate, or multiple cryotherapy sessions would have been required to decrease the temperature and the edema of deeper tissues.

In humans, phenylephrine has been reported to treat severe asthma and edema of the airways (20). Phenylephrine is a sympathomimetic amine with potent α-1 agonist effects, it is a potent vasoconstrictor and it is used for the beneficial effect of reducing mucosal thickness and plasma extravasation, thereby increasing airway calibre. To the authors’ knowledge, there are no clinical studies evaluating the use of phenylephrine in cats to address laryngeal edema. Experimental studies have used cats as a model to compare the effect of phenylephrine spray (0.3% to 1%) and a specific α-2-adrenoceptor agonist for treatment.
of nasal congestion. Both drugs demonstrated decongestant activity, mediated by vasoconstriction, with transient systemic cardiovascular effect (hypertension that lasted between 15 and 60 min) (21). In our case, phenylephrine spray was not available and a total dose of 0.01 mg/kg BW of injectable phenylephrine was topically applied directly on the larynx. It was impossible to evaluate the potential beneficial effect of phenylephrine in this case because immediate re-intubation was necessary. Hypertension was not observed. Considering the poor response to medical treatment and the severe respiratory distress shown by the cat after extubation, temporary tracheostomy was performed. Procedures for performing temporary and permanent tracheostomies have been described and are routinely used in dogs and cats (22,23). Temporary tracheostomies are normally used to bypass the obstruction for a short period of time. Indications for a temporary tracheostomy include UAO, trauma, and neoplasia or ventilated patients in intensive care setting. Compared to dogs, cats with tracheostomy are considered to be at higher risk of complications, due to a combination of increased pro-

tolysis or ventilated patients in intensive care setting. Compared for a temporary tracheostomy include UAO, trauma, and ne-


References


One Health and the expansion of veterinary education and practice in Canada

N. Ole Nielsen

Continuing demographic change in Canada, and the emergence of the concept of One Health as a priority for veterinary medicine more broadly (1), make it timely to consider expanding opportunities in veterinary education. New colleges were established in 1964 (WCVM), 1984 (AVC), and 2005 (UCVM). In the intervals between each of these events Canada's population grew by roughly 6 to 7 million. This expansion of veterinary medical education was driven by student demand and parity of opportunity, continuing importance of animal agriculture, emergence of aquaculture, increasing recognition of environmental and wildlife factors in disease and health, and the adoption of pets into the family. The latter has fueled the absorption of most of the graduates of the expansion of veterinary faculties, and there is no compelling reason to believe this demand will abate. Continuing pet population growth, increasing environmental concerns, as well as demographic changes are leading to the need for a 6th veterinary school, perhaps as soon as 2025, when Canada's population is projected to be about 40 million, an 8 million increase since 2005. Judging by past experience, it is not too early for those who would support such an objective to begin lobbying for such an outcome. The requirement for an additional school becomes even more pressing when new needs for veterinarians with adequate training in One Health are included.

The full incorporation of One Health into the substance of veterinary medicine can set the stage for a significant expansion of veterinary medicine for the benefit of society, if the profession and veterinary academia take up this daunting but exciting challenge (2). To be meaningful, One Health has to be more than the rebranding of public health and comparative research. The imperativeness of One Health arises from the unrelenting challenge it will have to adjust its policies and institutions that govern education and practice to be more flexible. Existing veterinary curricula simply cannot accommodate the volume of new material that will be necessary to educate veterinary students for direct entry to the emerging One Health field. The profession holds the key to enabling a One Health career track in DVM programs. It will have to fashion regulatory mechanisms that make designated licensing in specific fields of practice the norm, together with congruent education accreditation and practice licensing policies. In response veterinary academia's objectives for curricula, faculty competence and research will have the flexibility to adjust to fully encompass One Health and at the same time enable this new approach in professional education. The result should create a significant new demand for graduates in the One Health field in excess of that arising from present circumstances as well as providing all students with more extensive preparation for their chosen career path.

Fortunately the profession is in a position to allow consideration of such radical change because of the remarkable increase in the competence of private veterinary practices that has occurred over the past 50 years, driven in part by the emergence of specialty colleges and boards. As a result these practices have the potential to play a greater role in the clinical education of veterinary students, thereby releasing existing resources in academia for re-allocation to One Health.

If veterinary faculties could be selective in choosing designated fields of practice on which to concentrate, this could motivate more universities to consider establishing a faculty of veterinary medicine, especially those with a medical school. It would now be possible to focus on clinical fields that are essential elements of One Health or other fields considered societal imperatives, without having the expense and complexity of a creating a complete program tailored to meet today's broad DVM accreditation standards for traditional fields. One can also speculate that One Health might be the key to forging an enduring strong functional bond between medical and veterinary faculties, a development that has proven elusive in the past. It would be based on veterinary medicine's expertise in ecological medicine as well as comparative medicine, which by itself has not achieved this end.

It is also worth mentioning that designated DVM career tracks, and congruent licensure and accreditation policies would give the profession more ability to respond to societal needs, whether it be for more or fewer graduates, in particular career topics.
fields. Such a development would mirror the engineering profession which has been extraordinarily successful in responding to new and changing needs in society. The kind of change advocated above would be difficult, but if adopted by the profession as a clear strategic goal, to be achieved over a carefully thought out time period, one can be confident that its creativity, enthusiasm, and commitment to the best interests of society would make it eminently successful. It would accord with Calvin Schwabe's assertion that “the veterinarian… is, first of all, a biologist” (4).

References

Avian Medicine, 3rd edition

Avian Medicine, 3rd edition is a well-organized, quick reference text that would see a great deal of use in either a practice or a wildlife center that treats the occasional avian patient. There is a wealth of information regarding falcons and falconry medicine in this book, a topic that I haven’t seen in North American avian texts. There is a decent amount of wildlife information which is why I think it would be useful for a wildlife center to have on hand, particularly with its practical information regarding treatment of oiled birds and trauma management. I do not think this is a book that would be useful in a mainly avian practice, as I am certain this type of practice would have several texts that provide more detailed information in each section, such as cytology and ophthalmology. I also question whether this textbook would be useful for students; it does provide good practical information but does not go into great depth on most topics — the same reason I think this book would find a good home in a general practice. It provides useful information in an easy to find fashion, enough to either prompt your memory or to give some direction with case management. It is not detailed enough for more complicated cases, particularly for internal medicine cases. I do greatly appreciate the orthopedic section; however, the diagrams and radiographs are fantastic and very helpful, and the chapter is organized by fracture type which helps immensely when planning fracture repair. The photography and diagrams in this text are lovely, providing clarity to the written components and giving excellent visuals for both the procedures and ailments described in the book. The hematology and cytology chapters provide excellent full color pictures that are nice to have if you do not have a specific avian cytology book.

Overall I am pleased with this text; I think it accomplishes the goal of providing an easy reference for general practitioners or avian interest practitioners. It is not bogged down with intense, detailed descriptions, rather presenting the information in a distilled, clarified manner. You will likely be disappointed if you are an avian specialist. A wildlife center could also find use for this text, particularly if the staff are not veterinarians.

Reviewed by Savannah Howse, DVM, Rocky Rapids Veterinary Service, Drayton Valley, Alberta.
Antibiotic Alternative for Cattle Now Available to Canadian Veterinarians

NovaVive Inc., a Canadian immunobiology company, announced that its cattle immunotherapeutic — Amplimune™ — is now available to Canadian veterinarians. The product received approval from the Canadian regulatory authority (CFIA) this past spring. The product is also approved for sale in the U.S.

Amplimune is a potent immunomodulator that reduces the clinical signs and mortality associated with E. coli K99 diarrhea in neonatal calves. The product is an emulsion of mycobacterium cell wall fractions (MCWF) that enhances innate immu

immunity to fight bacterial infections without the use of antibiotics. The product can be administered by intravenous, intramuscular or subcutaneous injection. Veterinarians and cattle producers are under increasing pressure to reduce antibiotic therapies used in animals, particularly food producing animals like cattle. E. coli diarrhea in calves is typically treated with antibiotics.

There is a growing concern in Canada and globally about the dramatic increase of antibiotic resistance. In animals, this has primarily resulted from the indiscriminate use or overuse of antibiotics as preventative therapies or growth promoters. “The development of antimicrobial-resistant pathogens in animals can pose serious risks to human health when they are transmitted as food-borne or water-borne contaminants. Antimicrobial-resistant infections are associated with a greater risk of death, more complex illnesses, longer hospital stays and higher treatment costs.” (Health Canada)

“There is a growing need for effective antibiotic alternatives,” said Graeme McRae, President of NovaVive Inc. “Products like Amplimune that activate the body’s innate immune system to fight infection and disease are one such alternative. We are excited to be taking this proactive step to help Canadian cattle producers curb antibiotic use in their herds, and we look forward to working with producers and veterinarians to assess additional cattle diseases where antibiotic alternatives are needed.”

There are approximately 960,000 dairy cattle and approximately 3.8 million beef cattle in Canada (producing 3.9 million calves per year) (Statistics Canada).

Contact: NovaVive Inc., Belleville, Ontario; phone: (613) 391-3837; website: www.NovaVive.ca

Walkin’ Pets Rolls Out MINI Wheelchair for Smallest Pets

Walkin’ Pets by HandicappedPets.com announces its latest innovation in the pet wheelchair business: the MINI Walkin’ Wheels for disabled or injured pets. This fully adjustable wheelchair accommodates toy dog breeds, cats, and other small animals weighing from two to ten pounds. The MINI Walkin’ Wheels is simple to use, and its adjustability makes finding the right fit quick and easy.

The availability of an off-the-shelf, adjustable product, such as the MINI Walkin’ Wheels, makes it not only affordable, but possible to ship on the same day as ordered. The adjustability feature enables owners to place the order with just two measurements and the pet’s weight and breed/species. This type of product has revolutionized the world of pet care. A Walkin’ Wheels can provide an alternative to euthanizing pets, when just a little rear limb support might enable them to continue to live happy, healthy lives.

Walkin’ Pets ships internationally and has dealers in many countries throughout the world: www.handicappedpets.com/find-an-international-dealer

K9 Advantix® II approved for killing mosquitoes and reducing biting by mosquitoes and stable flies

Bayer Inc.’s K9 Advantix® II received approval from Health Canada for killing mosquitoes through contact and reducing bites from mosquitoes and stable flies in dogs. These new claims for K9 Advantix II are in addition to existing indications for the control of fleas, ticks and lice for at least four weeks. The coverage offered by K9 Advantix II against parasites gives dog owners peace of mind, allowing them to focus on enjoying the outdoors with their dogs during the summer.

Studies have shown that K9 Advantix reduces biting by mosquitoes by up to 95.2% and stable flies by up to 90.2%[1,2]. Mosquito feeding can produce itchy welts that result in self-trauma and distress[3]. Mosquitoes also spread potentially fatal diseases like heartworm when they bite, making them a big concern for dog owners. The bite of a stable fly is painful and can produce open wounds, attracting more flies causing further irritation to the pet. Stable flies will bite dogs and humans on warm days throughout the summer[4].

K9 Advantix® II is applied to the skin along the dog’s back. From there, it spreads evenly as a thin layer over the skin surface and is locked into the oils of the dog’s skin rather than being absorbed internally. When parasites such as biting flies (mosquitoes and stable flies), fleas, ticks, and lice come in contact with the skin of a treated dog, the parasites’ nervous system becomes affected. The parasites quickly become uncoordinated and stop feeding. K9 Advantix® II works through contact and does not require the pest to bite in order to be killed. Reducing biting keeps pets comfortable and can help to prevent disease transmission.

“Unlike many other parasite medications, the active ingredients in K9 Advantix® II stay on the outside of the pet where they can kill pests through contact.” Biting is not required said Dr. Tamara Hofstede, Senior Manager Veterinary Scientific Affairs, Bayer Inc. “Reducing biting is important for pet comfort and reducing the risk of disease transmission, providing pet owners with peace of mind.”


Contact: Bayer Inc., 2920 Matheson Boulevard East, Mississauga, ON L4W 5R5; website: www.animalhealth.bayer.ca

Raw fermented goats milk as a supplementary superfood for cats and dogs

Happy Days Dairy introduces what will be the first of several natural wholesome products focused on providing nutrition and immune system support with the highest quality standards. Raw Fermented Goats Milk a supplementary superfood for cats and dogs that claims to have the following benefits: boost immunity; is nutrient rich; builds bone density; improves digestion; offers detoxification support and is well received by the liver and liver conditions. Happy Days Dairy was founded in 1993 (24 years serving Canada) and they are Canadian Food Inspection Agency (CIFA) certified for human and pet products. Their goats are in open air barns and free to roam in beautiful British Columbia while being fed only the highest quality natural and hormone free feed.

At Happy Days Dairy, their mission is to provide only natural products that are of the highest quality to provide pets with wholesome nutrition. For more information or any technical questions on the product please contact them.

Contact: Dan, phone (440) 669-6748. To order product in Western Canada, contact Avafina Pet Products, phone: (604) 558-1155; in Eastern Canada, contact Natural Pet Distributors, phone: (905) 274-9331. Website: http://www.happydaysdairy.com
Is there an application for wireless capsule endoscopy in horses?

Julia B. Montgomery, Jose L. Bracamonte, Mohammad Wajih Alam, Alimul H. Khan, Shahed K. Mohammed, Khan A. Wahid

Abstract — This pilot study assessed wireless capsule endoscopy in horses. Image transmission was achieved with good image quality. Time to exit the stomach was variable and identified as one limitation, together with gaps in image transmission, capsule tumbling, and inability to accurately locate the capsule. Findings demonstrate usefulness and current limitations.

Wireless video capsule endoscopy is a diagnostic imaging technology routinely used in humans (1–3) for noninvasive direct assessment of the small intestine for various disorders, including inflammatory bowel disease (e.g., Crohn's disease) and gastrointestinal bleeding. More recently, wireless capsule endoscopy has received attention in small animal veterinary medicine (4–6). There is 1 published report of its experimental use in horses (7). Available endoscopy equipment allows for examination of the equine stomach, using a wired 3-meter endoscope (gastroscope). In smaller horses this endoscope can, at best, be advanced to the pylorus and proximal duodenum. Routine direct assessment of most of the small intestine is not possible in the standing, awake horse. The purpose of this prospective pilot study was to test a commercially available human wireless capsule endoscopy system in horses. Our objectives were to: i) determine ease of capsule administration and ability to receive images transmitted by the capsule after administration; ii) assess image quality; iii) determine limitations of using a human capsule in horses; and iv) develop algorithms for computerized image analysis.

Three healthy adult horses (1 Thoroughbred, 2 Quarter Horses) were used in this prospective pilot study. Horses used for this study were ages 4, 10, and 19 y with a mean weight of 507.7 kg (range: 468 to 575 kg). All animal procedures were approved by the University of Saskatchewan Animal Care and Use Committee.

Prior to administration of the capsule, horses were fasted for 24 h, during which they had access to water. The endoscopy capsule (PillCam SB2 and SB3; Medtronic, Brampton, Ontario) SB2 was used for horse 1 and SB3 for horses 2 and 3 (Figure 1a), was administered into the horses' stomach through a nasogastric tube under standing sedation with xylazine (Rompun 100 mg/mL; Bayer, Toronto, Ontario), 0.5 mg/kg body weight (BW). After administration of the capsule a dosing syringe was used to flush the tube with water. The data recorder was attached behind the shoulder of each horse with use of a surcingle (Figure 1f). In horse 1, the sensor pads (PillCam SB2, 8 total) were attached to the lateral body wall with Tensoplast® (BSN Medical, Pinetown, 3610, South Africa), 10 cm × 4.5 m; 4 sensors on each side. The hair was clipped in the area of the sensors. In horses 2 and 3 a newer sensor belt system was used and similarly attached. The belt was fastened around the abdomen of the horse midway between the thoracic and the pelvic limbs.

Horses were placed in a stall and constantly monitored for the duration of the 8-hour trial. Two hours after administration of the capsule the horses received free choice hay in a hay net. In each horse, the trial was continued for 8 h, the average battery life of the endoscopy capsule.

Among the capsules that are commercially available, PillCam SB capsules are mainly developed for use in the small bowel. A typical PillCam SB3 capsule (Figure 1a) consists of an 11 × 26 mm dome integrated with a camera, illumination...
Figure 1. a – PillCam SB3 Capsule; b – Data recorder with PillCam SB2 sensor pads; c – Data recorder with PillCam SB3 sensor belt; d – Data analyzed on a computer and viewed on RAPID software; e – General setup showing the placement of the wireless capsule endoscopy system components (data recorder and sensors) on the horse; f – Experimental setup in horse 1.
optics, transmitter, and batteries. The capsule sends continuous images acquired during its life to a data recorder through a sensing system consisting of sensor pads (Figure 1b) or a belt (Figure 1c). Finally, the data recorder is connected to a workstation where RAPID software (PillCam; Medtronic) is available for further analysis (Figure 1d). The setup of the data recorder with sensor arrays around the horse is shown in Figures 1e and 1f.

A typical endoscopy capsule when administered to humans works for 8 to 10 h, generating ~ 57 600 frames (8). Evaluating each frame individually would be tiresome and inefficient for a clinician. An automated detection algorithm may be able to mark suspected frames with abnormalities, allowing the clinician to evaluate only the suspected frames to make diagnostic decisions. Such an automated process is not intended to replace the role of the clinician. For the initial development of the algorithm

Figure 2. a to c – Images from the PillCam at various regions of the stomach and small intestine. d to e – PillCam image indicating presence of blood (marked using an auto-detection algorithm during offline diagnosis); f – Regular gastroscopy image with an ulcerous region marked (using automated segmentation algorithm during offline diagnosis).
to aid in computerized image analysis in future studies, equine gastroscopy images from clinical cases that have undergone wired gastroscopy were used. Several features of the images were used to classify the frames according to abnormalities (i.e., acute or chronic ulcers) as the images show different properties for different types of abnormalities. These properties were used as features of the classification procedure. The images were first labeled and cropped according to normal and ulcerous regions (Figure 2f). Each cropped image was read as RGB (Red, Green, Blue) and converted into HSV (Hue, Saturation, Value) plane. In both RGB and HSV planes, statistical features such as mean, standard deviation, kurtosis, skew, and entropy were assessed. Similar features were chosen in a recently published study (9) to detect bleeding in human endoscopy; these worked well. Initial development of the image analysis algorithm showed that not all of the features were significant to classify the abnormalities properly. The training accuracy values of the classifiers were in a range of 85% to 100%.

No complications were observed during or after administration of the capsule. After administration, the endoscopy capsule transmitted real-time images from the gastrointestinal tract of all 3 horses. In horse 1, the capsule entered the small intestine approximately 1 h and 11 min after administration and continued to transmit images until it reached the cecum. Gaps in image transmission were noted when the external sensors could not locate the capsule signal. The total recorded time was 8 h and 23 min, which confirms that the capsule was active for the full duration of its battery life, although significant gaps in image transmission were found. Here, “gap” means loss of communication. Depending on the position of the capsule inside the horse and the positions of the sensors on the belt, some loss of communication was observed. As a result, there were time gaps in the frames received. On an average, the total time-gap was found to be 67 min (131 min for horse 1, 45 min for horse 2, and 27 min for horse 3). It was further noted that some of the images were retrograde as a result of capsule tumbling within the small intestine. Quality of the acquired images was good (Figures 2a, b, c). Here, “good” means “acceptable quality for clinical diagnosis.” The images that were received had clear view of the intestine with good focus and lighting. Very few images were distorted (such as, missing pixels, freezing). Presence of blood (Figures 2d, e) was noted in the stomach of horse 1, which we believe was due to nasal bleeding caused during insertion of the capsule through the nasogastric tube.

The belt system used in horses 2 and 3 resulted in more consistent image transmission. However, the image transmission depends not only the belt but also on the location at which it is positioned and on the size of the horse. It is also to be noted that the capsule took longer to exit the stomach in horse 2 and did not leave the stomach in horse 3 for the duration of the study. The capsules acquired images for 8 h and for 6 h for horses 2 and 3, respectively. There were some missed signals during the experiment, which resulted in loss of frames and therefore, may have resulted in loss of important information as well.

This project was a joint effort between specialists in veterinary medicine and electrical and computer engineering. Results from our pilot study showed that a commercially available endoscopy capsule intended for use in humans (PillCam, Medtronic) was relatively easy to administer into the stomach of horses. When using the sensor pads, there were some gaps in image transmission, but image quality was good, allowing for identification of the section of gastrointestinal tract from which the image was acquired, as well as assessment of the gastrointestinal mucosa. The newer sensor belt technology allowed for almost continuous image transmission in 2 horses, and should be used over the older sensor pad system in future studies.

The main limitations identified included the inability to control the time at which the capsule exits the stomach and the occurrence of capsule tumbling in the small intestine, resulting in acquisition of some retrograde images. Horses were fasted to allow for visualization of the small intestinal mucosa, but this has the disadvantage that fasting reduces gastrointestinal motility. Fasting is required to enable visualization of the small intestinal mucosa; however, the impact of fasting on intestinal motility may be a limitation of wireless capsule endoscopy. Another limitation of the current technology is that the exact location of the endoscopy capsule within the small intestine cannot be determined. This means that even if an abnormality is detected, its exact location would not be known. Tumbling was observed because the horse’s small intestine has a wider diameter than the human small intestine. The small intestinal diameter is what prevents tumbling of the capsule in human patients.

The average recording time of the PillCam (Medtronic) capsule is 8 h. Since it is tiresome and inefficient for a physician or veterinarian to analyze all images that have been acquired we developed an algorithm for automatic detection of abnormalities in endoscopic images. Although PillCam (Medtronic) is able to provide us with a frame rate of 2 to 6 frames/second (fps), a higher frame rate of 10 fps is desired in order to detect the region of interest. Furthermore, the image resolution needs to be enhanced as the diagnostic ability using the algorithm is still error-prone due to the low resolution of the captured images. Using a higher number of image features will make the classification easier. However, a large number of features will make the classifier system bulky. For this reason, the system should be optimized using most significant features only.

Based on the findings from our pilot study, proposed modifications for use of endoscopy capsules in horses include a camera on each end of the capsule to correct for capsule tumbling within the small intestine and the ability to switch on capsule transmission once the capsule exits the stomach in order to save battery life while the capsule is in the stomach. Reduction in capsule size would enable the use of nasogastric tubes with a smaller diameter and therefore allow for use in smaller horses and possibly foals. In order to use wireless capsule endoscopy for routine diagnostic purposes in horses, the ability to accurately locate the position of the capsule within the small intestine would also be required.

A customized equine wireless endoscopy capsule would have several potential uses both for diagnostic and research purposes. It would enable assessment of normal and abnormal small intestinal motility and transit time, aid in the diagnosis of small intestinal problems such as inflammatory bowel disease, neoplasia or post-operative assessment of small intestinal anastomosis.
and would provide a platform for newer, minimally invasive, diagnostic tools such as optical biopsy. In addition to cameras, the capsule could be equipped with different biochemical sensors that detect specific biomarkers within the small intestine and transmit the data in real time. Examples of biomarkers include inflammatory mediators, products of intestinal metabolism, and analysis of microbial communities.

In conclusion, this initial trial of wireless capsule endoscopy in 3 horses demonstrated both its potential usefulness and limitations. An equine specific prototype intended to address some of these limitations is currently under development by one of the authors (KW).

Acknowledgments

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References


Books Available for Review

Livres disponibles pour compte rendu

The following books are yours to keep free of charge in exchange for a book review that will be published in *The CVJ*. To order a book and receive suggestions on how to do a book review, please contact Kelly Gray-Sabourin, Editorial Coordinator, Journals, Canadian Veterinary Medical Association, at kgray@cvma-acmv.org


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Unusual case of pyometra in a bichon frise dog

Katherine Malik

**Abstract** — An intact female bichon frise dog with anorexia and chronic vaginal discharge, was clinically diagnosed with an open pyometra upon workup and ovariohysterectomy. Two cystic structures were identified protruding from the uterine body and the wall appeared thickened. Histopathology revealed pyometra, cystic endometria hyperplasia, and adenomyosis with squamous metaplasia.

**Résumé** — Cas inhabituel de pyométrite chez une chienne Bichon frisé. Une chienne Bichon frisé intacte atteinte d’anorexie et de pertes vaginales chroniques a reçu un diagnostic clinique de pyométrite ouverte après le profil sanguin et l’ovariohystérectomie. On a identifié deux structures cystiques qui faisaient protrusion du corps utérin et la paroi semblait épaisse. L’histopathologie a révélé la pyométrite, l’hyperplasie de l’endométrite cystique et l’adénomyose avec une métaplasie squameuse.

(A traduit par Isabelle Vallières)

An 8-year-old intact female bichon frise dog, weighing 5.2 kg, was presented to Temiskaming Veterinary Services with a 3-day history of anorexia, yellow-gray vulvar discharge of at least 5-months’ duration, and an absence of estrus during the past 12 mo. The dog had no known mating history and no additional significant medical history.

Clinical examination included a physical examination, complete blood (cell) count (CBC) and manual differential blood cell count, comprehensive biochemistry profile, cytological examination of the vulvar discharge, and brief ultrasonography of the caudal abdomen. On physical examination, rectal temperature was 38.2°C, heart rate was 120 beats/min, respiratory rate was 28 breaths/min, mucous membranes were pink, and capillary refill time was < 2 s. The vulva was enlarged and a moderate amount of yellow-gray discharge was noted. The CBC revealed a marked leukocytosis [39.4 \( \times \) 10\(^9\)/L; reference interval (RI): 6.0 to 17.0 \( \times \) 10\(^9\)/L] with a left shift (neutrophils 35.9 \( \times \) 10\(^9\)/L; RI: 3.0 to 12.0 \( \times \) 10\(^9\)/L), normocytic hyperchromic moderate anemia (hematocrit 25.6%; RI: 37.0% to 55.0%), and a normal platelet count (363 \( \times \) 10\(^9\)/L; RI: 200 to 500 \( \times \) 10\(^9\)/L). The manual differential blood cell examination showed toxic changes to numerous neutrophils (90%) and codocyte (target cell) morphology of red blood cells. The total protein and globulins were both mildly elevated, at 87 g/L (RI: 54 to 82 g/L) and 58 g/L (RI: 23 to 52 g/L), respectively. The comprehensive biochemistry profile was otherwise unremarkable. Cytology of the vaginal discharge showed degenerative neutrophils, epithelial cells and rods, and cocci that were too numerous to count. Upon brief ultrasonography of the caudal abdomen, 2 anechoic structures were visualized, suspected to be uterine horns filled with purulent fluid. An ovariohysterectomy was recommended.

Lactated Ringer’s solution (LRS) IV fluids at 5 mL/kg body weight (BW) per hour and cefazolin (Cefazolin Sodium 100 mg/mL; Sandoz, Boucherville, Quebec), 22 mg/kg BW, IV, q90 min until surgical closure, were given 30 min before surgery. Under isoflurane (Isoflurane; Benson, Markham, Ontario) anesthesia the ovaries, uterine horns, and uterine body were removed in a routine ovariohysterectomy (Figure 1). The left ovary appeared cystic. The uterine body had 2 cystic structures that were 1 cm in diameter and protruded approximately 1 cm from the uterine body; the ligatures were placed distal to these structures (Figure 1). The uterine stump was lavaged using sterile saline and lap sponges to protect the remainder of the abdomen from bacterial contamination. A clinical diagnosis of pyometra was made when both uterine horns and body were incised and found to be filled with yellow-gray purulent material. The wall of the uterine body appeared thickened with a narrowed lumen, inconsistent with a typical pyometra (Figure 2).

The dog recovered unremarkably from anesthesia, remained hospitalized on LRS IV fluids at 3 mL/kg BW per hour for 24 h after surgery and was discharged 36 h following the surgery. Meloxicam (Rheumocam 1.5 mg/mL; Merck, Kirkland, Quebec), 0.1 mg/kg BW, PO, q24h for 3 d and amoxicillin/clavulanate potassium (Aventilac 125 mg; Aventix, Burlington, Ontario), 12.5 mg/kg BW, PO, q12h for 14 d were prescribed at the time the patient was discharged.
A portion of uterine horn and body was fixed in 10% formalin and histopathology was evaluated at the Animal Health Laboratory, University of Guelph, Guelph, Ontario. Histopathological examination confirmed a diagnosis of pyometra, cystic endometrial hyperplasia, and adenomyosis with squamous metaplasia.

**Discussion**

Pyometra is the result of hormonally induced changes in the uterus that allow a secondary infection to occur. Whether the pyometra is open or closed depends on the patency of the cervix (1). This dog had an open pyometra which allowed drainage of the purulent fluid. Cystic endometrial hyperplasia is the first step in development of pyometra. It is driven by prolonged exposure to progesterone after a period of priming by estrogen (1). Secondary ascending bacterial infections become established, with *Escherchia coli* being the most common organism isolated, in approximately 70% of cases (1,2). Exogenous estrogens potentiate the effects of progesterone on the uterus and cause a predisposition for pyometra (1). Clinical signs vary and can be nonspecific including lethargy, anorexia, dehydration, abdominal pain, vomiting, and diarrhea (2). The most distinctive clinical sign is vaginal discharge: characteristic of an open pyometra. Fever can be variable (1). This dog had obvious vaginal discharge which made the diagnosis easier than is the case with closed pyometra which would require reliance on other clinical signs.

Typical clinical pathology findings include an inflammatory leukogram with septic-toxic changes in neutrophils, a degenerative left shift, mild nonregenerative anemia, hyperproteinemia, hyperglobulinemia, and prerenal azotemia (1). The changes in this dog were consistent with these typical findings; however, she did not have a degenerative left shift or prerenal azotemia. It is important to differentiate pyometra from pregnancy on ultrasonography as fetal calcification occurs after 42 d in gestation and before that time pregnancy can appear ultrasonographically similar to pyometra (1). Ovariohysterectomy is the treatment of choice for pyometra, and was performed in this case. Prognosis is good if there is no uterine rupture or abdominal contamination; the mortality rate is < 10% (1). If pyometra is left untreated, a serious consequence of the uterine infection is endotoxemia, progression to systemic inflammatory response syndrome (SIRS), and death (2).

Differentiating mucometra (the accumulation of mucinous fluid in the uterus) from pyometra can be difficult. Pyometra is distinguished by inflammatory response, presence of bacteria, and more severe clinical signs. Uterine pathology such as cystic endometrial hyperplasia and mucometra are believed to predispose the uterus to infection and subsequent pyometra (2). It is hypothesized this dog had a long standing mucometra that had converted to pyometra.

Adenomyosis is hyperplastic overgrowth of the endometrial glands moving into the myometrium, the muscular wall of the uterus (3), usually from invagination rather than true invasion (4). The normal secretory epithelial lining of the glands undergoes squamous metaplasia (5), which is consistent with the history of this patient as these likely were longstanding lesions. Adenomyosis is rare and usually found as an incidental finding accompanied by endometritis, pyometra, or glandular-cystic hyperplasia. If adenomyosis occurs concurrently with pyometra, the inflammation may extend along these ectopic glands into the myometrium and even the uterine serosa. On gross examination, small subserosal abscesses are evident and these may spontaneously rupture leading to peritonitis (3). The lesions on this patient’s uterine body were unruptured.

Another differential for the appearance of the uterine body was uterine neoplasia; however, this was not supported by histology in this case. Uterine neoplasms are rarely reported in dogs, accounting for only 0.3% to 0.4% of all canine tumors (6). Leiomyoma is the most common uterine tumor,
adenocarcinomas are less frequent, and leiomyosarcomas are rare (6,7). Clinical signs of a uterine neoplasm depend on tumor size, presence of metastases, or concurrent disease such as mucometra or pyometra (7).

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The Art of Private Veterinary Practice
L’art de la pratique vétérinaire privée

Human-animal emotional contagion and client communication

Myrna Milani

Quality communication always plays a critical role in veterinary practice. This is especially true in companion animal practice in which clients’ relationships with their animals may arise from strong emotions, but little or no knowledge of animal behavior. At the same time though, the very nature of domestication is such that it enables members of domesticated species, including humans, to accept members of other species as one of their own more readily. And just as animals may pick up the emotions of other animals of the same species and group via a process of emotional contagion, humans and domestic animals may do likewise.

Those who work with herds of flocks of food animals or equines, in general, are more aware of this phenomenon than those who share their homes with dogs and cats. Since ancient times, successful farmers and herdsmen worldwide have perceived themselves as caretakers of their animals. Inherent in this role was the recognition of the inter- and intra-specific emotional contagion for highly practical reasons. Events that panic an entire herd or flock, or even just those key animals perceived as leaders by other animals in the group, could endanger all the animals’ well-being. That, in turn, could interfere with the amount and quality of any products the animals produced on which the farmers or herders depended for their livelihood. Equally important, failure to properly interpret the intra-species emotional contagion also could endanger the well-being of any humans working among these animals. Farmers and herdsmen who recognized the key role remaining calm played in calming fearful animals protected themselves as well as their livestock. Later, veterinarians and others who routinely worked with these animals similarly appreciated these highly practical physiological aspects of the bond.

Because adult domesticated animals retain certain juvenile traits, a condition known as neoteny, it seems reasonable that they as well as their young would be prone to view humans more as parental figures of their own species. In companion animal practice, clients and veterinary staff unwittingly may trigger an even greater potential for human-animal emotional contagion to occur when they support the perception of dogs and cats as family members. This also may contribute to communication breakdowns.

For example, Ms. Merriweather enthusiastically agrees that her dog, Boopsie, is not only a member of her family, but also a greatly treasured one. But in her zeal to create a perfect world for him since she got him as a young puppy, and barring any knowledge of canine behavior that would lead her to do otherwise, she treated him like a human baby. Or rather, she treated him the way she thought a human baby would like to be treated. Unfortunately, she possessed no previous experience with either babies or puppies. Consequently, her maternal approach was akin to that of an anxious, inexperienced human mother. This resulted in an equally anxious dog that made any dealings with Ms. Merriweather and Boopsie a trial for the veterinarian and staff.

“It seems like I spend most of my time trying to soothe Ms. Merriweather and Boopsie,” complained Dr. Phelan to his associate.

Worse, this anxiety-driven process commences the minute Ms. Merriweather and Boopsie arrive at the veterinary clinic, if not before. In a situation familiar to many experienced clinicians and their staffs, the fearful client arrived with her equally fearful dog and the two of them fed off each other’s fears. Sometimes these effects will be magnified if such human-animal pairs must sit in a waiting room for more than a few minutes. Additionally, their fears have the potential to affect the emotions and behaviors of other animals and people sharing that same space. Hence, the recommendation is to get these people and their animals into an examination room where they are met by a calm and relaxed veterinarian or technician as soon as possible.

However, some clinicians and their technicians may be more concerned about how the animal’s and owner’s anxiety will affect...
their own ability to interact successfully with the animal and the client in the examination room. Interestingly, veterinary staff and client fears often involve the same concerns as these clients. Like Dr. Phelan and his team, Ms. Merriweather also worries about how much her and Boopsie's anxiety will influence her dog's vital signs and mask or exaggerate any medical problems. Will his negative emotional response cause him to lash out or otherwise endanger those handling her pet? What can she do to minimize these effects? Would it be better if she stayed with Boopsie or if she left or waited in another room while Dr. Phelan and his technician examined her dog?

To relieve these anxieties, Dr. Phelan recommends he and Ms. Merriweather meet to discuss how to improve Boopsie's veterinary experiences. He also suggests she leave Boopsie with a trusted friend during their meeting and she agrees. The meeting occurs in the veterinarian's private office to ensure a more relaxed atmosphere. During their time together, Dr. Phelan offers what he considers viable anxiety-relieving options and requests similar input from Ms. Merriweather so they can find a solution that works for Boopsie as well as them.

In situations such as these, the best approach is always for the veterinarian and the client to keep the focus on ensuring as relaxed a veterinary experience for the animal as possible. If that means that all the people involved must get a grip on their own emotions during that interval, so be it. Because Ms. Merriweather recognized that Dr. Phelan wanted the same thing she did — a healthy dog — and was willing to help her, she learned to calm herself. And because the veterinarian wanted the veterinary experience to be as atraumatic as possible for his client, he learned to replace the frustration elicited by the Ms. Merriweathers and Boopsies of the world with a more calming and professional demeanor.

When Ms. Merriweather told her sister about the transformation, noting, “I can't say too much good about Dr. Phelan. He knew I couldn’t accept medicating Boopsie or abandoning him during his clinic visits and I knew how much this annoyed him. But he cared enough about Boopsie and me to help me work out an approach that worked for all of us.”

Other times clients and their veterinarians figure out what approach generates the best results for each human-animal pair via a process of trial-and-error. For example, a normally anxious Ms. Brown decides that observing her pet's distress at the veterinary clinic so upsets her that she and her dog would fare better if her more relaxed partner took the animal instead. As he often told her, the dog was fine with him. She also acknowledged that, even when the dog surprised her and behaved, her own anxiety with all its what-if scenarios made it difficult to process anything the veterinarian told her in a meaningful way. In this situation, the client so quickly came to the realization that her negative emotions were having a negative effect on her animal that all Dr. Phelan needed to do was commend her for putting her dog's needs first.

On the other hand, sometimes Mr. Greene appears totally relaxed during his cat's veterinary visits to the point that the cat is a joy to handle. Other times, Mr. Greene suddenly becomes anxious and his cat becomes resistant to handling and more likely to bite or scratch. When Dr. Phelan begins paying attention to what triggers these changes in the quality of the emotional contagion occurring between the client and his cat, he realizes that the only part of the examination that upsets Mr. Greene is the sight of the needle. When the veterinarian offers to take the cat into the treatment room for any procedures that involve injections, Mr. Green accepts this proposal enthusiastically.

“Thank you so much!” exclaimed the obviously relieved client. “I didn't know that was an option!”

Similarly, the Whites' out-of-control rescue dog frustrates and embarrasses them so much that they feel relieved and grateful when Dr. Phelan suggests that, for now, they sedate the animal prior to any veterinary visits. Additionally, the veterinarian also provides them with contact information for trainers in the area who have demonstrated skill working with dogs like theirs. In this case, the clinician’s ability to recognize how the emotional interplay between the clients and their animal is contributing to their dog's problem behavior causes him to take a combined approach. First, he provides a means to ensure the quality of any veterinary care in the immediate future. And second, he provides information regarding trainers qualified to help the clients and their animal achieve a long-term solution. Once again, just knowing that the veterinarian cares helps the clients relax.

Ideally, all clients recognize how their behavior may undermine that of their animals' in the veterinary setting. But if they do not, it is up to the practitioner to raise the subject for the sake of all involved in the process.
1. **D)** Heartworms live 2 to 3 years on average in the cat, and 5 to 7 years on average in the dog.

**D)** Les vers du cœur vivent en moyenne de 2 à 3 ans chez le chat et de 5 à 7 ans chez le chien.

2. **E)** Cytology offers the examiner the cell populations, their morphology, and the background in which they lie. True tissue architecture and *in situ* arrangement can only be appreciated in any detail using histologic evaluation. The remaining answers are indeed benefits that cytology holds over histology.


3. **A)** The correct answer is skin biopsy. Urticaria is a cutaneous reaction pattern; vasculitis is the most likely diagnosis for such lesions. A skin biopsy is the only diagnostic test that will rule in or rule out vasculitis as a cause and/or confirm the diagnosis of urticaria. The other diagnostic tests evaluate infectious or neoplastic causes for these lesions.

**A)** La bonne réponse est la biopsie cutanée. L’urticaire est une réaction cutanée; la vasculite est le diagnostic le plus probable pour de telles lésions. Une biopsie cutanée est la seule épreuve diagnostique pour confirmer ou infirmer la vasculite comme cause des lésions ou confirmer un diagnostic d’urticaire. Les autres épreuves diagnostiques évaluent les causes infectieuses ou néoplasiques de ces lésions.

4. **A)** Flumazenil is for reversal of benzodiazepines, atipamazole is for reversal of α-2 agents, and nalbuphine is for reversal of opioids.

**A)** Le flumazémil neutralise les benzodiazépines, l’atimazole neutralise les agents alpha 2 et la nalbuphine neutralise les opioides.

5. **C)** The use of human recombinant erythropoietin leads to dose-dependent increases in packed cell volume (PCV), but can also trigger an anemia secondary to the formation of antierthropoietin (EPO) antibodies.

**C)** L’emploi de l’érythropoïétine recombinante humaine conduit à une dose dépendante dans l’hématocrite, mais peut aussi provoquer une anémie secondairement à la formation d’anticorps antiérythropoïétique.
Business Directory

FMS Medical Systems Ltd.

1480 Pemberton Avenue
North Vancouver, BC V7P 2S1
Phone (604) 446-9099
www.fmsmeds.com
Email: info@fmsmeds.com

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