Acute surgical intervention for a depressed skull fracture causing a laceration to the brain parenchyma from a bite wound in a dog

Dietary imbalances in a large breed puppy, leading to compression fractures, vitamin D deficiency, and suspected nutritional secondary hyperparathyroidism

Can *Ureaplasma diversum* be transmitted from donor to recipient through the embryo? Two case reports outlining *U. diversum* losses in bovine embryo pregnancies

Hybrid surgical treatment for 2 feline cases of intrahepatic shunt

*Babesia odocoilei* as a cause of mortality in captive cervids in Canada

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Food Animal Abuse Not Widespread

Dear editor,

I would like to thank Dr. Evenson for his letter to the editor in the October issue of *The Canadian Veterinary Journal* (Can Vet J 2017;58:1017) concerning food animal abuse, as it is always an important issue. However, the letter leaves the incorrect impression that cruelty and abuse to food animals in Canada is widespread, is mainly associated with large commercial operations, and could only be effectively addressed by 24-hour video monitoring. This is not the case.

Most food animals in Canada are treated well, as good welfare is highly correlated with good production and most stock people do what they do because they like caring for animals. Excellent examples of animal welfare can be associated with all sizes of production units, as can egregious examples of animal abuse.

Problem areas which need to be addressed include end-of-production and transportation. However, the response should be targeted and science-based. There are other methods of resolution that do not include 24-hour video monitoring of all production units. For example, third party welfare auditing is becoming more common, especially in large systems, and can identify the units and production methods in which animal welfare is being carried out well and those in which improvements are necessary.

Temple Grandin often states that industry (and therefore society) only improves those aspects of animal welfare, including handling and slaughter, when they are measured. I would suggest that we take steps to celebrate our successes in improved animal welfare, such as the updated Codes of Practice and on-farm welfare assessment programs that are developed to adhere to it, regardless of the production type and size, in addition to continuing to identify those areas in which improvement is required.

Submitted by Dr. Dennis Will, on behalf of the SVMA Animal Welfare Committee, Saskatoon, Saskatchewan.

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- Optimizing the Life of an Indoor Cat

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Le mot de la présidente

Update on CVMA activities to support responsible and prudent use of antimicrobial drugs
Mise à jour sur les activités de l’ACMV pour appuyer une utilisation responsable et prudente des médicaments antimicrobiens

First of all, let me wish everyone a happy and healthy 2018! I thought I would summarize and update you on the Antimicrobial Guidelines and timelines. As many of you are already aware, the CVMA has invested resources in this area for the better part of 20 years. We have been working in close association with many stakeholders such as government, producer groups, CCVR (Canadian Council of Veterinary Registrars), and CAHI (Canadian Animal Health Institute) to name a few. In collaboration with the CCVR, the CVMA developed a framework entitled “Veterinary Oversight of Antimicrobial Use — Professional Standards for Veterinarians.” This framework provides a template of professional standards to be used by provincial and territorial veterinary regulatory bodies when developing their own regulations, guidelines, or by-laws. The CVMA’s Veterinary Pharmaceutical Stewardship Advisory Group (VPSAG) was instrumental in developing this framework. You can access this document on the CVMA website (www.canadianveterinarians.net) under Policy and Advocacy.

Finally, there is light at the end of the tunnel and the scheduled implementation of regulatory and policy changes is just around the corner including:

- Growth promotion claims will no longer be allowed on labels of veterinary products containing antimicrobials that are important to human medicine as of December 1, 2018. A voluntary logo will be included on labels to remind users of the importance of responsible use.

- By December 1, 2018, a veterinary prescription will be required for the purchase of antimicrobials in categories 1–3 that are important to human medicine. These drugs will cease to be available for purchase at livestock outlets, co-operatives, and anywhere else over-the-counter animal medications are sold. Producers will need a veterinary prescription to purchase these products through a veterinarian or pharmacist, which also means they will require a valid veterinary client-patient relationship (VCPR) (check with your individual

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veterinary association with respect to what the classification of a valid VCPR entails). Antimicrobials mixed in feed will still be available at feed mills and will require a prescription.

- As of November 13, 2017 the antimicrobials in categories 1–3 can no longer be imported for own use importation (OUI).
- There will be a new pathway for companies to import and sell veterinary health products for companion and food-producing animals as additional health management tools.
- There will be mandatory reporting of sales volume from manufacturers and importers.

As part of antimicrobial stewardship, the CVMA is renewing its 2008 Antimicrobial Prudent Use Guidelines (PUGS) for dairy, beef, poultry, swine, and developing guidelines for small ruminants and companion animals. (The CVMA previously developed a small animal smart app for urinary tract infection).

The deadline for these is March 31, 2018.

With the support of the Canadian Food Inspection Agency (CFIA) during 2017, the CVMA began work on development of an antimicrobial use (AMU) surveillance initiative at the veterinary prescription interface. It is proposed that this work continue in 2018. Reliable surveillance data will be important to foster responsible antimicrobial stewardship and keep market open. The OIE (World Organisation for Animal Health) is engaging in collecting and disseminating available country data internationally. On behalf of the VPSAG, preliminary guidelines for veterinary care of bees have also been developed.

Communication is key, and currently the CVMA is working with the Veterinary Drugs Directorate (VDD) to help develop consistent communication regarding the transition, stakeholder engagement, and execution of key timelines around the implementation and impacts of Health Canada’s moving all medically important antimicrobials to the Prescription Drug List. As part of this communications strategy, the VDD agreed to develop a web “landing page” allowing for up-to-date timelines and consistent messaging (www.canada.ca/en/public-health/services/antibiotic-antimicrobial-resistance/animals/actions/responsible-use-antimicrobials.html). On November 1, 2017, CAHI, which is the trade association representing the manufacturers and distributors of animal health products in Canada, sent a statement reminding farmers, feed mills, and veterinarians that as of December 1, 2018 a veterinary prescription will be needed to use medically important antimicrobials (antibiotics). For more on CAHI and the changes regarding veterinary use please see the website (www.cahi-icsa.ca). The CVMA will keep members updated on information according to timelines put in place. The website (www.canadianveterinarians.net), e-mails and social media will be used for updates.

In closing, “the development of antimicrobials has contributed enormously to improving the health and welfare of people and animals throughout the world. However, the increase in antimicrobial resistance and the spread of resistant bacteria poses a global threat to both human and animal health. A ‘One Health’ approach through cooperation with stakeholders is vitally important to be able to best address this growing problem. Continuous availability of a range of effective antimicrobials is vital, and preservation of antimicrobial efficacy through responsible and prudent use of antimicrobials is in the best interest of
both animal health and welfare, food safety, human health and the sustainability of our planet.” This statement is taken from the joint statement on Continuous Use of Antimicrobial Use and Antimicrobial Resistance (American Veterinary Medical Association/Canadian Veterinary Medical Association and the Federation of Veterinarians of Europe 2011).

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Ethical question of the month — January 2018

On dairy herd health visits you routinely bring your technician along to assist you with various procedures. This technician has worked with you for several years and you greatly respect his expertise, dedication, and animal care ethic. On your way back to the clinic after one such visit, the technician mentions to you that at the previous herd health visit he saw the owner’s son repeatedly use an electric prod on a cow that was down in the alleyway and could not get up. On this visit he witnessed the same young man kicking a cow in the head to get her to back out of a stall. You have had reservations about the son's husbandry skills for some time but have never observed any abusive behavior yourself. The owner is an outstanding dairyman. He has had some problems with this boy but hopes to have his son keep the farm in the family after he retires. Your technician expects you to act on behalf of the welfare of the cows. You suspect the boy has some mental health issues but this, of course, is not your area of expertise. How should you respond?

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.
Ethical question of the month — October 2017

Stockpeople raise livestock with the intention of producing meat, milk, fiber, and a range of other products. When an animal develops a problem such as lameness, producers wrestle with a sense of failure together with the belief that it is wrong to “waste” the animal’s life, i.e., euthanize an animal that has not fulfilled its purpose. The belief that an animal’s life should have a purpose is one reason that less than perfectly fit animals are sometimes transported to slaughter. When such animals appear at abattoirs or sales barns, producers may be accused of trying to extract the last bit of profit from an animal, or that they didn’t recognize that the animal had a problem. The producer on the other hand feels it is wrong that an animal that he or she has cared for over months or years should be killed and disposed of on the farm because it was not perfectly fit to travel. Is this belief that an animal should fulfill its intended purpose misguided? Is it wrong to “waste” an animal’s life?

An ethicist’s commentary on shipping sick animals as to not waste them

The producer rationale for shipping sick animals to slaughter recounted here, namely that “an animal’s life should have a purpose,” is one that I find extremely ironic. In my own theoretical account of human moral obligations to animals, the concept of telos, derived from Aristotle, figures very prominently. All living things, according to Aristotle, possess an inherent nature, or function, or purpose, or end, which is the particular form of life they are constituted to live. To put it simply, pigs possess a “pigness,” dogs a “dogness,” and good welfare for an animal consists in large measure in living a life to which it is suited by that nature.

As I argued in my recent book, A New Basis for Animal Ethics: Telos and Common Sense, this view of animals is built into a common sense view of the world — as the song goes, “fish gotta swim, birds gotta fly.” That is precisely why I based my ethical writings upon it, namely that it resonates so well with ordinary people and how they look at the world. Placing animals into situations in which they are forced to live is totally violative of their nature is abhorrent to common decency. That is why gestation crates are so jarring to ordinary people — such crates in which a sow spends her entire productive life, unable to move or even turn around, violate our moral sensibilities. Respect for telos is a fundamental principle of respect for animal welfare.

In my own work, I have used the concept of telos to make progress in improving numerous ways in which we keep animals to suit our purposes, not theirs. It is thus ironic to find producers claiming that we can cavalierly ignore a farm animal’s welfare and ship the animal to slaughter, so that it can fulfill its “nature.” In other words, in the misguided metaphysics of such a producer, the telos of the cow is to become a hamburger or a steak. I am reminded of the absurd advertisements for canned tuna where Charlie Tuna laments not being chosen to be packed in such a can! This is double talk, reminiscent of George Orwell.

The nature of the cow is to live as it is biologically determined to live. I am virtually certain that a sense of the foregoing principle is precisely why the public has tended to reject high confinement for farm animals, austere cages for zoo animals, small pools for keeping killer whales, and innumerable other traditional animal uses, even though many such uses were in some sense enjoyable and sanctified by historical tradition.

I can recall with some bitter amusement walking with my Great Dane in a New York City park and being accosted by a woman who chastised me for not having cropped my dog’s ears for ethical reasons. When I tried to explain, she shouted at me: “Don’t you understand? It belongs to the nature of the Great Dane to have cut ears!” My reply stopped her in her tracks: “If it belongs to the nature of the Great Dane to have cropped ears, then why don’t they come that way?”

The human ability to rationalize virtually any bizarre behavior is well-known. The argument employed by the producer in this case is a poster boy for such rationalization! We need not worry about the shipping of the sick animal and its suffering because such is the animal’s nature. God bless the North American public for seeing through such nonsense!

It has been suggested to me that the case was misstated; it should have said that the telos of the farmer is violated when he or she is unable to perform the function of producing food, and therefore is justified in shipping sick animals. There is a very simple response to that: in my view the telos of the farmer is to provide good husbandry for the animals raised, a point that I have raised repeatedly in this column. And a crucial part of good husbandry is assuring that the animals not suffer. As one husbandry-based cattle rancher said to me, “we should not ship our mistakes; we should eat them.”

Bernard E. Rollin, PhD
1. Which of the following is true regarding the bone marrow reserve available to respond to inflammation?
   A. Dogs have a limited bone marrow reserve and have a limited degree of neutrophilia in response to inflammation.
   B. Cats have a limited bone marrow reserve and have a limited degree of neutrophilia in response to inflammation.
   C. Cattle have a limited bone marrow reserve and have a limited degree of neutrophilia in response to inflammation.
   D. Horses have no bone marrow reserve and have no neutrophilia in response to inflammation.

2. "Thrush" is an infection of the oral cavity and esophagus caused by which of the following?
   A. Streptococcus pyogenes
   B. Corynebacterium pyogenes
   C. Actinobacillus lignieresii
   D. Candida albicans
   E. Histophilus somni

3. Which of the following is INCORRECT regarding feline toxoplasmosis?
   A. Serum immunoglobulin M (IgM) titer is a poor test for diagnosis of an active infection.
   B. Transplacental infection can occur.
   C. Intestinal replication and oocyst shedding occur only in cats.
   D. In the bradyzoite state, large tissue cysts form in muscle, brain, and other organs.
   E. Toxoplasmosis can cause blindness, uveitis, and chorioretinitis.

4. Which of the following is NOT true regarding valvular endocardiosis in dogs?
   A. It is an age-related condition.
   B. It most frequently involves the mitral valve.
   C. It is associated with a systolic murmur.
   D. It causes smooth, nodular thickening of affected valves.
   E. It is associated with bacterial infection.

1. Lequel des énoncés suivants est vrai à propos de la réserve de moelle osseuse disponible pour répondre à l'inflammation?
   A. Les chiens possèdent une réserve de moelle osseuse limitée et ont une faible quantité de neutrophiles en réponse à l'inflammation.
   B. Les chats possèdent une réserve de moelle osseuse limitée et ont une faible quantité de neutrophiles en réponse à l'inflammation.
   C. Les bovins possèdent une réserve de moelle osseuse limitée et ont une faible quantité de neutrophiles en réponse à l'inflammation.
   D. Les chevaux ne possèdent pas de réserve de moelle osseuse et n’ont pas de neutrophiles en réponse à l’inflammation.

2. Le muguet est une infection de la cavité buccale et de l’œsophage causée par lequel des organismes suivants?
   A. Streptococcus pyogenes;
   B. Corynebacterium pyogenes;
   C. Actinobacillus lignieresii;
   D. Candida albicans;
   E. Histophilus somni.

3. Lequel des énoncés suivants est INCORRECT à propos de la toxoplasmose féline?
   A. Le titre d’immunoglobuline M (IgM) sérique constitue une mauvaise épreuve pour le diagnostic d’une infection active.
   B. L’infection transplacentaire peut se produire.
   C. La réplication intestinale et l’excrétion des oocystes se produisent seulement chez le chat.
   D. À l’état de mérozoïte, de gros kystes tissulaires se forment dans les muscles, l’encéphale et les autres organes.
   E. La toxoplasmose peut causer de la cécité, de l’uvéite et de la chorioretinité.
5. Which of the following is the correct term for a normal behavior of stallions that involves curling the upper lip and dropping the penis?
   A. Bruxism
   B. Dominance aggression
   C. Aerophagia
   D. Flehmen response

4. Lequel des énoncés suivants N'EST PAS vrai à propos de l'endocardiose valvulaire chez le chien?
   A. C’est une affection reliée à l’âge.
   B. Elle implique plus fréquemment la valve mitrale.
   C. Elle est associée au souffle systolique.
   D. Elle cause un épaississement nodulaire lisse des valves atteintes.
   E. Elle est associée à une infection bactérienne.

5. Lequel des termes suivants est le terme exact pour désigner le comportement normal des étalons lorsqu’ils retroussent la lèvre supérieure et rabattent le pénis?
   A. bruxisme;
   B. agressivité de dominance;
   C. aérophagie;
   D. flehmen.

(See p. 81 for answers./Voir les réponses à la page 81.)
Apply for a Sponsorship to Attend the 2018 Emerging Leaders Program!
Présentez une demande de commandite pour assister au Programme des futurs leaders 2018!

As part of the 70th CVMA Convention to be held in Vancouver, British Columbia, the Canadian Veterinary Medical Association (CVMA) will hold the 9th edition of the CVMA Emerging Leaders Program (ELP).

The Program, sponsored by Virox Animal Health, is a highly interactive 8-hour workshop spread across 2 1/2-days. In addition to the 8-hour ELP session, participants are also invited to attend some of CVMA’s signature events including the CVMA Summit and the CVMA Annual General Meeting and Awards Luncheon.

We would like to remind CVMA members who graduated within the last 10 years (2007 or later) to apply for a full sponsorship to participate in the 2018 Emerging Leaders Program that helps Canadian veterinarians, technicians and technologists to identify and develop leadership skills while building a leadership network within the profession.

Sponsored participants will receive the following:
• Travel to and from Vancouver, British Columbia
• Two nights’ accommodation at the JW Marriott Vancouver Hotel
• Eight-hour workshop with Dr. Rick DeBowes
• Complimentary registration for the 2018 CVMA Convention (value $645).

Up to 2 sponsored participants per province will be selected.

We encourage you to take advantage of this generous opportunity made possible by Virox Animal Health.

Applications are due to Sarah Cunningham (scunningham@cvma-acmv.org) on March 28, 2018.

Dans le cadre du 70e congrès de l’ACMV qui aura lieu à Vancouver, en Colombie-Britannique, l’Association canadienne des médecins vétérinaires (ACMV) tiendra la 9e édition du Programme des futurs leaders (PFL) de l’ACMV.

Le programme, qui est commandité par Virox Animal Health, est un atelier hautement interactif d’une durée de huit heures qui est réparti sur deux demi-journées. En plus de l’atelier de huit heures du PFL, les participants seront aussi invités à assister à des événements phares de l’ACMV, dont le Sommet de l’ACMV ainsi que l’Assemblée générale annuelle de l’ACMV et déjeuner de remise des prix.

Nous aimerions rappeler aux membres de l’ACMV qui ont obtenu leur diplôme au cours des dix dernières années (2007 ou ultérieurement) de présenter une demande de pleine commandite pour participer au Programme des futurs leaders 2018 qui aide les vétérinaires, les techniciens et les technologues canadiens à identifier et à développer des compétences en leadership tout en créant un réseau de leadership au sein de la profession.

Les participants commandités bénéficieront des avantages suivants :
• Trajet aller-retour à Vancouver, en Colombie-Britannique
• Deux nuitées à l’hôtel JW Marriott Vancouver
• Atelier de huit heures avec le Dr Rick DeBowes
• Inscription gratuite au congrès 2018 de l’ACMV (valeur de 645 $).

Jusqu’à deux participants commandités par province seront choisis.

Nous vous encourageons à profiter de cette généreuse occasion rendue possible par Virox Animal Health.
Les demandes doivent être acheminées à Sarah Cunningham (scunningham@cvma-acmv.org) d’ici le 28 mars 2018.

«Au cours des cinq dernières années, j’ai eu l’immense plaisir d’être associé avec des collègues extraordinaires de toutes les régions du Canada qui se réunissent pour participer au Programme des futurs leaders. J’enseigne depuis 39 ans aux étudiants en médecine vétérinaire et aux collègues inscrits aux études supérieures et je peux vous dire que ces personnes figurent parmi les plus brillantes que l’on pourrait rencontrer. C’est une expérience gratifiante et une leçon d’humilité que de constater l’énergie, l’enthousiasme et l’intelligence qu’ils contribuent à nos séances d’apprentissage interactives. Un nombre important poursuivent ensuite le travail en améliorant les équipes de leur pratique ou ils assument un rôle dans la médecine vétérinaire organisée à l’échelle provinciale ou nationale... en donnant toujours l’exemple. Si vous désirez prospérer dans notre profession aujourd’hui et faire une différence positive pour les autres, je vous recommande sans réserve de considérer le Programme des futurs leaders de l’ACMV!» — Dr. Rick DeBowes

2018 CVMA Convention
July 5 to 8, 2018
Ignite Your Passion!

The CVMA Convention returns to downtown Vancouver after 10 years at the newly-opened JW Marriott Parq Vancouver located next to the BC Place Stadium. The CVMA Convention is organized in partnership with the CVMA-SBCV Chapter and in collaboration with the Registered Veterinary Technologists and Technicians of Canada (RVTTC).

Interested in a unique opportunity to learn and network with Canada’s veterinary community? Then join us for the 2018 CVMA Convention where you and your colleagues can participate in a selection of over 105 continuing education (CE) sessions featuring 25 renowned speakers from Canada and the United States and afterwards, socialize in our popular social evening event. Eager for more knowledge? Additional wet labs will be offered that require early registration: Basic and Advanced Dental Extractions with Dr. Kevin Stepaniuk and Dr. Sue McTaggart; and Common Surgical Procedures of the Canine Abdomen with Dr. Ameet Singh. Finally, Tara Evans of Serona Animal Health will host a special workshop to demonstrate the proper handling of dental instruments.

Continuing Education tracks at the 2018 CVMA Convention include: Business Management, Companion Animal, Ruminant, Equine, Animal Welfare, and more.

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Les volets de formation continue du congrès 2018 de l'ACMV incluent notamment la gestion commerciale, les animaux de compagnie, les ruminants, les équidés, le bien-être animal et plus encore.

Lors de l’atelier de la gestion commerciale, vous entendrez la Dr. Heather Romano parler de la nouvelle génération de culture où vous découvrirez comment mettre à contribution les compétences des milléniaux qui entrent sur le marché du travail afin d’améliorer votre pratique en attirant et en conservant les employés les plus talentueux de cette nouvelle génération. La Dr. Romano a travaillé pendant plus de vingt ans dans une clinique vétérinaire avant l’obtention de son poste actuel auprès de iVET 360, dont quatorze années passées en gestion vétérinaire.


Les ateliers de formation continue sur les ruminants porteront sur la médecine et la chirurgie bovines, les maladies du système reproducteur et les maladies respiratoires des bovins de boucherie.

Le volet des équidés portera sur l’anesthésie et l’analgésie sur le terrain, la chirurgie de la tête pour le praticien sur le terrain, la médecine et chirurgie et l’ophtalmologie.

Le volet sur le bien-être animal se penchera sur la violence envers les animaux et un autre volet examinera le bien-être au travail. Pour la première fois en 2018, un atelier abordera le sujet des animaux destinés à l'alimentation — Abeilles domestiques pour Dummies : Une introduction pour les vétérinaires.

In the Business Management session, you will listen to Dr. Heather Romano discuss The New Generation of Culture where you will gain insights on how to leverage the skillset of millennials entering the workforce to improve your practice by attracting and retaining the best talents of this new generation. Dr. Romano brings over 20 years of veterinary hospital experience to her current position with iVET 360, including 14 years in veterinary management.

The Companion Animal CE sessions will focus on: Anesthesia and Pain Management, Dentistry, Dermatology, Dental Instrument Care, Diagnostic Imaging, Endocrinology and Gastroenterology, Feline Medicine, Pain Management, Ophthalmology, Soft Tissue Surgery, and Senior Cat Preventative Care and Management.

The Ruminant CE sessions will focus on Bovine Medicine and Surgery, Beef Cattle Reproductive and Respiratory Diseases. The Equine stream will discuss Field Anesthesia and Analgesia, Head Surgery for the Field Practitioner, Medicine and Surgery, and Ophthalmology.

The Animal Welfare stream will examine animal abuse, and another stream will look at workplace wellness. New in 2018, a session will focus on food producing animals — Honey Bees for Dummies: A Primer for Veterinarians.

In addition to the CE sessions, the CVMA Convention offers an Exhibit Hall with over seventy exhibit spaces showcasing the latest in veterinary products and services.

The CVMA Convention also features several signature events that you can partake in, including: the CVMA Summit facilitated by CVMA’s president-elect, Dr. Terri Chotowetz; the CVMA National Issues Forum; and the Emerging Leaders Program. These signature events offer unique networking, engagement and learning opportunities.

Invest in yourself and re-ignite your passion for veterinary medicine for as little as $70 per CE hour. More news and updates on the CVMA website (www.canadianveterinarians.net).
Chaque année, dans le cadre du programme national des prix vétérinaires nationaux de l’Association canadienne des médecins vétérinaires (ACMV), des vétérinaires sont honorés pour leurs contributions exceptionnelles à la médecine vétérinaire. Nous vous encourageons à mettre en candidature des collègues méritants pour leur travail ardu et leur dévouement envers la profession.

Les candidats (sauf ceux mis en candidature pour le titre de membre honoraire) doivent être des membres en règle de l’ACMV pour être admissibles à la mise en candidature. Cependant, ils peuvent être mis en candidature par des non-membres de l’ACMV.

Les Prix de l’ACMV seront présentés durant le congrès de l’ACMV, qui se déroulera à Vancouver, en Colombie-Britannique, du 5 au 8 juillet 2018. Des mises en candidature seront acceptées jusqu’au 31 janvier 2018 pour les prix suivants :

- **Prix humanitaire de l’ACMV** (Sponsored by Merck Animal Health)
- **Prix vétérinaire Merck** (Sponsored by Merck Animal Health)
- **Prix du praticien des petits animaux** (Sponsored by Petsecure Pet Health Insurance)
- **Prix de la pratique de l’année de l’ACMV** (Sponsored by Scotiabank)
- **Prix de l’industrie de l’ACMV**
- **Membre à vie de l’ACMV**
- **Membre honoraire de l’ACMV**

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 au 339, rue Booth, Ottawa (Ontario) K1R 7K1.

Les trousses de mise en candidature doivent inclure un formulaire de mise en candidature rempli, une description sommaire des principales réalisations professionnelles du candidat et des lettres d’appui (des articles de journaux et des articles écrits par le candidat peuvent être inclus si applicable).


E ach year, through the Canadian Veterinary Medical Association’s (CVMA’s) national veterinary awards program, veterinarians are honored for their exceptional contributions to veterinary medicine. We encourage you to nominate deserving colleagues for their hard work and dedication to the profession.

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.

CVMA Awards will be presented during the CVMA Convention, which takes place in Vancouver, British Columbia from July 5 to 8, 2018. Nominations will be accepted until January 31, 2018 for the following awards:

- **CVMA Humane Award** (Sponsored by Merck Animal Health)
- **Merck Veterinary Award** (Sponsored by Merck Animal Health)
- **Small Animal Practitioner Award** (Sponsored by Petsecure Pet Health Insurance)
- **CVMA Practice of the Year Award** (Sponsored by Scotiabank)
- **CVMA Industry Award**
- **CVMA Honorary Membership**

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

Nomination packages must include a completed nomination form, an outline of the nominee’s key professional accomplishments, and letters of support (newspaper articles and articles written by the nominee can be included if applicable).

Please visit the **CVMA Awards section**, under **About CVMA**, on the website (www.canadianveterinarians.net) for complete nomination guidelines, award descriptions, nomination forms, and past award recipients.
Future Veterinarian Support Continues from Scotiabank, a Preferred Partner of the CVMA

La Banque Scotia, un partenaire privilégié de l’ACMV, continue d’offrir du soutien aux futurs vétérinaires

 Scotiabank is proud to continue to support the efforts of The Canadian Veterinary Journal (The CVJ) to keep students informed about the industry during their studies. Since 2015, Scotiabank has supported the program, offering veterinary students across Canada subscriptions to The CVJ at no cost.

Scotiabank understands the importance of staying informed about current issues and topics for professionals within the veterinary community across the country. By providing access to these journals Scotiabank demonstrates an ongoing commitment to the profession.

As the CVMA’s preferred financial services provider, Scotiabank designed the Scotia Professional Student Plan program to meet the unique needs and goals of professional students. For practicing professionals, the Scotia Professional Plan provides everything needed to help them become financially better off, both personally and professionally.

Additional resources are provided to veterinary students at each faculty across Canada under Scotiabank’s Faculty Representative Program; this program ensures a dedicated advisor is assigned to address the needs of veterinary students at each university. The Scotiabank faculty representatives are listed below and will be working with the CVMA to distribute the journals at each of the veterinary colleges.

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<td>Inder Arora</td>
<td><a href="mailto:inder.arora@scotiabank.com">inder.arora@scotiabank.com</a></td>
<td>403-801-6509</td>
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<td>Saskatchewan</td>
<td>Trevor Humphries</td>
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<td>306-668-1419, x7000</td>
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<td>Guelph</td>
<td>Jason Roberts</td>
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<td>519-766-7663, x4200</td>
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<td>Montreal/Montréal</td>
<td>Vivian Contonis</td>
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<td>Caroline Nicholson</td>
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<td>902-629-7755, x3001</td>
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For more information about Scotiabank’s services for professional students, please visit (www.Scotiabank.com/studentprofessional) or meet with a Scotiabank small business advisor today.

Pour en savoir davantage à propos des services de la Banque Scotia pour les étudiants professionnels, veuillez visiter (http://www.scotiabank.com/ca/fr/0,,2580,00.html) ou prenez rendez-vous avec un conseiller en petites entreprises de la Banque Scotia dès aujourd’hui.
Veterinary oversight of antimicrobial use is being strengthened in Canada as part of the fight against antimicrobial resistance. This means important changes for you as a practitioner and for the veterinary profession.

Health Canada is moving a number of Medically Important Antimicrobials (MIAs) approved for veterinary use before 2004 to the Prescription Drug List (PDL). With this change, Health Canada will establish the same level of oversight for those MIAs approved before 2004 as for those approved after.

As of December 1, 2018, all MIAs for veterinary use will be on the PDL and must be sold by prescription only.

Changes being made by Health Canada include:

- Ending Own Use Importation of MIA by animal owners effective November 13, 2017;
- Increased oversight and new rules for the handling of Active Pharmaceutical Ingredients for veterinary use starting May 17, 2018;
- Mandatory reporting of sales information related to MIA; and
- New requirements and the expansion of the veterinary health products program to increase access to alternative animal health products for production animals.

Questions about the regulatory and policy changes? Visit the Responsible use of Medically Important Antimicrobials in Animals section on the Government of Canada website (www.vetdrugs-medsvet@hc-sc.gc.ca).

If you have questions on the management of the inventory of medically important antimicrobials during the transition to December 1, 2018, visit (www.cahi-icsa.ca/antimicrobial-stewardship).

The Canadian Veterinary Medical Association (CVMA), along with the Canadian Animal Health Institute (CAHI) and other stakeholders, have been engaging with Health Canada's
Veterinary Drugs Directorate about these changes and will continue to support these actions with ongoing engagement and communication.

After broad stakeholder consultation and discussion, the Canadian Council of Veterinary Registrars and the CVMA’s Veterinary Pharmaceutical Stewardship Advisory Group developed Veterinary Oversight of Antimicrobial Use — A Pan-Canadian Framework for Professional Standards for Veterinarians regarding veterinary oversight of antimicrobial use; it is available for download on the CVMA website (www.canadianveterinarians.net).

Si vous avez des questions à propos de la gestion de l’inventaire des antimicrobiens importants sur le plan médical durant la transition jusqu’au 1er décembre 2018, visitez (www.cahi-icsa.ca/antimicrobial-stewardship).

L’Association canadienne des médecins vétérinaires (ACMV), l’Institut canadien de la santé animale (ICSA) et d’autres intervenants ont interagi avec la Direction des médicaments vétérinaires de Santé Canada à propos de ces changements et ils continueront d’appuyer ces actions dans le cadre d’engagements et de communications.

Après de vastes consultations et discussions avec les intervenants, le Conseil canadien des registraires vétérinaires et le Groupe consultatif sur la gouvernance des produits pharmaceutiques vétérinaires de l’ACMV ont créé le document Surveillance vétérinaire de l’utilisation des antimicrobiens — Un cadre de travail pancanadien pour les normes professionnelles régissant les médecins vétérinaires sur la surveillance vétérinaire de l’utilisation des antimicrobiens; ce document peut être téléchargé sur le site Web de l’ACMV (www.veterinairesaucanada.net).

Canadian Biosafety Guideline
Veterinary Practices: Physical Design and Operational Practices for Diagnostic Activities

Ligne directrice canadienne sur la biosécurité
Conception physique et pratiques opérationnelles dans le cadre d’activités de diagnostic en établissement vétérinaire

In Canada, facilities that conduct controlled activities, such as handling or storing human and zoonotic pathogens or toxins, are regulated under the Human Pathogens and Toxins Act (HPTA) and the Human Pathogens and Toxins Regulations (HPTR). As such, they require a Pathogen and Toxin Licence unless they meet the exclusion criteria specified in the HPTA or are exempted from the licensing requirement under the HPTR. The Veterinary Practices: Physical Design and Operational Practices for Diagnostic Activities guideline (the Guideline) describes biosafety precautions and recommendations for veterinary activities that are regulated by the HPTA and HPTR.

Au Canada, les installations qui effectuent des activités réglementées (p. ex., la manipulation ou l’entreposage) avec des agents pathogènes humains et zoonotiques ou des toxines sont réglementées en vertu de la Loi sur les agents pathogènes humains et les toxines (LAPHT) et le Règlement sur les agents pathogènes humains et les toxines (RAPHT). Ces installations nécessitent un permis, sauf si elles répondent aux critères d’exclusion spécifiés dans la LAPHT ou qu’elles sont exemptées de l’obligation d’obtenir un permis en vertu du RAPHT. La ligne directrice sur la Conception physique et pratiques opérationnelles dans le cadre d’activités de diagnostic en établissement vétérinaire (Ligne directrice) décrit les précautions et les recommandations pour les établissements vétérinaires où des activités de diagnostic qui ne requièrent pas de permis en vertu du RAPHT sont exécutées.

This Guideline provides biosafety precautions and recommendations for the safe handling of samples collected from animals that may be infected with Risk Group 2 (RG2) human pathogens. These will help to manage risks associated with personnel exposure to infectious material, and prevent the release of pathogens into the environment. Elements provided in the Guideline are based on the minimum physical containment and operational practice requirements for Containment Level 2 facilities specified in the Canadian Biosafety Standard, 2nd edition (2015). These elements encompass the principles of biosafety and biosecurity that veterinary practices can follow to demonstrate that all reasonable precautions have been taken to protect the health and safety of the public against the risks associated with the material in their possession, in accordance with the HPTA. Additional guidance can be found in the Canadian Biosafety Handbook, 2nd Edition (2016).

The Guideline is divided into 5 chapters:

• Chapter 1 provides background information, criteria for exclusion from the HPTA, and examples of situations that exempt veterinary practices from the requirement for licensing.
• Chapter 2, Physical Design Features, defines the basic facility design and engineering controls that may be established to minimize personnel exposure to potentially infectious material and to limit the spread of pathogens.
• Chapter 3, Operational Practices, refers to the administrative and procedural controls that may be put in place to prevent inadvertent exposure of the public and release into the environment of pathogens and potentially infectious material. This chapter also offers recommendations for the safe handling, use, storage, and disposal of RG2 pathogens and other biological material that may contain RG2 pathogens in veterinary practices.
• Chapters 4 and 5 are the Glossary and Resources, respectively.

The Centre for Biosecurity’s consultation included the Canadian Food Inspection Agency, provincial and territorial veterinary medical associations through the Canadian Veterinary Medicine Association (CVMA), and veterinarians. All comments were taken into considerations for the final version of this Guideline, which was published on the Government of Canada’s website and can be found at (www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance.html).

The Centre for Biosecurity would like to thank the CVMA for their collaboration and for coordinating the consultation with veterinary medical associations and veterinarians. We welcome your feedback and suggestions for the continual improvement of future editions of this guideline. Please contact us at (PHAC.pathogens-pathogens.ASPC@canada.ca).
Dr. Joyce Van Donkersgoed Named the 2017 Metacam® 20 Bovine Welfare Award Winner

The Dr. Tim Nickel of Boehringer Ingelheim Canada Ltd. presents the Prix Metacam MD 20 pour le bien-être des bovins

Dr. Joyce Van Donkersgoed of Coaldale, Alberta was named the recipient of the 2017 Metacam® 20 Bovine Welfare Award for her leadership in the Canadian beef industry to improve the welfare of feedlot animals.

The award is presented annually by the Canadian Association of Bovine Veterinarians (CABV) in partnership with Boehringer Ingelheim (Canada) Ltd.

“We are honored to announce Dr. Van Donkersgoed as the recipient of the 2017 Metacam® 20 Bovine Welfare Award,” said Dr. Germain Nappert, president of the CABV/ACVB. “Her leadership in the area of animal welfare is exemplary, and her work will help improve the welfare of feedlot animals across the country.”

Dr. Van Donkersgoed operates a private feedlot practice in southern Alberta where she provides emergency, herd health and production services, and research and regulatory services to her clients. She was instrumental in the development of the Canadian Feedlot Animal Care Assessment tool for auditing animal welfare, which has been certified by the Professional Animal Auditor Certification Organization. It is the first certified audit designed for the feedlot segment of the food production industry.

“You can’t manage what you don’t measure, which includes animal welfare, and we must continually strive to improve,” Dr. Van Donkersgoed said. “Beef veterinarians have a key ethical and moral responsibility to ensure animal welfare whilst balancing the needs of their clients. It isn’t always simple or easy to do but persistence does pay off over time if you don’t give up and are doing the right thing for the animals, which is ultimately best for the client.”

The Metacam® 20 Bovine Welfare Award is given annually to a DVM or animal scientist working in Canada, a faculty member or a graduate student of a Canadian university to recognize his/her achievements in advancing the welfare of animals via leadership, public service, education, research/product development, and/or advocacy.

The well-being of the animals is the ultimate best for the clients, which is best for the client.

La Dre Joyce Van Donkersgoed est nommée lauréate du Prix 2017 MetacamMD 20 pour le bien-être des bovins

La Dre Joyce Van Donkersgoed est nommée lauréate du Prix 2017 MetacamMD 20 pour le bien-être des bovins pour son leadership dans l’industrie bovine canadienne afin d’améliorer le bien-être des animaux des parcs d’engraissement.

Le prix est décerné annuellement par l’Association canadienne des vétérinaires bovins (ACVB) en partenariat avec Boehringer Ingelheim (Canada) Ltée.

« Nous sommes honorés d’annoncer que la Dre Joyce Van Donkersgoed est la récipiendaire du Prix 2017 MetacamMD 20 pour le bien-être des bovins », a dit le Dr Germain Nappert, président de l’ACVB. « Son leadership dans le domaine du bien-être animal est exemplaire et son travail aidera à améliorer le bien-être des animaux des parcs d’engraissement partout au pays. »

La Dre Joyce Van Donkersgoed gère une pratique privée pour les parcs d’engraissement situés dans le sud de l’Alberta où elle offre à ses clients des soins d’urgence ainsi que des services liés à la santé du troupeau et à la production, à la recherche et à la réglementation. Elle a joué un rôle crucial dans l’élaboration de l’outil servant à l’évaluation des soins aux animaux dans les parcs d’engraissement canadiens, en vue de vérifier le bien-être animal, qui a été certifié par la Professional Animal Auditor Certification Organization. Il s’agit du premier outil conçu pour le segment des parcs d’engraissement de l’industrie de la production alimentaire.

« On ne peut pas gérer si on ne mesure pas, ce qui comprend le bien-être animal et nous devons continuellement travailler afin de nous améliorer », a dit la Dre Van Donkersgoed. « Les vétérinaires bovins ont une responsabilité éthique et morale afin d’assurer le bien-être animal tout en tenant compte des besoins de leurs clients. Ce n’est pas toujours simple mais la ténacité est récompensée à la longue si l’on persévère et que l’on travaille dans l’intérêt supérieur des animaux, ce qui, en bout de ligne, sera aussi la meilleure option pour le client. »

Le Prix MetacamMD 20 pour le bien-être des bovins est décerné annuellement à un médecin vétérinaire ou à un expert des sciences animales travaillant au Canada, à un professeur ou à un étudiant diplômé d’une université canadienne afin de reconnaître ses réalisations pour l’avancement du bien-être des animaux en faisant preuve de leadership, en travaillant pour le bien commun, par l’éducation, la recherche et le développement de produits et/ou en défendant des intérêts.

Dr. Tim Nickel (Boehringer Ingelheim Canada Ltd.) presents the Metacam 20 Bovine Welfare Award to Dr. Joyce Van Donkersgoed.

Le Dr Tim Nickel (Boehringer Ingelheim Canada Ltée) présente le Prix Metacam 20 pour le bien-être des bovins à la Dr Joyce Van Donkersgoed.
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Case Report Rapport de cas

Acute surgical intervention for a depressed skull fracture causing a laceration to the brain parenchyma from a bite wound in a dog

Natasha Hodgson, Andrea Walters, Corinne Lawson, Devon Hague, Stephen Joslyn, Maureen McMichael

Abstract — A 5-month-old spayed female mixed breed dog was attacked by another dog causing multiple fractures of the left calvarium with a fragment penetrating through the gray matter of the parietal lobe. Surgery was performed to remove the bone fragment. A 6-month follow-up showed dramatic improvement in neurologic status.

Résumé — Intervention chirurgicale d’urgence pour une fracture du crâne pénétrante causant une laceration au parenchyme du cerveau suite à une morsure chez une chienne. Une chienne stérilisée de race croisée âgée de 5 mois a été attaquée par un autre chien causant des fractures multiples de la voûte crânienne gauche avec un fragment pénétrant dans la matière grise du lobe pariétal. La chirurgie a été réalisée pour enlever le fragment d’os. Un suivi de 6 mois a démontré une amélioration spectaculaire de l’état neurologique.

(T raduit par Isabelle Vallières)

T raumatic brain injury (TBI) is defined as an alteration in brain function caused by an external force (1). It is a common presentation in the veterinary emergency room and can be caused by dog bites, motor vehicle accidents, falls from heights, crush injuries, and human assaults (2). Most cases of TBI are treated medically; however, surgical intervention may become necessary. A major indication for surgery in human medicine is a poor response to medical treatment resulting in worsening intracranial pressure (ICP) measurements (3). Since ICP monitoring is rarely performed, reasons for surgery in veterinary medicine are controversial and may include inadequate response to medical therapy, depressed or open skull fractures, ongoing hemorrhage, foreign bodies, or hematomas (2,4). There is little in the veterinary literature about surgical intervention for TBI, and currently it is unknown even in human medicine if there is a positive correlation between surgery and clinical outcome (5). To the authors’ knowledge this is the first veterinary report describing acute surgical intervention (< 24 h) for depressed skull fractures with fragment penetration into the brain parenchyma resulting from a bite wound.

Case description
A 5-month-old 2.0-kg spayed female mixed breed dog was examined 3 h after being attacked by another dog. On presentation to the Veterinary Teaching Hospital emergency service, the dog had evidence of 2 open bite wounds on the dorsal aspect of her head with no other obvious external injuries. The dog had a heart rate of 164 beats/min with occasional ventricular prematurity complexes recorded on electrocardiogram examination. The dog was hypertensive with a systolic blood pressure measured at 210 mmHg with Doppler. On neurologic examination, the dog had an obtunded mentation and a rotary nystagmus in both eyes. No other cranial nerve deficits were identified. The dog was non-ambulatory tetraparetic with decreased withdrawal reflexes in both thoracic limbs. The modified Glasgow Coma Score (MGCS) was 13 (6). The patient’s injury was neurolocalized to the central vestibular areas (brainstem) due to the mentation change, ambulation deficits, and nystagmus. The decreased withdrawals could be explained by damage to the vestibulospinal tracts causing inhibition to the gray matter interneurons and/or injury to the medial longitudinal fasciculus, affecting the dorsal ventral funiculus of the cervical and cranial thoracic spinal cord segments.

Initial stabilization included intravenous boluses of Lactated Ringer’s solution (Hospira, Lake Forest, Illinois, USA), total dose of 45 mL/kg body weight (BW), mannitol (Nova-Tech, Grand Island, Nebraska, USA), 1 g/kg BW, IV bolus, pain management with fentanyl citrate (Hospira), 6 mg/kg BW bolus then 3 to 5 μg/kg BW/h, IV, and supplemental oxygen via an oxygen chamber (FiO₂ of 40%). The wounds on the head were clipped and cleaned aseptically with chlorhexidine gluconate solution (Hibiclens; Mölnlycke Health Care, Norcross, Georgia, USA) and antibiotics were started (ampicillin/sulbactam; Auromedics Pharma, Dayton, New Jersey), 30 mg/kg BW, IV, q8h.
An initial STAT blood profile (Critical Care Express; Nova Biomedical, Waltham, Massachusetts, USA) did not identify significant abnormalities. Following initial triage, the dog became stuporous. After stabilization treatments, the dog’s mental status changed from stuporous to dull. Using minimal sedation with fentanyl citrate, 5 μg/kg BW per hour, IV, and midazolam (West-Ward, Eatontown, New Jersey, USA), 0.1 mg/kg BW, IV, computed tomography (CT; GE Lightspeed 16-slice; GE Healthcare, Milwaukee, Wisconsin, USA) scan of the skull, cervical spine, and thorax was performed using the VetMouse Trap (Universal Medical Systems, Solon, Ohio, USA). Multiple fractures of the left calvarium involving the left frontal, parietal, and occipital bones were found (Figures 1A, B). The fractures were open with a small gas pocket seen within the calvarium indicating pneumocephaly. One bony fragment was noted to be displaced toward the left lateral ventricle, penetrating through the gray matter of the parietal lobe. The fractures and overall compression of the left hemisphere were also associated with mid-line shift of the falx cerebri and reduced attenuation of the left cerebral white matter consistent with white matter edema (Figures 2A, B). The patient was hospitalized overnight with supportive care and monitoring.

During the first 12 h of hospitalization, the dog returned to a stuporous state. The dog developed an intermittent rotary and vertical nystagmus OU, miotic pupils OU, and absent menace OU. The dog continued to be non-ambulatory tetraparetic. Her MGCS at this time decreased to 11. An additional dose of mannitol (0.5 g/kg BW, IV) was administered. Her mentation improved to an obtunded state, with normalization of pupil size and a MGCS of 15 was recorded. Although her mental status improved with the additional dose of mannitol, a craniectomy was advised due to concerns of increased intracranial pressure secondary to the compressive fracture.

Based on the location of the depressed skull fracture on CT and history of a bite injury with concern for bacterial infection, surgical intervention was recommended to remove the bone fragment and lavage the wound. Levetiracetam (Keppra; Hospira), 30 mg/kg BW, IV, q8h, was started before surgery, which was
Conducted 16 h after presentation to the emergency room. The dog was premedicated with midazolam, 0.2 mg/kg BW, IV, lidocaine (Hospira), 2 mg/kg BW, IV, and fentanyl citrate, 10 µg/kg BW, IV, and induced with propofol (Hospira), 1 mg/kg BW, IV. A continuous rate infusion of propofol, fentanyl citrate, and lidocaine was continued during surgery. Inhalant anesthetics were not used during the procedure.

The patient was positioned in sternal recumbency with the head elevated. The hair over the calvarium was shaved and the area prepared with chlorhexidine gluconate solution. A ~4-cm skin incision was made along the midline over the frontoparietal region. The underlying musculature was bruised and edematous. The interscutularis, frontalis, and temporalis muscles were sharply dissected with a #15 blade and reflected away from the skull using a periosteal elevator.

The fractured parietal bone was observed upon exposure of the skull. A depressed fracture fragment in the parietal lobe was penetrating deep into the brain parenchyma. This bone fragment was removed and measured ~1.5 cm in diameter. There was a mild amount of hemorrhage and a small area (~2 cm) of damaged cerebral cortex from which the bone fragment had been removed. The remaining cortex appeared grossly normal. The surgical site was flushed and lavaged with 0.9% sodium chloride irrigation (Hospira) to minimize contamination from the bite wound. An aerobic culture was negative for growth. The surgical site was flushed and lavaged with 0.9% sodium chloride irrigation (Hospira) to minimize contamination from the bite wound. An aerobic culture was negative for growth. There was no evidence of gross hemorrhage or brain swelling prior to closure. A thin piece of gelfoam was placed over the bite wound. An aerobic culture was negative for growth.

The dog continued to be intermittently obtunded. The dog had a more significant rotary nystagmus, developed anisocoria, and had a persistent profound vestibular ataxia and tried to roll to the left continuously. She had excellent withdrawals and motor activity in all limbs.

The one wk recheck from discharge showed slow improvement. The dog continued to be dull, but was more responsive and aware of her surroundings. The nystagmus was no longer present. The dog was also regaining some vision in her left eye and had an intermittent menace response, but continued to have an absent menace response in the right eye. She was experiencing profound vestibular ataxia and tried to roll to the left continuously. She had excellent withdrawals and motor activity in all limbs.

The dog continued to improve, and at her 2 mo recheck she was bright and alert and had a menace response in both eyes. There was a mild left head tilt and she was ambulatory with moderate vestibular ataxia. In addition, according to the owners, she had regained most of her pre-injury personality. At 6 mo, the owners stated that they were very happy with the dog’s continued improvement with only minor vestibular ataxia still present.

**Discussion**

This case report describes the use of a craniectomy within 24 h of TBI to remove a penetrating bone fragment causing a parenchymal brain lesion. There is a paucity of literature in veterinary medicine on craniectomies in the setting of TBI. There have been case reports of dogs and cats having craniectomies performed; however, these reports describe debridement of an abscess associated with a previous injury or removal of a partial bullet fragment a few days after the incident in a patient with normal mental status (7–11). The present case specifically reports surgery in the acute setting (<24 h) after a laceration causing a compressive fracture within the brain parenchyma.

Based on the examination, this patient’s injury was neurolocalized to the central vestibular portions of the brain based on her mentation (abnormal level of consciousness), gait abnormalities (tetraparesis and vestibular ataxia), and cranial nerve deficits (nystagmus). The progressive changes on her neurologic examination, specifically the miosis and worsening mentation change, indicated compression of the midbrain or a severe prosencephalic lesion. The progression to a deficit with the menace response most likely indicated a lesion affecting the central visual cortex.

The CT imaging showed evidence of the fracture affecting the skull and causing damage to the cerebral cortex. There are 2 potential explanations for her neurologic clinical symptoms.
One possibility is that the cerebral edema also caused secondary edema and swelling leading to increased intracranial pressure causing transtentorial and/or transforaminal herniation, which is likely, given the clinical signs of a Cushing's reflex, changes in mentation, and pupil size. The brainstem is difficult to accurately image via CT (due to beam hardening artifact). The other possibility is that the traumatic injury caused a lesion in the conscious vestibular pathway. This is less well-described in the canine species and is likely associated with the conscious auditory pathway projecting from the thalamus (via the medial geniculate nucleus), through the internal capsule, to the vestibular cortex in the temporal lobe (12).

There is substantial controversy about the use of medical versus surgical management for stabilization of veterinary patients with TBI after primary injury (which is the acute, direct injury causing mechanical damage). Medical management is aimed at minimizing secondary injury, which occurs minutes to days following the trauma and consists of systemic physiologic insults that may decrease cerebral perfusion pressure, worsen cerebral edema, increase intracranial pressure, and damage the blood-brain barrier (2). Our patient, therefore, received 16 h of medical management with intravenous fluids to prevent hypotension, oxygenation to prevent hypoxia, and mannitol to decrease presumed spikes in intracranial pressure from cerebral edema (13). Seizures have also been reported to worsen secondary injury, and given that this patient had several risk factors for the development of early seizures after TBI (13), the dog was started on prophylactic anti-convulsants (14,15). During this stabilization period, the neurologic status of the patient was assessed serially to determine the efficacy of treatments (including motor activity, brain stem reflexes, and level of consciousness to calculate the MGCS score) (6).

While medical therapy remains the mainstay of TBI in veterinary medicine, surgery may be warranted in cases of failed medical treatment, ongoing hemorrhage, hematomas, foreign bodies, pneumocephalus, depressed skull fractures, midline shift of falx cerebri, and compression of the basilar cisterns (2,4,9,16,17). These recommendations are based on little data, as the only experimental surgical study involved healthy dogs and concluded that ICP decreased after the combination of surgical therapies (including craniectomy and durotomy) and medical therapies (including ICP lowering treatments). This study, however, did not differentiate medical versus surgical management (18). In our patient, although medical management was associated with an overall neurological improvement based on MGCS, surgery was recommended due to the presence of depressed skull fractures, suspected contamination from the bite wound, pneumocephaly, and a midline shift on CT. After surgery, the dog displayed continued evidence of neurological improvement, and at 6 mo after surgery the dog only had mild neurological deficits.

There have been numerous human studies investigating the role of decompressive craniectomies in TBI with varying outcomes. Some of the evidence indicates that they may be useful in patients with severe TBI refractory to medical therapy. A 2012 comprehensive review in human medicine showed that decompressive craniectomies may be associated with reduced ICP values at 24 h and 48 h, although the correlation of ICP with clinical outcome is uncertain (19). In addition, the RESCUicp study concluded that patients undergoing surgery had lower mortality rates at 6 mo after injury, but higher vegetative states, compared with standard medical therapy (5). The DECRA trial, evaluating early decompressive craniectomy (< 72 h) versus standard medical therapy in patients with severe diffuse TBI and refractory intracranial hypertension, showed that although surgery decreased ICP and length of stay in the intensive care unit, it was associated with more unfavorable outcomes (20). Similarly, a 2016 systematic review concluded that decompressive craniectomy was not associated with a favorable outcome compared to medical therapy (1). Due to a lack of consensus on treatments for TBI in humans, the Brain Trauma Foundation recommendations were modified to include decompressive craniectomies for the management of severe TBI, but recognize that there is insufficient high-quality body of evidence on the topic (21).

Another ongoing debate in both human and veterinary medicine involves the ideal timing of surgical intervention after TBI. Given that there have been several reports of intracranial abscess formation after a bite wound in dogs (7,8,10), it was presumed in this case that early surgical intervention and debridement of an open wound to the brain parenchyma would minimize the risk of bacterial proliferation and decrease patient morbidity. As such, surgery was recommended as soon as the dog was stable (< 24 h after injury), and was associated with a favorable outcome. There is no veterinary evidence to support ideal timing of surgical debridement, and human literature is contradictory on this matter. The time frame to the development of generalized infection is considered to be approximately 6 h, and thus prompt wound management would be ideal (22). Culture results from samples obtained during surgery were negative, although only aerobic cultures were submitted. Aerobic bacteria are most common in nonpurulent dog bite wounds which is the reason, in addition to cost, anaerobic culture was not performed. It is possible that anaerobic bacteria were present (23). One prospective human study showed that there was no detriment in outcome in patients who received early (< 24 h) surgical management with a decompressive craniectomy compared to those who had longer medical management and stabilization (> 24 h) before surgery (17). Another human study, however, concluded that a higher survival rate was found in the group of patients who had late decompressive craniectomies (after > 24 h of stabilization or clinical deterioration), compared to early surgical intervention (< 24 h after injury) (24).

Finally, recommendations about treatment may be centered on certain prognostic indicators associated with TBI. In humans, poor prognostic indicators on advanced imaging include compressed or absent basilar cisterns, the presence of subarachnoid hemorrhage, midline shift, and intracranial hemorrhagic lesions (25). In addition, skull fractures in certain anatomical locations, brain herniation, and an increased size of the intraparenchymal lesion, have also been associated with a poor prognosis in dogs and humans (26–29). In dogs with head trauma (but not specifically TBI), prognosis was also associated linearly with a decreased MGCS, poor perfusion (evidenced by
metabolic acidosis and hyperlactatemia) and decreased SpO₂, severe concurrent injuries, or a requirement for hypertonic saline or endotracheal intubation (30). In the present case, the dog displayed many of these negative prognostic indicators on presentation, including intracranial hemorrhage, midline shift, and skull fractures. Despite these, the dog showed some improvement with pre-operative medical management (oxygen, intravenous fluids, and mannitol therapy), and remained stable during the peri- and post-operative periods. Although it is unknown whether medical management would have resulted in the same outcome, this case report may provide evidence to support that the use of early aggressive medical management and surgical intervention can be associated with a positive outcome, even in severely affected dogs.

We describe a canine patient with a compressive skull fracture and penetration into the brain parenchyma after a traumatic injury and subsequent surgical removal of the fragment. After surgery the patient continued to improve, with only mild neurologic abnormalities remaining. Surgical intervention may be indicated in patients with contaminated, compressive, and open fractures into the brain parenchyma. Based on the insufficient data on decompressive craniectomies in dogs with TBI, additional studies are necessary to determine which patients will benefit from medical or surgical therapy, the ideal timing of surgery, and prognostic indicators. Until then, every TBI case should be assessed individually and the clinician should determine whether medical therapy alone is sufficient, or if the case should be assessed individually and the clinician should determine whether medical therapy alone is sufficient, or if the patient may benefit from surgical intervention, based on their type and severity of injuries, level of contamination, response to medical management, and ability to withstand anesthesia. As seen in this case report, aggressive therapy may yield a good prognosis despite severe parenchymal lesions of the brain.  

References
Dietary imbalances in a large breed puppy, leading to compression fractures, vitamin D deficiency, and suspected nutritional secondary hyperparathyroidism

Moran Tal, Jacqueline M. Parr, Shawn MacKenzie, Adronie Verbrugghe

Abstract — A 6-month-old intact female giant schnauzer dog fed a nutritionally unbalanced homemade diet was evaluated because of a 1-month history of lameness and difficulty walking. Abnormalities identified on ancillary tests, in conjunction with the dog’s clinical improvement following diet change, suggested a diagnosis of vitamin D deficiency and nutritional secondary hyperparathyroidism. This report underlines the importance of appropriate feeding management, especially during the vulnerable growth phase.

Abstract — Des équilibres alimentaires chez un chiot de grande race causant des fractures de compression, une carence en vitamine D et de l’hyperparathyroïdisme souffrante secondaire à la nutrition. Une chienne Schnauzer géante intacte âgée de 6 mois qui consommait une diète maison qui n’était pas équilibrée sur le plan nutritionnel a été évaluée en raison d’une anamnèse de 1 mois de boiterie et de difficultés ambulatoires. Les anomalies identifiées sur des tests anciens, de concert avec l’amélioration clinique du chien après le changement de diète, suggéraient un diagnostic de carence en vitamine D et d’hyperparathyroïdisme nutritionnel secondaire. Ce rapport souligne l’importance d’une gestion appropriée de l’alimentation, particulièrement durant la phase de croissance vulnérable.

Growing dogs have more demanding dietary requirements than adult dogs (1–3). Unbalanced diets can lead to nutritional deficiencies or excesses, resulting in detrimental health consequences, especially during growth (4). These clinical consequences are most evident in puppies (5,6) with adult weights > 23 kg (6), as they are more prone to diseases related to rapid growth. Such diseases include developmental orthopedic diseases (DODs) (e.g., osteochondritis dissecans, osteochondrosis, retained cartilaginous core, panostitis, hypertrophic osteodystrophy, canine elbow dysplasia, and hip dysplasia) (7) and dilated cardiomyopathy (8). Previous studies demonstrated malnutrition, especially energy excess, suboptimal concentrations of dietary vitamin D (VitD), calcium (Ca), and phosphorus (P), as well as a reverse Ca to P ratio (Ca:P), to be a risk factor for the development of DODs in large breed puppies (6,9). This report presents an assessment of the nutritional requirements for growth of a large breed puppy as a result of clinical implications due to feeding an unbalanced homemade diet and accounts for the key nutritional factors mentioned above when designing the diet plan.

Case description

A 6-month-old, intact female giant schnauzer dog was presented to the Ontario Veterinary College Health Sciences Centre (OVC-HSC) Neurology Service with an acute onset of generalized pain and paraparesis. Medical history revealed lameness, difficulty walking, and yelping/painful episodes with an unknown trigger during the past 2 mo. One month before consultation at the OVC-HSC, the dog was presented to the referring veterinarian with suspected urinary infection — a urine pH of 8, elevated leukocytes (30 to 50/400 field), and a small amount of bacteria on urinalysis (urine sample collected via free catch). The patient was treated initially with amoxicillin/clavulanic acid [12.5 mg/kg body weight (BW), PO, q12h], based on a first-line drug choice approach (10). On physical examination at the OVC-HSC the puppy was tense and rigid, especially on hind limb palpation. Lumbar pain was noted particularly with
Table 1. Serum biochemical panel and vitamin D profile of a 6-month-old intact female giant schnauzer dog at presentation and before discharge.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blood analysis</th>
<th>Reference range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.6</td>
<td>5.39 to 9.22</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Total protein</td>
<td>61</td>
<td>45 to 73</td>
<td>g/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.7</td>
<td>2.6 to 3.7</td>
<td>g/L</td>
</tr>
<tr>
<td>Globulins</td>
<td>24</td>
<td>22 to 35</td>
<td>g/L</td>
</tr>
<tr>
<td>ALP</td>
<td>473</td>
<td>126 to 438</td>
<td>U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>14</td>
<td>≤ 32</td>
<td>U/L</td>
</tr>
<tr>
<td>CK</td>
<td>1058</td>
<td>40 to 192</td>
<td>U/L</td>
</tr>
<tr>
<td>GGT</td>
<td>4</td>
<td>≤ 4.3</td>
<td>U/L</td>
</tr>
<tr>
<td>Amylase</td>
<td>1238</td>
<td>≤ 1683</td>
<td>U/L</td>
</tr>
<tr>
<td>Lipase</td>
<td>28</td>
<td>≤ 139</td>
<td>U/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>8.8</td>
<td>2.6 to 12.9</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2.05</td>
<td>0.17 to 2.22</td>
<td>μmol/L</td>
</tr>
<tr>
<td>BUN</td>
<td>4.2</td>
<td>3.3 to 13.3</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>83.1</td>
<td>23.9 to 77.8</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>119</td>
<td>99 to 120</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.8</td>
<td>1.4 to 5.2</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.8</td>
<td>5.6 to 9.6</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.9</td>
<td>3.9 to 6.1</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>156</td>
<td>139 to 159</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Total calcium</td>
<td>2.1</td>
<td>2.5 to 3.3</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Vitamin D profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iCa</td>
<td>1.5</td>
<td>1.25 to 1.45</td>
<td>mmol/L</td>
</tr>
<tr>
<td>PTH</td>
<td>4.6</td>
<td>0.50 to 5.80</td>
<td>pmol/L</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>8</td>
<td>60 to 215</td>
<td>pmol/L</td>
</tr>
</tbody>
</table>

ALP — alkaline phosphatase; ALT — alanine aminotransferase; GGT — gamma-glutamyl transpeptidase; BUN — blood urea nitrogen; CK — creatine kinase; iCa — ionized calcium; PTH — parathyroid hormone; 25(OH)D — 25-hydroxyvitamin D.

Values at presentation. Reference range: puppies 4 to 6 months old (Reference 15).

Values at discharge (2 days following presentation). Reference range: DCPAH — Michigan State University, Diagnostic Center for Population & Animal Health.

The dog was tachypneic and in pain upon abdominal palpation. She was non-ambulatory in her hind legs, lacked proprioception, had a hunched posture, and was knuckling bilaterally.

Nutritional screening evaluation was performed according to the World Small Animal Veterinary Association nutritional assessment guidelines (11). Growth, feeding with an unconventional diet, and the medical condition of the puppy were immediately identified as nutritional risk factors. The owner had fed the puppy a homemade diet since it was 2 mo old. The homemade diet was based on instructions provided on the Canine Life website (http://www.theskyesthelimit.com/). Some alterations were made by the owner. The recipe contained a combination of ingredients; i.e., 2 lbs of regular ground beef, 2 cups of grated carrots, 2 cups of green beans and broccoli, 2 to 3 tbsp of refined sunflower oil, 2 red apples, 3 eggs, 2 cloves of garlic, 3 to 4 cups of brown rice flour, and 1/2 a cup of oat bran per day. All ingredients were mixed together and baked into muffins. Batches of raw ingredients were prepared weekly by the owner and stored in the freezer. Four meals were fed per day. In addition to this daily portion, the dog received 1/2 to 1 cup of cooked white organic rice, apples and carrots, one capsule of cod liver oil, and 1 tsp of bone meal. The brands and manufacturers of the supplements were unknown to the owner. The patient weighed 20.36 kg with a normal body condition score (BCS) of 5/9 (12) and mild muscle wasting, likely due to disuse and potentially malnutrition (13). Previous information regarding the puppy’s BCS or weight was not obtainable. Therefore, although growth standards for various dog breeds are becoming more available, to compare an individual’s growth to a healthy reference population (14), this method was not applicable in the current case. An extended nutritional evaluation was indicated (11). The owner consented to further diagnostics and assessment of the homemade diet. The complete blood (cell) count (CBC) was unremarkable. When compared to a reference range for similarly aged dogs (15), serum total Ca and P concentrations were decreased, and serum alkaline phosphatase (ALP) and creatine kinase (CK) were elevated on the biochemistry profile (Table 1). A sterile urine sample was obtained through cystocentesis. Urinalysis showed elevated leukocytes (200 to 250/400× field), pH = 7.5, specific gravity = 1.020. Urinary culture revealed Escherichia coli, susceptible to various antibiotics including cefazolin. Treatment with cefazolin (TEVA cefazolin; Teva Canada, Scarborough, Ontario), 22 mg/kg body weight (BW), IV, every 12 h was initiated.

Differential diagnoses included nutritional VitD deficiency, nutritional secondary hyperparathyroidism (NSH), diseospondylitis, autoimmune meningoymelitis, spinal empyema, trauma (causing fracture/luxation), and parasitic myelitis (toxoplasma, neospora). Primary hyperparathyroidism was less likely, as hypocalcemia was evident, and not hypercalcemia (16). As there was no evidence of renal dysfunction on urinalysis or blood analysis, renal secondary hyperparathyroidism was considered unlikely (17).

For further evaluation of the acute neurological abnormalities, thoracolumbar spinal radiographs were taken and magnetic resonance imaging (MRI) was performed. Diagnostic imaging tests were focused on the thoracic and lumbar spine based on localization of neurological deficits and normal findings on physical and orthopedic examination of the front and hind limbs. On orthogonal radiographs of the thoracic and lumbar spine the entire axial skeleton had a reduced opacity with thinning of the cortices and reduced trabecular markings. Multiple vertebral bodies were misshapen, being shortened and more cuboidal than rectangular in shape. The L2 vertebral body had a trapezoidal shape and centered at the L1-L2 intervertebral disc there was mild kyphosis (Figure 1). Magnetic resonance imaging of the thoracic and lumbar spine was carried out to further evaluate the abnormalities seen on spinal radiographs and to determine the sites of and degree of spinal cord compression contributing to the acute neurological deficits (Figure 2). Sequences acquired for images included: T2*-weighted single shot fast-spin echo (FSE) in a sagittal plane; T2-weighted FSE in sagittal, dorsal, and transverse planes; T1-weighted FSE in transverse plane; short tau inversion recovery (STIR) in a sagittal plane; T1-weighted FSE in transverse and sagittal planes following intravenous contrast administration. The MRI showed compression fractures of multiple vertebrae (chronic fractures) and an intramedullary spinal cord lesion from L2 to L5 (Figure 2A). A vertebral compression at L4 was associated with marked narrowing of the vertebral canal, attenuation of the subarachnoid space, and loss of the epi-dural fat (Figure 2B). The muscles surrounding the L4 vertebral body had increased signal intensity on T2-weighted and STIR
images, and were moderately contrast enhancing. There was no evidence of spinal cord compression at the T8 or L2 vertebrae (Figure 2C). Given the severity of the changes at this level an acute fracture was suspected and the paraparesis was attributed to this lesion. The growth plates appeared normal for a dog this age, with no indication that the compression fractures involved the endplates. The differential diagnosis for the intramedullary lesion included edema or syringohydromyelia.

The diet was analyzed using computer software (Balance IT online formulation and supplements, West Sacramento, California, USA) and a homogenized sample of the diet (obtained from the owner) was sent for proximate analysis and analyses of minerals (SGS Agri-food Laboratories, Guelph, Ontario) and VitD (NF EN 12821: Determination of vitamin D by high performance liquid chromatography (HPLC) — Measurement of cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) (Table 1). Both analyses included the main recipe, the additional rice portion, and the 2 supplements. For the software analysis, since the owner was not familiar with the brands or manufacturers, commercially available supplements were added to match the recipe as closely as possible, so that it could be compared to the laboratory analysis. The computer-generated diet analysis represents an estimation of the dietary nutrient profile based on ingredient composition available in an online nutrient database [United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference Release]. However, the laboratory diet analysis represents the actual nutrient composition of this puppy’s diet (all ingredients and supplements, per owner). The findings of this laboratory analysis were not available until 10 d after presentation. Therefore, an initial computer-generated diet analysis was performed and revealed deficiencies in protein, Ca, chloride, copper, iodine, iron, P, potassium, sodium, selenium and zinc, vitamin E, choline and riboflavin (the most relevant nutrients are shown in Table 2).

Based on the dog’s clinical presentation, initial diagnostics (abnormalities on blood analysis, radiographs and MRI), and the presence of nutrient deficiencies assessed by computer software (Table 2), the presumptive diagnosis was NSH (18,19). The prognosis for this patient was guarded to poor, and euthanasia was considered. However, the owner decided to pursue treatment.

Previous studies have demonstrated malnutrition, especially energy excess, suboptimal dietary VitD, Ca, and P content, as well as a reverse Ca:P ratio, to be a risk factor for DODs in large breed puppies (6,9). Accordingly, on the day of presentation the puppy was immediately transitioned to a balanced commercial large breed puppy dry food (Table 2), accounting for the key nutritional factors mentioned. The daily energy requirement (DER) was calculated, based on a current ideal body weight of 20.36 kg, to be 1476 kcal/day using a DER factor of 2.2 [DER = 70 × (20.36)^0.75 × 2.2 = 1476 kcal/day] (20,21).
The DER factor was chosen for a growing puppy that reached more than 50% of adult weight, but accounted for the dog being cage rested for 6 wk due to vertebral compression fractures, and was therefore lower than the recommended 2.5 for this growth period (20).

Prior to discharge, 2 d following admittance, a blood sample was taken for analysis of parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], and ionized Ca (iCa) (Diagnostic Centre for Population and Animal Health, Lansing, Michigan). The serum PTH concentration was within the reference range, the serum 25(OH)D concentration was low, and the serum iCa concentration was slightly elevated (Table 1). At the time the blood sample was taken, the dog had been consuming a commercial puppy food for 48 h.

The results of the laboratory diet analysis became available 10 d after presentation. This analysis focused on fewer nutrients due to costs, so comparison to the computer-generated diet analysis was restricted to the nutrients assessed by the laboratory. Differences between the 2 analyses included higher Ca, P, Ca:P, Na, VitD and lower protein with the computer software analysis compared with the laboratory analysis. These differences resulted from average nutrient profiles of the ingredients based on information from an online nutrient database (USDA — National Nutrient Database for Standard Reference Release) used within the computer software, and the addition of generic supplements to the recipe by the authors, as detailed information from the owner was lacking. Still, similar end results were obtained (Table 2), i.e., minimal dietary Ca and P concentrations, reversed Ca:P ratio, and dietary VitD content that was below the detection level of 100 IU/kg DM. These results suggested the diagnosis of NSH and also that the nutritional VitD deficiency most likely resulted in rickets. This was based on the dog’s young age and the consumption of an unbalanced diet since 2 months of age, although X-rays of the long bones were not performed to confirm this clinical diagnosis (4,18).

Two months after discharge, the puppy was re-checked at the OVC-HSC Neurology Service and the Clinical Nutrition Service. The puppy was bright, alert, and responsive, and the owner noted the positive trend for her recovery approximately 3 wk after discharge. At that time, neurological examination revealed marked improvement with only mild residual ataxia. The dog had also regained strength and had improved activity. On screening nutritional assessment (11), the patient weighed 24 kg and had a BCS of 6/9 (12) with mildly underdeveloped muscle (13). The owner confirmed feeding of the recommended commercial large breed puppy dry food (Table 2). Treats and unbalanced human foods (e.g., salmon skin, apple, carrot, and table scraps) were also given, but the exact amounts were unknown. The risks of excessive energy intake resulting in rapid growth rate and/or obesity during the growth period were explained to the owner, and the link to DODs was emphasised (22). Also, the risks of unbalanced nutrition were reiterated to the owner. The owner agreed to reduce the treats and unbalanced human foods to no more than 10% of the recommended DER (23). The dog’s DER was recalculated to be 1446 kcal/day based on the estimated ideal body weight, which was reassessed to be 22.5 kg [24 kg × 75% lean mass]/80% lean mass] (24). The DER factor was reduced from 2.2 to 2 because of inactivity and overweightness [DER = 70 × (22.5)^0.75 × 2.0 = 1446 kcal/day] (20). The diet remained the same, and a gradual increase in activity was recommended to improve muscle mass.

Follow-up with the owner 1 year after discharge revealed that the dog was doing well, although she became tired easily. Once rested, she resumed her normal activity. Her hunched posture was permanent. The owner was still feeding her the recommended commercial large breed puppy dry food (Table 2); however, he was about to transition her to an adult food, from the same manufacturer. The dog still received various treats in small amounts (e.g., cooked turkey necks, vegetables, fruits, and some of the muffin mixture that was given to her before

### Table 2. Nutrient profiles of an unbalanced homemade diet, fed to a 6-month-old intact female giant schnauzer, and of the commercial puppy dry food given to this puppy following admission. The homemade diet was assessed by both computer software and laboratory analysis. The nutrient profiles of both diets are compared to the National Research Council (2) recommended allowance for growth and American Association of Feed Control Officials (AAFCO) (3) minimum requirements for growth.

<table>
<thead>
<tr>
<th>Homemade diet</th>
<th>Softwarea</th>
<th>Laboratoryb</th>
<th>Puppy dry foodc</th>
<th>NRC (2)</th>
<th>AAFCO (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>58.99</td>
<td>54.07</td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (% DM)</td>
<td>13.18</td>
<td>18.48</td>
<td>30.60</td>
<td>17.50</td>
<td>22.00</td>
</tr>
<tr>
<td>Ca (% DM)</td>
<td>0.54</td>
<td>0.09</td>
<td>1.11</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td>P (% DM)</td>
<td>0.59</td>
<td>0.37</td>
<td>0.84</td>
<td>1.00</td>
<td>0.80</td>
</tr>
<tr>
<td>Ca:P ratio</td>
<td>1:1.09</td>
<td>1:4</td>
<td>1:3.2:1</td>
<td>1.2:1c</td>
<td>1 to 2:1c</td>
</tr>
<tr>
<td>Na (% DM)</td>
<td>0.15</td>
<td>0.11</td>
<td>0.56</td>
<td>0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>K (% DM)</td>
<td>0.37</td>
<td>0.76</td>
<td>0.84</td>
<td>0.44</td>
<td>0.60</td>
</tr>
<tr>
<td>Mg (% DM)</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>VitD (IU/100 g DM)</td>
<td>58</td>
<td>&lt; LODd</td>
<td>100</td>
<td>55.20</td>
<td>50.00</td>
</tr>
<tr>
<td>ME (kcal/100 g DM)</td>
<td>431.3</td>
<td>NA</td>
<td>398.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DM — dry matter; Ca — calcium; P — phosphorus; Na — sodium; K — potassium; Mg — magnesium; VitD — vitamin D; ME — metabolizable energy; NA — not available; LOD — limit of detection.

a BalanceIT, West Sacramento, California.

b SGS Agri-Food Laboratories, Guelph, Ontario.

c Royal Canin Maxi Puppy dry food; formulated to meet the nutritional levels established by AAFCO (3) dog food nutrient profiles for growth.

d Upon laboratory analysis, the VitD content of the homemade diet was below the LOD of 100 IU/kg DM.

e The ratio was calculated using the recommended allowance according to the NRC (2).
the initial admittance to the hospital). The owner was well aware of the risks for unbalancing the nutrient profile of the diet with excessive treats and the risks associated with feeding cooked bones. A recheck appointment with both the OVC-HSC Neurology and Clinical Nutrition Services was recommended, but the owner declined.

**Discussion**

Nutritional recommendations for large breed puppies take into consideration key nutritional factors, such as energy, VitD, Ca, P, and Ca:P ratio (7). Energy excess results from offering food free choice or above caloric needs, especially when a diet has a high fat content, and therefore high energy density. Excess energy during growth not only promotes obesity (25) and reduced longevity (26), but also promotes rapid growth, and hence DODs (27), such as hip dysplasia and subsequent osteoarthritis (28). Therefore, reduced energy density (350 to 400 kcal/100 g) and a moderate fat level [< 12% on a dry matter (DM) basis], are recommended (6,24).

Passive Ca absorption occurs from 6 wk to 6 mo of age, and corresponds directly to dietary Ca intake (29). When the hormonal and intestinal regulation mature, active Ca absorption represents 90% of Ca uptake (30). Excessive Ca intake was shown to promote occurrence of DODs, even with normal Ca:P ratio (6). Also, 1,25-dihydroxyvitamin D [1,25(OH)₂D] may play a role in passive absorption of Ca in the small intestines and modulates active intestinal Ca absorption, as well as renal Ca reabsorption (18,30–32). Other calcitropic hormones, PTH, growth hormone, and calcitonin, are associated with active Ca absorption, and bone mineralization and remodelling (18). In puppies, excessive dietary Ca intake is negatively correlated to P absorption (29). Contrarily, a diet deficient in Ca, but high in P causes a reverse Ca:P ratio, resulting in NSH (18,22).

Unbalanced homemade diets that contain a significant amount of meat commonly have high P concentrations (33). For growing puppies 0.8% to 1.2% Ca on DM is recommended (2,3), and P is based upon Ca concentrations, to achieve an appropriate Ca:P ratio (22), of 1:2:1 calculated based on the National Research Council (NRC) recommended allowance for both minerals (2), or 1 to 2:1 based on the American Association of Food Control Officials (AAFCO) recommendations for growth (3). For large breed puppies the Ca:P ratio becomes more narrow at 1.1 to 1.3:1 (6). Therefore, if a homemade diet contains insufficient Ca and a reverse Ca:P ratio (above 1:2) occurs (4), this leads to NSH, demineralization of the bones, and skeletal deformity (34). Skeletal abnormalities manifest in 1 to 3 mo, affecting cancellous bone located in the epiphyseal plate of long bones or within the center of the vertebral bones. These areas become very thin, allowing the spiculae to collapse, causing compression fractures (18). Vitamin D and calcitropic hormones, such as PTH, and calcitonin, strongly influence bone and Ca metabolism in the growing dog, Vitamin D₃ is formed in most mammals in the skin, under UV-B radiation. However, in dogs, it is an essential vitamin, absorbed from the diet (VitD₃ — cholecalciferol, animal origin; VitD₂ — ergocalciferol, plant origin), bound to VitD binding protein and transported to the liver for its first hydroxylation into 25(OH)D. A second hydroxylation occurs in the kidneys into either the most biologically active metabolite, 1,25(OH)₂D, or 24,25-dihydroxyvitamin D (18). Impaired metabolism or inappropriate dietary intake of VitD will lead to low concentration of serum 25(OH)D and its metabolites. Nutritional VitD deficiency combined with dietary Ca deficiency can manifest as both rickets and fibrous osteodystrophy. In fibrous osteodystrophy, fibrous connective tissue replaces mature bone due to osteoclastic resorption (35).

However, in young mammals with open epiphyseal plates, these deficiencies will cause improper bone formation (defective mineralization/calcification), potentially leading to deformities and fractures, i.e., rickets (18).

In NSH, an increase of 1,25(OH)₂D is expected to promote intestinal absorption and renal reabsorption of Ca and P (18). However, if the diet contains insufficient amounts of VitD₂ or VitD₃, then low concentrations of serum VitD metabolites are inevitable, worsening the concurrent NSH. Nutritional VitD deficiency can also initiate NSH on its own, due to the reduced Ca absorption caused by hypovitaminosis D (18,36).

In this report, a 6-month-old large breed puppy was presented with vertebral compression fractures, suspected rickets, and NSH due to nutritional deficiencies caused by consumption of an unbalanced homemade diet, confirmed by computer-generated and laboratory diet analyses. The computer-generated diet analysis gives an estimation of nutrient composition based on average ingredient composition available in an online nutrient database. In addition, since no information was available from the owner regarding the VitD and bone meal supplements he used, common commercial supplements were added according to the amounts the owner gave, in order to have an approximate initial assessment of the dog’s nutrient intake. This type of analysis was helpful as an initial assessment, and assisted in convincing the owner to have the laboratory analysis performed. The laboratory diet analysis assessed the actual nutrient profile of this puppy’s diet. Although the software analysis overestimated the content of Ca, P, Ca:P, Na, and VitD, and underestimated protein content (Table 2), both agreed on imbalances of the nutrients of concern. Serum total Ca measured on the day of presentation was lower than the reference range, and could be indicative of low dietary intake or reduced absorption, as a high correlation exists in puppies younger than 6 mo (Table 1) (29). Alkaline phosphatase is a membrane-associated enzyme which can originate from several tissues, including bone, and indicates damage. Creatine kinase (CK) is a cytosolic enzyme, highly active in muscular tissues, brain, and nerves. Increased serum CK concentrations are indicative of muscle damage, and if persistent, indicate on-going muscle damage (37), which in this case, could occur due to the vertebral fractures and the patient’s decreased mobility. An increase in creatinine was observed likely for the same reasons. The hypophosphatemia detected in the serum is a result of insufficient dietary calcium for intestinal passive absorption, reverse Ca:P ratio, and insufficient VitD, which resulted in deficient 1,25(OH)₂D levels, and NSH; hence bone resorption (18). Unfortunately, it was difficult to convince the owner that further diagnostics should be done; therefore, serum samples for analysis of PTH, 25(OH)D, and iCa were not submitted on the day of presentation. A tentative diagnosis
of nutritional VitD deficiency and NSH was made based on abnormalities on serum biochemical profile, the presence of vertebral compression fractures, muscle damage, and generalized osteopenia found on radiographs and MRI, and further supportive findings resulting from the computer-generated diet analysis (Table 2). In this case, the spinal radiographs supported the diagnosis of NSH, but the degree of spinal cord compression associated with the mild vertebral malalignment could not be determined on radiographs alone. An MRI examination was performed as a more sensitive and specific diagnostic test to determine the site and degree of spinal cord compression. The MRI was also used to assess for any lesions that could require surgical decompression. Since no clinical signs of swelling or discomfort were observed in the long bones, which are commonly affected by NSH, no further diagnostics were performed to evaluate them.

On the day of discharge (48 h after presentation), the owner agreed to perform more in-depth blood analysis (Table 1). Serum iCa concentrations at discharge were mildly higher than the reference range, while serum PTH was within the reference range. Serum 25(OH)D concentrations were low compared with the reference range. In puppies younger than 6 mo, Ca absorption is in direct proportion to the Ca in the diet (29) and passive absorption can represent up to 70% of Ca uptake (30). It is most likely that serum iCa and PTH concentrations normalized (Table 1) by the time of discharge due to passive Ca absorption, especially since the dog was fed a commercial large breed puppy diet following admission (Table 2). However, 25(OH)D might have been too depleted to respond in just 2 d (Table 1). Continuing this diet was recommended as it seemed sufficient to normalize the serum Ca concentration without additional Ca supplementation. The dog continued to improve and recover on this diet after hospitalization, which was observed at the follow-up appointment.

This patient’s debilitating diet-induced diseases and permanent hunched posture emphasize the importance of not only nutritional assessment but also of client compliance, based on good client communication. The client had fed the puppy an unbalanced homemade diet since she was 2 mo old, believing that it would improve the dog’s health and strengthen their bond, as many pet owners do. Compassionate communication is required to relay medical findings to the owner, allowing the owner a better understanding of the diet-induced diseases, without blame, anger, or guilt (38). It was also important to set expectations that the dog would be permanently crippled, but could maintain a good quality of life if her pain could be managed. Once the owner understood the severity of the diet-induced diseases, he was willing to cooperate and change the dog’s diet based on the OVC-HSC Clinical Nutrition Service’s recommendations. At the time of the recheck, and also during the follow-up telephone conversation, the owner was satisfied with the puppy’s improvement; however, it was still difficult for him to maintain a strict regimen for treats, snacks, and human foods of no more than 10% of her daily energy requirements (23).

In conclusion, this report presented a case of nutritional VitD deficiency and NSH causing vertebral compression fractures, due to a deficient homemade diet fed to a growing large breed puppy. Successful treatment was attributed to an appropriate nutritional assessment and diagnosis. However, a key to this success was the client’s compliance, which resulted from good communication and empathy, encouraging the owner not to focus on guilt and self-blame, but rather on promoting the dog’s recovery.

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Can *Ureaplasma diversum* be transmitted from donor to recipient through the embryo? Two case reports outlining *U. diversum* losses in bovine embryo pregnancies

M. Bronwyn Crane, Colleen A. Hughes

Abstract — Two bovine embryo recovery results are outlined from different herds. Both cases involve significant late gestational loss from embryos relating back to a single donor. *Ureaplasma diversum* was confirmed in 3 of 4 cases submitted for postmortem examination. Natural infection originating from the donor and transmitted to the recipient has not previously been documented.

Mycoplasmas and ureaplasmas are recognized as 2 of the few potential pathogens that are not removed from embryos through the recommended International Embryo Transfer Society (IETS) washing procedures (1,2). *Ureaplasma diversum* is a widespread commensal and pathogenic bacterium associated with vulvitis, endometritis, abortion, and infertility in cattle (3,4). When endemic within a herd, the typical clinical presentation of *U. diversum* infection includes infertility and vulvitis (5). This article reports pregnancy results from 2 herds experiencing late gestational losses due to *U. diversum*, in which both herds had the same underlying and unique history of the affected pregnancies all originating from 1 embryo donor. This clinical presentation suggests that it may be possible for this bacterium to be transmitted by embryo transfer. Furthermore, this route of infection and the insidious nature of this bacterium may be more likely to result in late term pregnancy loss. *Ureaplasma diversum* is likely an unrecognized factor contributing to gestational and neonatal losses in pregnancies from embryo transfer.

**Case descriptions**

**Herd 1: Donor 1A, 1B, and 1C**

The first case occurred in a herd of approximately 60 lactating Holstein cows in Nova Scotia. This herd was under regular veterinary care and had a comprehensive vaccination protocol in place for protection against infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) viruses. Frozen embryos from 3 donors (Donors 1A, 1B, and 1C) were purchased from a single herd in Ontario. The frozen embryos from each donor were from single collection procedures and therefore the same donor-sire matings. Frozen-thawed embryos were transferred into virgin heifer recipients. Transfers took place between September 2012 and May 2013 and occurred on 5 separate occasions when recipients were synchronized. Recipients that were not pregnant after embryo transfer were bred by artificial insemination (AI) and did not receive a second embryo. The transferred embryos from Donor 1A had an initial pregnancy rate of 7/7, or 100%. Embryos from Donor 1B had an initial pregnancy rate of 4/9, or 44%. Embryos from Donor 1C had an initial pregnancy rate of 5/7, or 71%. The pregnancy outcomes for embryos from Donor 1A are outlined in Table 1. Preganacies resulting from embryos from Donors 1B and 1C were normal and all calves survived. One of those 9 births occurred early at 255 d; despite this, the calf did survive. Only 1 of the 7 pregnancies from Donor 1A resulted in a full-term surviving calf. The remaining 6 pregnancies were associated with pregnancy loss, stillbirths, premature births, and neonatal loss (Table 1). Each case was either suspicious of, or confirmed, as having *U. diversum* infection. During this time period, pregnancies from AI in heifers from the same groups as the recipients
were normal and none aborted, were stillborn, or experienced neonatal loss.

The first 3 losses from Donor 1A were not submitted for necropsy. From the 3 calves submitted for necropsy, all polymerase chain reaction (PCR) tests for BVD, IBR, and Neospora caninum were negative and all aerobic bacterial cultures were negative. In addition, when fungal cultures, Coxiella burnetti PCR tests, or Chlamydia spp. PCR tests were performed, all were negative. Samples were submitted for mycoplasma/ureaplasma culture from all 3 calves and there was growth in 2 of the 3 cases. In the case with no mycoplasma/ureaplasma growth (Table 1; Embryo #4), a PCR test for *U. diversum* was not performed. In the case of Embryo #7 (Table 1), in which only a few colonies were detected by culture, the PCR test for *Ureaplasma diversum* was also positive.

The first postmortem (PM) examination (#1) was performed on a fresh premature fetus with a placenta that was aborted at 250 d gestation (PM #1, Table 1). This postmortem examination revealed focal placentitis and fetal pneumonia. The fetal pneumonia consisted of inflammatory cells in the alveoli as well as small lymphoid cell aggregates seen near small bronchioles and blood vessels. The final diagnosis was an abortion of infectious pneumonia, with 3 occurring late in gestation (Table 2). Only 5/9 pregnancies resulting from these embryos are known. Fresh embryos were transferred into synchronized recipient heifers from the same herd. Augustine stimulation in the lung, conjunctivitis, and late term ureaplasma abortion.

Herd 2: Donor 2

The second case occurred in a herd of approximately 100 lactating Holstein cows in Prince Edward Island. This herd was also under regular veterinary care and had a comprehensive vaccination protocol in place for protection against IBR and BVD. Pregnancy diagnosis typically occurred between 28 and 42 d and again between 60 and 75 d after embryo transfer. On this farm, embryo collections from various donors were regularly performed (1 to 2/month). Embryo transfers and the resulting pregnancies were generally successful and uneventful.

Although this herd rarely purchased new animals, the owners purchased a virgin heifer (Donor 2) at 11 mo old and entered her into the herd in April 2014 with the intent of having her become an embryo donor. Three embryo recoveries were performed between May and September 2014 (Table 2; Flush #1 to #3). A significant proportion of frozen embryos from each of these flushes was sold and the outcomes associated with these embryos are unknown. Fresh embryos were transferred into synchronized recipient heifers from the same housing group as the donor. Although not all the pregnancy outcomes are precisely known, 5/9 pregnancies resulting from fresh embryos from the first 3 flushes were lost after pregnancy diagnosis, with 3 occurring late in gestation (Table 2). Only

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**Table 1.** Details and outcomes from the 7 embryos transferred that originated from Donor 1A in Herd #1. All 7 embryos resulted in pregnancies.

<table>
<thead>
<tr>
<th>Embryo number</th>
<th>Transfer date</th>
<th>Gestation length</th>
<th>Outcome</th>
<th>Postmortem diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 15, 2012</td>
<td>Full term</td>
<td>Stillborn</td>
<td>None performed</td>
</tr>
<tr>
<td>2</td>
<td>November 15, 2012</td>
<td>250 days</td>
<td>Aborted</td>
<td>None performed</td>
</tr>
<tr>
<td>3</td>
<td>February 6, 2013</td>
<td>270 days</td>
<td>Survived 4 days</td>
<td>None performed</td>
</tr>
<tr>
<td>4</td>
<td>February 6, 2013</td>
<td>250 days</td>
<td>Aborted</td>
<td>PM #1 — Placentitis and fetal pneumonia, no <em>U. diversum</em> growth, no <em>U. diversum</em> PCR performed</td>
</tr>
<tr>
<td>5</td>
<td>February 6, 2013</td>
<td>268 days</td>
<td>Dymature bull calf euthanized</td>
<td>PM #2 — Placentitis, conjunctivitis, lung pathology, <em>U. diversum</em> confirmed by culture (heavy growth), no PCR performed</td>
</tr>
<tr>
<td>6</td>
<td>February 6, 2013</td>
<td>Full term</td>
<td>Live bull calf</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>March 5, 2013</td>
<td>250 days</td>
<td>Aborted</td>
<td>PM #3 — No placenta submitted, conjunctivitis, lung pathology, <em>U. diversum</em> confirmed by culture (few colonies) and PCR positive</td>
</tr>
</tbody>
</table>
1 postmortem examination was conducted from these losses. During this same time period, heifers in the same group as the recipients that received embryos from other donors or were bred by AI did not experience gestational losses.

The specimen submitted from herd 2 for postmortem examination was a fresh female fetus, 250 d gestation with the placenta. The final diagnosis was placentitis and bronchopneumonia. The moderate to severe placentitis was described as necrosuppurative, multifocal, and acute. The moderate to severe bronchopneumonia was described as neutrophilic, diffuse, and acute. Lesions were consistent with a bacterial infection, but bacterial cultures were non-diagnostic (mixed growth), fungal culture was negative and viral PCR tests were all negative. At the submitting clinician’s request, samples were sent for ureaplasma evaluation, which required diagnosis at an outside laboratory and cost approximately an additional $200. *Ureaplasma diversum* was cultured from the fetal lungs (few, 2+) and the fetal abdominal fluid (few, 2+). These findings explained the inflammatory changes observed in the placenta and fetal lungs, confirming that *U. diversum* was the cause of abortion in this case.

Donor 2 delivered a full term viable calf in June 2015 and was flushed 3 more times during her first lactation (Table 2: Flush #4 to #6). There were fewer fresh transfers with these flushes, yet 1 out of 2 pregnancies resulted in a mid-gestation loss.

### Discussion

The cases described in this report suggest that there is the potential for *Ureaplasma diversum* to infect embryos and then for these embryos to result in gestational losses in the recipient heifers. The postmortem results were conclusive for *U. diversum* infection as the cause of the abortion in 3 of the 4 submitted cases (Tables 1 and 2). Although *U. diversum* most commonly presents as vulvitis, endometritis, and infertility, it has been shown to cause sporadic abortions at any stage of gestation (4), but most commonly mid to late gestation (5). *Ureaplasma diversum* infection also results in stillborn and weak calves (4,6). Based on studies of aborted fetuses, the prevalence of ureaplasma abortion is approximately 1.7% to 3% (7,8). The 2 herds described in this report experienced a cluster of losses among the embryo pregnancies that were suspicious of or confirmed as *U. diversum* infection in 67% (12/18) of the losses. In these 2 herds, infertility was not the presenting complaint among the recipient heifers, rather pregnancy loss and stillbirths were the prominent clinical problems. It is possible that if embryos are the source of the *U. diversum* infection, abortions, stillbirths, and weak neonates could be how the disease is manifested. By the time the problem was recognized in these 2 cases, the embryos had been dispersed and it was too late to evaluate the embryos themselves as a source of the disease cluster.

Embryo processing techniques have been developed to reduce the risk of transfer of infectious disease by *in vivo*-derived bovine embryos (9). The standard International Embryo Transfer Society (IETS) recommended washing procedure includes at least 10 washes with serial 1:100 dilutions of embryo holding medium and 2 trypsin treatments (10). This protocol removes almost all of the important pathogenic viruses and bacteria (11). However, mycoplasmas and ureaplasmas are not removed from embryos with this protocol (12). In addition, standard antibiotics present in embryo processing media are not effective against ureaplasmas and mycoplasmas (11). Previously published data on the prevalence of *U. diversum* has identified the organism in vulvo-vaginal swabs in 38.8% of cows and heifers with granular vulvitis (13), 44% of beef heifers (14), and 11% to 100% of cows’ vaginas (4). Therefore, it is possible that *U. diversum* present on the vulva may contaminate the flush catheter or solutions and exist in bovine embryo recoveries at a similar prevalence.

Typical characteristics of an abortion caused by *U. diversum* include a relatively fresh fetus and a placenta that is frequently retained and exhibits a placentitis (15). Lung pathology of the

### Table 2. Details and outcomes from the embryos originating from Donor 2 in Herd #2.

<table>
<thead>
<tr>
<th>Flush 1</th>
<th>Total viable embryos</th>
<th>Number pregnant/Number fresh transfers</th>
<th>Fresh embryo pregnancy losses/Number pregnant</th>
<th>Loss at 30 to 75 days</th>
<th>Loss at 75 days to full term</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 27, 2014</td>
<td>22</td>
<td>2/5</td>
<td>1/2</td>
<td>0</td>
<td>1 (150 days)</td>
</tr>
<tr>
<td>Flush 2</td>
<td>12</td>
<td>3/6</td>
<td>1/3</td>
<td>0</td>
<td>1 (Stillborn)</td>
</tr>
<tr>
<td>July 3, 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flush 3</td>
<td>13</td>
<td>4/7</td>
<td>3/4</td>
<td>2^</td>
<td>1 (250 days)</td>
</tr>
<tr>
<td>September 9, 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flush 4</td>
<td>5</td>
<td>0/1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>September 11, 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flush 5</td>
<td>12</td>
<td>1/1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>November 5, 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flush 6</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
<td>N/A</td>
<td>1 (100 days)</td>
</tr>
<tr>
<td>December 9, 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>65</td>
<td>11/21</td>
<td>6/11</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

^1 Preganacies were thought to be lost in this time frame because there was no record of live births or late term abortions from these recipients.

^2 Calf submitted for necropsy (see PM report).

N/A — Not available.
fetus and conjunctivitis are often reported as well (6). The only postmortem examination in this report that did not result in a positive *U. diversum* culture did have a diagnosis of placentitis and fetal pneumonia (Table 1), which is highly suggestive that *U. diversum* infection was the cause of the abortion. A PCR test for ureaplasmas was not part of this postmortem examination. A PCR assay is a more sensitive test than culture because *U. diversum* requires special media to grow and it can be hard to isolate from clinical samples. In addition, the sample needs to be received at the laboratory quickly after collection and examined by personnel with expertise in ureaplasma and mycoplasma diagnoses (16). Most veterinary diagnostic laboratories have a standard diagnostic protocol for bovine specimens for postmortem investigation, but PCR testing for ureaplasmas is not usually included as a routine test. This occurred in the case of Herd 2, in which a PCR test for ureaplasmas had to be requested in addition to the standard postmortem examination. Therefore, it is possible that *U. diversum* abortions are underreported.

Despite the standard procedures put in place to prevent disease transmission, there is still a risk of introducing *U. diversum* from a donor’s vulva into her uterus during artificial insemination. In fact, donor cows often undergo multiple inseminations after being super-ovulated. If this bacterium was transmitted into the uterus at the time of breeding, the uterus may have an established infection by the time of embryo collection resulting in adherence of the *U. diversum* to the developing embryos. Also, certain donor cows may carry a higher load of this bacterium than others, as evidenced in a study that cultured ureaplasmas from cows’ vulvas for up to 3 mo following a herd outbreak of granular vulvitis (17). This would potentially explain why even if the donor cow appeared clinically normal at the time of insemination, she could still have *U. diversum* present in her vulva, which could therefore contaminate the embryos and embryo flush fluid during embryo recovery. It is also theoretically possible that these clusters are connected due to a cause other than the donor cow, such as through a contaminated embryo transfer (ET) gun. However, if the technician’s ET gun was contaminated with this pathogen in these cases, there should have been an equal chance of infection among all embryos transferred, not only those from a single donor. In addition, in the case of Herd 2, different practitioners transferred the embryos on different locations with the losses spread across both locations. Intravaginal device applicators could be another potential source of transmission among a herd, but the cows would most likely present with a vaginitis and possible infertility if these devices were the source. In the cases reported, intravaginal devices were not used. In both of these herds, the reproductive losses could all be linked to 1 donor cow, so contamination from ET guns or intravaginal devices was unlikely to be the cause.

In conclusion, this case report suggests that practitioners need to consider *U. diversum* as a possible infectious cause of bovine abortion in embryo pregnancies and pursue this differential diagnosis despite additional costs and testing. To the authors’ knowledge, these cases are unique in that there have been no other published reports describing a single donor cow as a potential cause of natural *U. diversum* infection originating from her transferred embryos. This report indicates the need to continue investigation into the potential for natural infection of *U. diversum* by adherence to the embryo.

**References**

Hybrid surgical treatment for 2 feline cases of intrahepatic shunt

Akiko Uemura, Telma Mary Nakata, Ryou Tanaka

Abstract — Intrahepatic portosystemic shunt was encountered in 2 cats (10 and 5 months old) exhibiting neurological symptoms and general deterioration. Both cats were treated with coil embolization using a hybrid surgical technique combining conventional open surgery and interventional radiology techniques, achieving good postoperative outcomes (follow-up: 22 and 10 months, respectively).

Résumé — Traitement chirurgical hybride pour deux cas félin de shunt intrahépatique. Un shunt portosystémique intrahépatique a été observé chez deux chats (âgés de 10 et de 5 mois) manifestant des symptômes neurologiques et une détérioration générale. Les deux chats ont été traités par une embolisation utilisant des spires à l’aide d’une technique chirurgicale hybride combinant une chirurgie ouverte conventionnelle et des techniques de radiologie d’intervention afin d’obtenir de bons résultats postopératoires (suivi : 22 et 10 mois, respectivement). (Traduit par Isabelle Vallières)

Unlike cases of portosystemic shunt (PSS) in canines, cases of feline PSS are rare (1). Ideal methods of treatment have yet to be established in dogs or cats. Feline PSS is usually identified at a young age, with a mean age at diagnosis of 4 to 9 mo (1–3). The mean body weight (BW) at diagnosis is also low, reportedly ranging from 1.2 to 2.9 kg for intrahepatic cases (mean: 2.3 kg) (4,5) and from 1.1 to 6.2 kg for extrahepatic cases (mean: 2.9 kg) (6,7). Because cats with PSS and neurological symptoms treated medically are reported to die within 2 y following diagnosis (8), surgery is often required at an early age.

The main method of treatment for feline PSS is surgical ligation (3), but dissection of the liver parenchyma to identify the shunting vessel can be challenging with intrahepatic PSS (IHPSS), in which a direct approach to the shunting vessel is anatomically difficult. Such operative stresses can occasionally prove fatal for kittens (9). Previous methods of treating extrahepatic portosystemic shunts in cats have included the use of surgical ligation (1,3,10,11), ameroid constrictors (12), and cellophane banding (7,13). With the exception of cellophane banding, these methods are also used for intrahepatic shunt vessels (1,3,4,14).

Microhepatia is seen in half of feline cases of intra- and extrahepatic PSS (2), and large-diameter shunts usually show a higher volume of blood flow. Attempting to achieve complete occlusion of the shunt in a single procedure under these circumstances sometimes causes portal hypertension, which in turn can cause ascites, postoperative development of multiple shunts, intestinal injury, or even death. Stepwise closure of the shunting vessel is therefore recommended in such cases (12). In our experience, when attempting surgical ligation, multiple approaches to the shunting vessel are sometimes difficult because of adhesions caused by the earlier approaches. An approach other than conventional surgery is thus desirable.

Percutaneous transvenous coil embolization (PTCE) has been used for both dogs (11,15) and cats (5) in an attempt to resolve the issues associated with conventional surgery. In those previous reports, jugular, femoral, or saphenous veins were used as approach sites. Size of the device in cats is restricted by the smaller body conformation and vascular diameter, so PTCE has been applied in only a limited number of cases. In addition, based on our personal experience, measuring portal vein (PV) pressure and performing angiography after shunt vessel occlusion in PTCE is sometimes difficult in cats.

The hybrid surgical technique (HST) represents a combination of conventional open surgery and interventional radiology. This approach was chosen for the following reasons based on our experience with canine and feline cases of PSS requiring stepwise closure. The HST requires a surgical approach only around the main trunk of the PV or mesenteric vein. The incision for the surgical site is relatively small, and the risk of adhesion is minimal. In addition, no dissection of the hepatic parenchyma is required. The distance between the sheath and shunt vessel is short, facilitating more accurate maneuvering of the catheter tip. Because this procedure is conducted under direct visualization of the organs inside the abdomen, intestinal color and peristaltic...
movement after coil implantation are easily evaluated, and liver biopsy can be readily and safely conducted. In addition, the sheath acts not only for placement of the device, but also as a catheter for the measurement of PV pressure and the injection of contrast agent.

Case descriptions

Case 1
A 10-month-old neutered male exotic shorthaired cat was referred to the Tokyo University of Agriculture and Technology Animal Medical Center (TUATAMC) because of drooling after meals that had appeared 2 mo previously. Two days before the initial hospital visit, the animal exhibited loss of both vigor and appetite, altered consciousness, and convulsive seizures. Plasma ammonia (PA) concentration taken at the referring clinic had been elevated (> 587 μmol/L), and the animal was brought to TUATAMC for further investigation (Day 1).

On initial examination, decreased level of consciousness and generalized seizures were evident. Body weight (BW) was 2.8 kg. The irises of both eyes showed a copper coloration, which has previously been reported in cats with PSS (16). Systolic/diastolic (mean) arterial pressure was 109/63 (80) mmHg. Complete blood cell count revealed anemia (hematocrit: 21.1%), and PA was 72.8 μmol/L. Plasma albumin concentration (29 g/L) was within normal limits. Computed tomography (CT) revealed the tortuous passage of a PV in the left hepatic segment through the liver before forming a direct shunt into the caudal vena cava via the hepatic vein (Figure 1). This shunt represented left divisional intrahepatic portosystemic shunt, which has been reported previously (17). The vascular diameter of the shunt was 5.5 to 6.8 mm, with a maximum vascular length of approximately 15 mm. Only 1 shunt vessel was present, and no other intrahepatic vascular anomalies were identified.

An attempt was made to control the symptoms with pharmacotherapy using levetiracetam (Otsuka Pharmaceutical, Tokyo, Japan), 20 mg/kg BW, q12h, and zonisamide (Sumitomo Dainippon Pharma, Tokyo, Japan), 2.5 mg/kg BW, q12h. However, no clear improvements were identified, and the PA concentration remained elevated (73.4 μmol/L). Coil embolization of the shunt vessel by HST was performed on Day 21. First, ampicillin sodium (injectable ampicillin Na; Kyoritsu Seiyaku, Tokyo, Japan), 30 mg/kg BW, IV, buprenorphine hydrochloride (Leptan injection 0.2 mg; Otsuka Pharmaceutical), 0.01 mg/kg BW, IV, and atropine sulfate (Atropine Sulfate Injection 0.5 mg; Mitsubishi Tanabe Pharma, Osaka, Japan), 0.05 mg/kg BW, SC, were administered as premedications, and anesthesia was induced by inhalation of 5% isoflurane (Isoflurane for Animal; Intervet, Tokyo, Japan). After tracheal intubation, inhalation anesthesia was maintained using 2.0% to 3.5% isoflurane. After surgical preparation, a midline incision was made in the abdomen (~ 8 cm in length), and a 3-Fr sheath was inserted in the mesenteric vein. A 0.018-inch guide wire and microcatheter were inserted into the sheath, and the microcatheter was placed at the narrowest part of the shunt vessel under fluoroscopic guidance. A detachable coil [Platinum Coil Vascular Occlusion System (Interlocking Detachable Coil); Boston Scientific Japan, Tokyo, Japan] with a diameter of 7 mm and a length of 10 mm was deployed under fluoroscopic guidance and then detached. Systolic/diastolic PV pressure was 5/2 (mean: 3) mmHg before coil deployment and 14/11 (mean: 12) mmHg after coil occlusion. Before coil occlusion, an injection of iopamidol contrast agent (Oypalomin; Konica Minolta Japan, Tokyo, Japan) via the mesenteric vein revealed the flow of contrast agent directly into the caudal vena cava from the shunt vessel. Shunt flow was decreased after coil deployment, but as expected, some residual flow remained (Figure 2). No additional coil implantation was considered during this operation. After removal of the sheath, the hole in the mesenteric vein was closed with continuous sutures using synthetic absorbable monofilament (6/0 Prolene; Johnson & Johnson, Tokyo, Japan). The procedure took 71 min to complete.

The patient recovered from anesthesia uneventfully, and showed clinical improvement in the postoperative period. On Day 154, BW had increased to 4.2 kg, no clinical symptoms were apparent, and PA concentrations had decreased to within normal limits (37.6 μmol/L) without the use of either oral

Figure 1. Computed tomography in Case 1 reveals tortuous passage of a portal vein in the left hepatic segment through the liver before forming a direct shunt into the caudal vena cava via the hepatic vein.

Figure 2. Shunt flow is decreased after coil deployment in Case 1, but is still present.
medication or dietary therapy. On Day 191, ultrasonographic examination showed that shunt flow had completely disappeared (Figure 3).

Case 2

A 5-month-old intact male Russian blue cat was referred to TUATAMC because of loss of appetite that had appeared 2 mo previously, high serum levels of total bile acid (137.0 µmol/L preprandially, 108.2 µmol/L postprandially), and a high PA concentration (133.8 µmol/L).

On initial examination (Day 1), loss of appetite and mild wasting were observed (body condition score: 2/5). Body weight was 980 g. The mucous membranes were slightly pale, and mild dehydration was evident. The superficial lymph nodes were not enlarged and no heart murmurs were audible. Blood tests revealed mild anemia (hematocrit: 26.7%), hyperammonemia (163.8 µmol/L), hypoalbuminemia (20 g/L), and low urea nitrogen (5.9 mmol/L). Contrast-enhanced CT revealed marked microhepatia and passage of a PV branch in the left hepatic segment through the liver toward the cranial side before forming a direct shunt into the caudal vena cava. This shape of the shunt suggested patent ductus venosus, but we could not confirm the origin. The vascular diameter of the shunt ranged from a minimum of 4.1 mm to a maximum of 4.8 mm. No other vascular abnormalities were evident.

Given the underdeveloped state of the liver and the large vascular diameter of the shunt, stepwise embolizations of the shunt vessel were planned. On Day 13, general anesthesia was induced using the same method applied in Case 1. After tracheal intubation, inhalation anesthesia was maintained with 1.0% isoflurane. After surgical preparation, a midline incision was made in the abdomen, and a 4-Fr sheath was inserted into the main trunk of the PV. Under fluoroscopic guidance, a 0.035-inch guidewire and a 4-Fr multipurpose catheter were inserted into the sheath and placed at the cranial side of the shunt vessel. An anchor (Coil Anchor II S; Medikit, Tokyo, Japan) was deployed in the shunt vessel under fluoroscopic guidance, and coil implantation was planned for after stabilization of the anchor against the vessel wall. This coil anchor proved very useful in preventing migration of the coils added later. Unlike stents for PTCE, this coil anchor can be placed in a small space. After removal of the sheath, the hole in the PV was closed with polypropylene synthetic non-absorbable sutures (8-0 PROLENE; Johnson & Johnson). The PV pressure was 4/1 (mean: 2) mmHg before coil anchor deployment and 6/3 (mean: 4) mmHg after coil anchor deployment. After this initial anchor implantation, the appetite of the animal improved and BW increased to 1.1 kg, but PA concentration remained high (300.5 µmol/L on Day 21). On Day 71, the patient showed neurological symptoms (apparent loss of vigor after meals). We attempted medical control with phenobarbital (1.0 mg/kg BW, q12h) and lactulose (1 mL, q12h). A second HST for coil implantation was then carried out on Day 78. Sutures from the initial procedure were visible on the PV, but no adhesions were evident. A 4-Fr sheath, 0.035-inch guidewire, and 4-Fr multipurpose catheter were therefore inserted into the main trunk of the PV in a similar fashion to that of the first HST. Under fluoroscopic guidance, a pushable coil (Trufill Pushable Coil Selection Complex; Johnson & Johnson) (diameter: 7 mm; length: 60 mm) was deployed to coil around the coil anchor already in place. The PV pressure was 8/5 (mean: 4) mmHg before coil implantation and 7/5 (mean: 5) mmHg after coil implantation. Although a residual shunt was observed around the coil, thrombus formation around the coil was expected, and no additional coil implantation was conducted during the procedure. The cat recovered uneventfully from the anesthesia. A third HST was conducted on Day 102 because PA concentrations remained high (281.8 µmol/L on Day 85) and neurological symptoms were becoming worse, with the appearance of convulsions. Anesthesia and the approach to the shunt vessel were conducted as previously described, except that this procedure used a 3-Fr sheath, 0.018-inch guidewire, and microcatheter. An additional pushable coil (Trufill Pushable Coil Selection Complex; Johnson & Johnson) (diameter: 7 mm; length: 60 mm) was implanted in the PV slightly caudal to the coil deployed in the second HST (Figure 4). Because residual shunt was still observed, another pushable coil (diameter: 5 mm; length: 40 mm) was added. The PV pressure was 5/3 (mean: 3) mmHg before additional coil implantation and increased to 27/24 (mean: 26) mmHg after additional coil implantation. Fluoroscopic observation revealed that the velocity of shunt flow was markedly reduced, as determined from the flow speed.
of contrast medium at the coil site. The cat recovered from the procedure uneventfully; the PA concentration was 35.2 µmol/L preprandially and 58.7 µmol/L at 2 h postprandially. Mild temporary ascites was seen after the third HST, but the general condition of the animal was markedly improved; the cat appeared energetic, showed good appetite, and was able to walk independently. Procedure time was 61 min for the first HST, 73 min for the second, and 62 min for the third. By Day 126, clinical symptoms had resolved without pharmacotherapy, and PA levels remained at approximately 58.7 µmol/L. Color Doppler ultrasonographic examination showed no blood flow around the coil in the shunt vessel on Day 144.

The interval between the first and second interventions was as planned. However, the interval between the second (Day 78) and third interventions (Day 102) was short, because the cat showed neurological symptoms on day 71. At first (Day 71), loss of vigor after meals was the only neurological symptom. However, general physical condition and neurological symptoms gradually worsened, to the point where convulsions developed. We attempted medical control with phenobarbital and lactulose, but clinical symptoms did not improve. We therefore made the decision to perform a third intervention within the short period of 3.5 mo.

**Discussion**

Both animals in these cases were in poor general condition at presentation. An attempt was made to control the condition of the cat in Case 1 with medication, but this did not result in any apparent improvement. Waiting for the animal to gain BW was impossible in Case 2. The use of PTCE for a cat with PSS that weighed 2.9 kg at 4 mo of age has been reported (5), but the cat in Case 2 weighed only 980 g and the diameter of the jugular vein was small, so physical insertion of the catheter into this vessel for PTCE would have been difficult. Because both animals had been diagnosed with left-sided intrahepatic PSS, surgical shunt vessel ligation was also a treatment option. However, both animals were in poor condition and anemic, so HST was chosen as a less-invasive procedure to limit bleeding and avoid the need for hepatic parenchymal dissection. Bleeding mainly occurred when the mesenteric vein or PV was punctured to insert the device and during withdrawal, and hemostatic control was good. To avoid troublesome device manipulation, the sheath was inserted directly into the PV, providing good maneuverability and enabling use of a large device not only in Case 1, in which the shunt vessel followed a complex, tortuous course, but also in Case 2, in which the animal was extremely small. In Case 2, the broad shunt vessel and severe microhepatia meant that a need for staged occlusion was anticipated, but even though HST was actually performed 3 times, no problems were identified with postoperative adhesions within the peritoneum or at the PV suture site where the sheath had been inserted. The HST is thus useful for avoiding the risk of approaching an adhesion site and averting problems such as prolonged operating time and excessive bleeding that would arise from the need to detach adhesions created during multiple surgeries.

The cat in Case 2 was initially treated in an unusual stepwise manner using an anchor. The purpose of the first intervention (on Day 13) was to create an anchor, not to initiate thrombosis. Although postoperative outcomes were uneventful in both cases reported here, issues warranting further investigation were also apparent. The thrombogenic speed of coils is difficult to predict, and protocols for staged embolization have yet to be established. In Case 2, the thrombogenic speed of coils immediately after the third HST was rapid, and mild postoperative retention of ascites occurred. Due to the rise in intraoperative PV pressure after coil deployment and the marked weakening of blood flow in the area around the coil observed on postoperative abdominal ultrasonography, the cause of ascites was inferred to have been a transient increase in PV pressure resulting from rapid thrombus formation with the introduction of the coils. By the time of the third HST (Day 102), neurological symptoms had worsened. We considered immediate occlusion of the shunt.
as necessary, even though the patient faced a growing risk of portal hypertension. Even though the cat in Case 2 remained in good general condition, with good appetite and energy, and the amount of ascites retained was small, this represents an important issue in HST that requires further investigation. Venous shunt vessels are also highly elastic, making the maximum diameter during coil embolization more difficult to predict than with arterial vessels. No coil standards defined by diameter of the shunt vessel have been established, and the optimal method for measuring PV pressure after coil embolization also remains to be determined.

The HST enables direct insertion of a device into the portal or mesenteric vein via open surgery, mitigating the restrictions on usable device size, permitting easy device manipulation, and allowing device manipulation while directly observing the peritoneal organs. We believe that HST offers a potentially useful new method to extend the range of options available for treating feline PSS.

Acknowledgments
We thank Drs. Toshiharu Fukayama, Kazumi Shimada, and Seijirow Goya for their assistance in providing anesthesia for these cases.

References
Babesia odocoilei as a cause of mortality in captive cervids in Canada

Amélie Mathieu, Adriana R. Pastor, Charlene N. Berkvens, Carolyn Gara-Boivin, Michel Hébert, Alexandre N. Léveillé, John R. Barta, Dale A. Smith

Abstract — Nine cases of fatal infection with Babesia odocoilei were confirmed in reindeer (Rangifer tarandus tarandus) and elk (Cervus canadensis) housed in zoological institutions located in southern Quebec, Ontario, and Manitoba, Canada between 2013 and 2016. All animals died of a hemolytic crisis. Frequent postmortem findings were extensive hemorrhage, pigmenturia, and intrahepatic cholestasis. The described ante- and postmortem signs are consistent with those of previously reported cases in the United States. Diagnosis was confirmed in all cases by polymerase chain reaction performed on DNA extracted from whole blood or frozen spleen. We propose that babesiosis is an emerging disease of cervids in multiple Canadian provinces, most likely as a result of climate change and the northward range expansion of Ixodes scapularis, the primary tick vector for B. odocoilei. The role of captive animals as sentinels for wildlife health is also highlighted.

Résumé — Babesia odocoilei, une cause de la mortalité chez les cervidés captifs au Canada. Entre 2013 à 2016, neuf cas d’infection fatale par Babesia odocoilei ont été détectés chez des caribous (Rangifer tarandus tarandus) et des wapitis (Cervus canadensis) gardés dans des établissements zoologiques situés dans le sud du Québec, de l’Ontario et du Manitoba, Canada. Les animaux sont morts suite à une crise hémolytique. Hémorragies, pigmenturie et cholestase intra-hépatique ont fréquemment été identifiées à l’examen postmortem. Les signes ante- et postmortem décrits correspondent avec ceux des cas précédemment signalés aux États-Unis. Le diagnostic de babésiose fut confirmé par réaction en chaîne par polymérase sur l’ADN extrait de l’échantillon de sang ou de rate congelée. Nous proposons que la babésiose des cervidés est une maladie émergente au Canada, et ce probablement en conséquence du réchauffement climatique et du mouvement vers le nord de la tique Ixodes scapularis, le principal vecteur de B. odocoilei. La valeur des animaux captifs comme sentinelles pour la santé de la faune est également discutée. (Traduit par les auteurs)

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Introduction

Babesia odocoilei (Apicomplexa, Pirolasmodia, Babesiiidae) is a tick-borne intraerythrocytic protozoal parasite originally described in white-tailed deer (Odocoileus virginianus) (1,2). Ixodes scapularis is the organism’s only proven vector (3–5). Dermacentor albipictus, Amblyomma americanum, and Ixodes pacificus ticks have been found on, respectively, elk (Cervus elaphus canadensis), white-tailed deer, and bighorn sheep (Ovis canadensis nelsoni) infected with B. odocoilei, but have not been confirmed as vectors (1,6,7).

Babesiosis has been described in wild white-tailed deer and desert bighorn sheep, as well as in captive elk, woodland caribou (Rangifer tarandus caribou), reindeer (Rangifer tarandus tarandus), muskoxen (Ovibos moschatus), markhor (Capra falconeri), yaks ( Bos grunniens), and muntjacs (Muntiacus reevesi) in the United States (1,6–12) (Figure 1). However, clinical disease has only been reported in elk, reindeer, and caribou (6–8). Clinical signs reported in these cervids include lethargy, pyrexia, icterus, hemoglobinuria, and sudden death (8,13,14). Disease can develop following infection of naïve animals or can manifest...
as a recrudescence of a latent infection in persistently infected animals (13). The stressors responsible for this recrudescence are often unknown, but may include concurrent disease, poor nutrition, rutting season, calving, high population density, and transportation (13,15,16). In persistently infected immunocompetent white-tailed deer, a mild transient decrease in hematocrit or clinical disease manifested by pyrexia, anemia, and emaciation can occur in association with low-grade parasitemia (11).

Knowledge of the epidemiology of *B. odocoilei* in North America is limited despite a number of published reports. Insufficient targeted surveillance for this pathogen in wildlife might explain the lack of geographic continuity between reported endemic regions; only sporadic reports exist regarding *B. odocoilei* infections in wildlife despite the widespread distribution of *Ixodes* spp. ticks in the eastern half of the United States (4). Serological evidence of *B. odocoilei* has been demonstrated in free-ranging white-tailed deer in southern Virginia, eastern Texas, Oklahoma, and Saskatchewan, as well as in desert bighorn sheep in southern California (1,2,7,11,16,17). *Babesia odocoilei* DNA was also detected in *I. scapularis* ticks found in Maine, Massachusetts, and Wisconsin (3). Reports of disease in captive susceptible hosts in ranches, farms, or zoos may provide further insight into the geographic range of the disease. Captive cervids and bovids infected with *B. odocoilei* have been identified in New Hampshire, New York, Pennsylvania, Indiana, Texas, Minnesota, and Wisconsin (6,7,13,14). Cervid babesiosis was reported for the first time in Canada in ranched elk in central Saskatchewan in 2012 (18). Although the source of the infection has rarely been investigated, several authors have proposed that cases in captive animals reflect endemicity in local wildlife or tick populations (8,13,18,19).

This is an overview of the diagnosed cases of cervid babesiosis in Canada following its first recognition in 2012 until 2016. This report highlights the emergent nature of the disease in Canada, as well as the role of captive animals in wildlife disease surveillance.

**Materials and methods**

**Case histories**

Medical files of cervid babesiosis cases diagnosed by the authors were gathered and data regarding signalment, antemortem clinical signs, clinical pathology findings, ancillary diagnostic test results, and postmortem findings were collated. Clinical presentation was defined as peracute or acute. Animals that died...
without overt prodromal signs were classified as peracute cases, while animals that showed clinical signs for a few days before death were classified as acute. Tissue and blood samples were collected during necropsy and held at −20°C for later DNA extraction. Data were reviewed for trends and patterns, but no statistical analysis was performed.

DNA Extraction, piroplasm-specific PCR and sequencing

Molecular genetic analysis of the cervid spleen and blood samples collected during necropsies at the Toronto Zoo was performed by the Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, using the following protocol. DNA was extracted from the samples using a DNAzol kit according to the manufacturer’s protocol (Molecular Research Center, Cincinnati, Ohio, USA). After isolation, DNA was quantified spectrophotometrically using a Nanodrop 2000 instrument (Thermo Fisher Scientific, Wilmington, Delaware, USA). Standard polymerase chain reaction (PCR) was performed in a T100 thermal cycler (Bio Rad, California, USA) in a 50-µL reaction containing 1× PCR buffer, 2U Platinum® Taq polymerase (Invitrogen, Carlsbad, California, USA), 0.8 mM dNTPs, 3 mM MgCl₂, 0.5 µM of each amplification primer (Table 1) and 100 to 200 ng DNA template (mixed cervid/parasite DNA). The PCR reaction conditions consisted of an initial melt at 94°C for 3 min followed by 35 amplification cycles (denature at 94°C for 30 s, anneal at ~59°C for 45 s, extend at 72°C for 1.5 min), and then terminate with a final extension of 72°C for 5 min to complete any partial products. Annealing temperatures were chosen based on Primer3 implemented from within the Geneious bioinformatics software (Version 6.1 and later, available from http://www.geneious.com) (22). Polymerase chain reaction (PCR) products were separated electrophoretically using a submarine 1.4% agarose gel with 1× TAE buffer (100 mL) and 4 µL of ethidium bromide dye (10 mg/mL, w/v). The GeneRuler 1 kb Plus DNA size ladder (Thermo Fisher Scientific) was used to determine product fragment lengths. Gels were examined using an ultraviolet transilluminator and DNA bands of expected sizes were excised using a sterile scalpel. DNA was extracted from the gel slice using the QIAquick Gel Extraction Kit (QIAGEN, Toronto, Ontario) according to the manufacturer’s instructions. Purified PCR amplicons were then submitted for sequencing in both directions with forward and reverse amplification primers using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, California, USA) by the Molecular Biology Unit of the Laboratory Services Division, University of Guelph, Guelph, Ontario. Chromatograms received from sequencing reactions were imported into Geneious for analyses.

Molecular genetic analysis of the blood and spleen samples collected from the elk housed at the Parc Safari in Hemmingford, Quebec was performed by the Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA using primers and methods described by Schoelkopf et al (7).

The sample of spleen collected from the female reindeer that died in November 2014 at the Toronto Zoo (Z167-14) was submitted to the Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, and molecular genetic analysis was performed following the protocol described by Pattullo et al (18). The blood sample collected from the reindeer housed at the Assiniboine Park Zoo in Winnipeg, Manitoba, was sent to the Vector Borne Disease Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA, for molecular genetic analysis. The DNA extraction was performed using QIAshredder SP (Qiagen, Valencia, California, USA) with QIAshredder DNA Mini Kit (192) (Qiagen). Standard PCR was performed in a thermal cycler (Mastercycler EP gradient aluminum block thermocycler, Eppendorf North America, Hauppauge, New York, USA) in a 25-µL reaction containing 12.5 µL of MyTaq Red Mix 2X (Bioline, London, UK), 0.5 µM of each amplification primer (Table 1) and 150 ng DNA template (mixed cervid/parasite DNA). The PCR reaction conditions consisted of an initial melt at 94°C for 5 min followed by 35 amplification cycles (denature at 95°C for 20 s, anneal at 58°C for 30 s and extend at 72°C for 1 min) and then terminate with a final extension of 72°C for 5 min to complete any partial products. The PCR products were separated by electrophoresis through a 2% (w/v) agarose gel. The unpurified PCR product was directly sent for Sanger sequencing through GENEWIZ (Research Triangle Park, North Carolina, USA). Sequences were aligned and compared with GenBank sequences using the AlignX software (Vector NTI Suite 6.0, InforMax, Bethesda, Maryland, USA).

### Results

Nine confirmed cases of fatal infection by *B. odocoilei* were identified in captive reindeer and elk in 3 Canadian provinces

### Table 1. Polymerase chain reaction (PCR) amplification primers used for amplification of *Babesia odocoilei* from cervid tissue samples.

<table>
<thead>
<tr>
<th>Amplified fragment</th>
<th>Product size (bp)</th>
<th>Primer names</th>
<th>Primer sequences (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribosomal 18S rDNA</td>
<td>1687</td>
<td>Medlin A (F)</td>
<td>AACCTGGTTGATCCTGACCAGT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piro_18S_1688_R</td>
<td>CGA CCTTCCTTTCTTTGAAGATAAAG</td>
</tr>
<tr>
<td>Ribosomal 18s rDNA</td>
<td>681</td>
<td>Piro_144_S</td>
<td>ACCGTGCTATTTGTÁGGGCTAATAACA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCOMMONON2R</td>
<td>TGTCTTGGCAGTAGTTGTGTC</td>
</tr>
</tbody>
</table>

* PCR performed by the Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.
* Primer A of Medlin et al (20) less polylinker region.
* Equivalent to primer BN1700 of Ramos et al (21).
* PCR performed by the Vector Borne Disease Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA, using primers described by Schoelkopf et al (7).
lesions described included hepatic necrosis (1/9), centrilobular hepatocyte degeneration (1/9), hepatic lipidosis (1/9), extravascular erythrophagia (2/9), and reactive spleen (2/9).

Diagnosis of *B. odocoilei* infection was confirmed in all cases using PCR performed on whole blood or frozen spleen (Table 3). Partial 18S rDNA sequences from ID #Z176-12 and D24259, 2 American elk, and from ID #Z147-15, a European reindeer, were submitted to GenBank under accession numbers AB12345.6, MF045131.1 and AB12345.6, respectively. All 18S rDNA sequences had 100% sequence identity to one another and to a number of *B. odocoilei* sequences submitted previously to GenBank from various hosts and geographic locations: white-tailed deer in Texas (AY046577; U16369.2); elk in New Hampshire (AY661503.1), Wisconsin (AY294206.1) and Saskatchewan (KC460321); and muskox from Minnesota (K00075).

Discussion

Between 2012 and 2016, there were at least 11 confirmed deaths in captive cervids in 4 Canadian provinces as a result of infection by *B. odocoilei* (18). Although this list is likely not exhaustive, the cases reported here suggest that cervid babesiosis is an emerging disease in Canada. Prior to the first report of the disease in a Saskatchewan elk herd, cervid babesiosis was not recognized as a clinical problem in Canada, and surveillance for the pathogen in free-ranging wildlife was not of high priority.

In part, this was likely a result of the presumptive absence of the pathogen in free-ranging wildlife was not of high priority. The main tick vector, *I. scapularis* (4). While endemism in wild

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**Table 2.** Signalment and geographic location of captive elk (*Cervus canadensis*) and reindeer (*Rangifer tarandus*) that died of infection with *Babesia odocoilei*.

<table>
<thead>
<tr>
<th>ID</th>
<th>Species</th>
<th>Gender</th>
<th>Age class</th>
<th>City</th>
<th>Province</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Date of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z165-12</td>
<td>Elk</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>October 2012</td>
</tr>
<tr>
<td>Z175-12</td>
<td>Elk</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>November 2012</td>
</tr>
<tr>
<td>Z176-12</td>
<td>Elk</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>November 2012</td>
</tr>
<tr>
<td>Z174-13</td>
<td>Elk</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>October 2013</td>
</tr>
<tr>
<td>K00075</td>
<td>Reindeer</td>
<td>Male</td>
<td>Adult</td>
<td>Winnipeg</td>
<td>Manitoba</td>
<td>49° 52'</td>
<td>97° 14'</td>
<td>June 2014</td>
</tr>
<tr>
<td>D24259</td>
<td>Elk</td>
<td>Female</td>
<td>Adult</td>
<td>Hemmingford</td>
<td>Quebec</td>
<td>45° 02'</td>
<td>73° 31'</td>
<td>June 2014</td>
</tr>
<tr>
<td>Z167-14</td>
<td>Reindeer</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>November 2014</td>
</tr>
<tr>
<td>Z147-15</td>
<td>Reindeer</td>
<td>Female</td>
<td>Adult</td>
<td>Scarborough</td>
<td>Ontario</td>
<td>43° 49'</td>
<td>79° 11'</td>
<td>November 2015</td>
</tr>
</tbody>
</table>

* Over 2.5 years of age.

**Table 3.** Major clinical and pathologic findings in captive elk (*Cervus canadensis*) and reindeer (*Rangifer tarandus*) that died of infection with *Babesia odocoilei*.

<table>
<thead>
<tr>
<th>ID</th>
<th>Species</th>
<th>Clinical syndrome</th>
<th>Piromplasms on blood smear</th>
<th>Hemorrhage</th>
<th>Pigmenturia</th>
<th>Intrahepatic cholestasis</th>
<th>Tissue(s) PCR positive for <em>B. odocoilei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Z165-12</td>
<td>Elk</td>
<td>Acute</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Spleen</td>
</tr>
<tr>
<td>Z175-12</td>
<td>Elk</td>
<td>Peracute</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Spleen</td>
</tr>
<tr>
<td>Z176-12</td>
<td>Elk</td>
<td>Peracute</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Spleen</td>
</tr>
<tr>
<td>Z174-13</td>
<td>Elk</td>
<td>Peracute</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Spleen</td>
</tr>
<tr>
<td>K00075</td>
<td>Reindeer</td>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Whole blood</td>
</tr>
<tr>
<td>D24259</td>
<td>Elk</td>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Spleen</td>
</tr>
<tr>
<td>Z167-14</td>
<td>Reindeer</td>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Whole blood, spleen</td>
</tr>
<tr>
<td>Z147-15</td>
<td>Reindeer</td>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Whole blood, spleen</td>
</tr>
</tbody>
</table>

<sup>a</sup> Absent.  
<sup>b</sup> Present.
Canadian cervids has yet to be conclusively demonstrated, it is reasonable to assume that in Canada, as in the United States, the white-tailed deer is a natural reservoir of infection (1,5,18). Research is currently underway in Saskatchewan and Ontario to establish its prevalence in farmed and wild cervids (17).

The antemortem clinical syndromes and postmortem findings reported here are consistent with previously documented cases of cervid babesiosis. Until recently, infection with Babesia odocoilei in reindeer and caribou was only known to manifest as a rapidly fatal acute disease. However, subclinical infections were recently demonstrated in reindeer at the Toronto Zoo (23). Disease manifestation in elk is more variable, and ranges from peracute to asymptomatic (6,13). The variability of clinical manifestations of infection with B. odocoilei is believed to be linked to differences in host susceptibility, levels of host infection and degree of stress-induced immunosuppression (13). Documented postmortem findings in peracute and acute cases of cervid babesiosis are consistent with a hemolytic crisis, and often include marked icterus, extensive multifocal petechial hemorrhages, splenomegaly, and pigmenturia. Commonly observed histologic lesions are hemoglobinuric nephrosis with tubular degeneration, splenic hemosiderosis, and hepatic centrilobular degeneration (6,8,13,19).

Adult males are overrepresented in the literature, with most fatal cases of cervid babesiosis occurring during the rutting season (i.e., between the months of September and November) (7,8,10,13,14,19). In the present report, cases occurred mainly in adult females, and were not restricted to the rutting season. The apparent gender predilection in our study is likely biased as herds of cervids in zoos typically are mostly composed of females, but does also support the premise that stressors other than rut may be implicated in the pathogenesis of cervid babesiosis. No particular predisposing factors were identified in the cases presented here. The absence of cases in juvenile cervids may also be biased as a result of low numbers of young animals in captive collections that were available to become infected, or may be a result of an inverse age resistance, as described with bovine babesiosis (24). Cervid babesiosis has been described in woodland caribou as young as 6 to 8 mo of age (10).

The seasonality of mortality correlates with that of the tick vector’s life cycle. The development cycle of I. scapularis is controlled by temperature and day-length, with the maximum activity of nymphs and adult ticks from mid-summer through autumn in the northern United States and southern Ontario (25–28). Transtadial survival of B. odocoilei from nymph to adult, and transmission from deer to deer by the adult tick were demonstrated for I. scapularis under laboratory conditions (5). Transmission by nymphs has not been shown, but is plausible given the fact that a related Babesia species, Babesia divergens, can be transmitted to hosts by all tick life stages (29).

The recent increase in detection of B. odocoilei in Canada is the combined result of the progressive incursion of its tick vector and of an increase in disease recognition and reporting by veterinarians. Movement of asymptomatic carriers between institutions may have contributed to the spread of the pathogen. Although most animals included in this report were born on-site, all 3 institutions occasionally acquire animals from other institutions, making the introduction of infected animals from endemic areas possible. Disease vectors are sensitive to climatic factors, and the effects of global warming on the extension of vector range from tropical to more temperate areas are well-documented with other vector-borne diseases such as malaria, Lyme borreliosis, and dengue fever (30). Scanning and targeted surveillance for I. scapularis shows that its range has been expanding northward from the northern United States into southern Manitoba, Ontario, Quebec, and the Maritimes; with detection clusters around Winnipeg, Toronto, and Montreal (30,31). The locations of the cervid babesiosis cases reported here coincide with the described geographic distribution of I. scapularis. Although the presence of B. odocoilei has yet to be demonstrated in I. scapularis in Canada, carriage of the pathogen is assumed. Interestingly, I. scapularis has been identified in Saskatchewan on northern pocket gophers (Thomomys talpoides) and in a handful of submissions by the public, but there are no known established populations in this province (30,32). It was hypothesized that the ticks responsible for transmitting B. odocoilei to the Saskatchewan elk herd had been carried by birds that migrated northward from American states or westward from Canadian provinces that support endemic I. scapularis and B. odocoilei populations (18,31,33). Preliminary results of ongoing research indicate that the tick is found sporadically in Saskatchewan cervids, but no endemic foci have been identified so far (17). These findings support the hypothesis that migratory birds are randomly transporting I. scapularis along with B. odocoilei into the province. As the tick vector range continues to expand northwards, it is likely that additional cases of babesiosis will emerge in captive cervids, and possibly other ungulate species, at higher latitudes than described here, and that B. odocoilei may eventually pose a risk to the health of the wild elk and caribou populations.
Conducting unbiased disease surveillance in free-ranging populations can be challenging. Population-based surveillance, which involves the collection of data through screening and targeted surveillance activities, is a powerful tool for monitoring infectious diseases, but is often labor-intensive and costly to implement and maintain. In the context of identifying disease in free-ranging wildlife, scanning surveillance relies on the opportunistic detection of disease events through the observation of diseased or dead animals, while targeted surveillance is based on the purposeful searching for evidence of disease or pathogens in animal populations. Sentinel surveillance can enhance detection of diseases and improve the cost-effectiveness of surveillance. In-depth data collection is performed on selected subpopulations, and the resulting analysis is used to signal disease outbreaks, identify epidemiologic trends, and monitor the burden of disease in the overall targeted population. Zoos and other collections of captive animals can be useful sentinel units for wildlife health, especially in regions in which minimal economic and human resources are dedicated to wildlife disease surveillance, or when population-based surveillance of wildlife health is limited to known enzootic pathogens (34). The finding of Cervid babesiosis should be included as a differential diagnosis for peracute or acute hemolytic crisis in captive cervids in Canada. Serological surveys of captive cervids suggests local endemicity in wild cervid or tick endemic wildlife pathogens (34). The finding of Babesia odocoilei in captive cervids suggests local endemicity in wild cervid or tick populations, and reinforces the value of captive collections as sentinel units for wildlife health.

Cervid babesiosis should be included as a differential diagnosis for peracute or acute hemolytic crisis in captive cervids in Canada. Although further research is needed to clarify the epidemiology of Babesia odocoilei in Canadian wildlife, this case series suggests that the parasite has been introduced to southern Quebec, Ontario, and Manitoba. Cervid babesiosis is expected to become increasingly prevalent in Canada as global warming continues to alter the geographic and seasonal distributions of tick vectors.

### Acknowledgments

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### References

Zoonotic Tuberculosis: *Mycobacterium bovis* and Other Pathogenic Mycobacteria, 3rd edition


The 3rd edition of this book updates the current status of *M. bovis* in industrialized and developing countries from the 2006, 2nd edition, includes new chapters on One Health, and covers 6 additional African countries. The book is well-organized with individual chapters building on each other while also being able to stand alone as review of a specific topic or location.

The book starts with the discussion on a One Health approach to manage zoonotic tuberculosis, followed by a chapter on its significance to public health. Subsequent chapters describe pathogen and disease specifics, including pathogenesis, macro- and molecular epidemiology, and current approaches for isolation, identification, and genotyping of *M. bovis*. These chapters are comprehensive and well-referenced. I found the molecular epidemiology section especially interesting as this technology is becoming more affordable and the information gained is so valuable in tracing the origin of an outbreak in either humans or animals.

These overview chapters are followed by chapters describing zoonotic tuberculosis in a specific country or region, as well as a chapter on zoonotic tuberculosis in nonhuman primates. Given the variation in the epidemiology, wildlife reservoirs, and management of zoonotic tuberculosis, country-specific programs have significant similarities and differences.

The book describes the long history of zoonotic tuberculosis occurrence and management, and the role Pasteurization has played in food safety and public health. This book would be helpful for veterinarians, students, and allied health professionals working in government, academia, research, or private practice in regions of Canada impacted by zoonotic tuberculosis, or those who work importing animals or managing endangered species or zoo populations.

Reviewed by Judy Hodge, BSc, DVM, MPH, Winnipeg, Manitoba.
A clinical trial investigating the impact of in-feed flavophospholipol on *Salmonella* shedding and antimicrobial resistance in pigs

Saranya Nair, Abdolvahab Farzan, Terri L. O’Sullivan, Robert M. Friendship

**Abstract** — A clinical trial was conducted to assess the effectiveness of in-feed flavophospholipol in reducing *Salmonella* shedding and antimicrobial resistance (AMR) associated with *Salmonella* and generic *Escherichia coli* in naturally infected grower-finisher pigs. Pigs were obtained from a farm with a history of salmonellosis and were housed at a research facility. Over the span of 10 weeks the pigs received either a feed containing 4 ppm of flavophospholipol (treatment, \(n = 25\)) or a non-medicated feed (control, \(n = 20\)). Weekly fecal samples were collected and cultured for *Salmonella* and generic *E. coli*. A subset of *Salmonella* and *E. coli* isolates were tested for antimicrobial susceptibility. A multilevel mixed-effects logistic regression model was used to compare the prevalence of *Salmonella* shedding and AMR in *Salmonella* and *E. coli* isolates in treatment and control groups. Overall, the prevalence of *Salmonella* shedding \((P > 0.05)\) and AMR in *Salmonella* \((P > 0.01)\) and *E. coli* \((P > 0.005)\) isolates was not different between the treatment and control groups.

**Résumé** — Essai clinique étudiant l’impact du flavophospholipol dans les aliments sur l’excrétion de *Salmonella* et l’antibiorésistance chez les porcs. Un essai clinique a été réalisé pour évaluer l’efficacité du flavophospholipol dans les aliments pour réduire l’excrétion de *Salmonella* et l’antibiorésistance associée à *Salmonella* et à *Escherichia coli* générique chez les porcs d’engraissement naturellement infectés. Les porcs obtenus provenaient d’une ferme ayant des antécédents de salmonellose et ils ont été logés à un établissement de recherche. Pendant 10 semaines, les porcs ont reçu soit des aliments contenant 4 ppm de flavophospholipol (traitement, \(n = 25\)) ou des aliments non médicamenteux (témoin, \(n = 20\)). Des échantillons fécaux hebdomadaires ont été prélevés et soumis à des cultures pour *Salmonella* et *E. coli* générique. Un sous-groupe d’isolats de *Salmonella* et d’*E. coli* ont été testés pour la susceptibilité antimicrobienne. Un modèle de régression logistique à effets contrastés à plusieurs niveaux a été utilisé pour comparer la prévalence d’excrétion de *Salmonella* et de l’antibiorésistance dans les isolats de *Salmonella* et d’*E. coli* dans le groupe de traitement et le groupe témoin. Dans l’ensemble, la prévalence d’excrétion de *Salmonella* \((P > 0.05)\) et de l’antibiorésistance des isolats de *Salmonella* \((P > 0.01)\) et d’*E. coli* \((P > 0.005)\) n’était pas différente entre le groupe de traitement et le groupe témoin.

(Traduit par Isabelle Vallières)

**Introduction**

On-typhoidal *Salmonella* spp. (*Salmonella*) are commonly recovered in the feces of swine but are rarely found to cause clinical disease (1). Studies have revealed that *Salmonella* are present on most Ontario swine farms (2, 3). The most common serotype recovered from Ontario pigs is *Salmonella* Typhimurium DT 104 (2), a multi-drug resistant serotype that is a common human pathogen (4). *Salmonella* can be difficult to control and there is little financial incentive for Canadian pork producers to institute costly on-farm measures to reduce *Salmonella* because the presence of *Salmonella* is not usually associated with recognizable clinical disease. However, flavophospholipol (ambermycin, flavomycin, moenomycin), an inexpensive antibiotic used as a growth promoter, may be an attractive method for swine producers to reduce on-farm *Salmonella* and the associated antimicrobial resistance (AMR) in pigs.

Flavophospholipol is an antibiotic without any therapeutic applications in human or veterinary medicine due to its poor pharmacokinetic and pharmacodynamic features (5, 6). Moreover, resistance to this phosphoglycolipid antimicrobial agent amongst bacterial populations is only slowly developed through mutations in the bacterial chromosome and is non-transferable (5, 7). Research suggests that flavophospholipol may alter the microflora in favor of beneficial bacteria inhibiting the colonization of *Salmonella* in animals (8). The inhibition by flavophospholipol is likely due to competition among pathogenic and beneficial bacteria for intestinal binding sites or may be due to reduced intestinal pH (8). Furthermore, flavophospholipol,
by inhibiting R-plasmid carrying bacteria in a process referred to as a “plasmid-curing effect,” may reduce AMR (9–12). Although the impact of flavophospholipol on Salmonella and AMR in pigs has been previously studied by experimental challenge studies, use of flavophospholipol under commercial pig farming conditions in which pigs are naturally infected with Salmonella has not been thoroughly investigated. The objectives of this study were to evaluate whether the in-feed use of flavophospholipol can reduce Salmonella shedding in naturally infected pigs and if flavophospholipol can influence AMR associated with Salmonella and generic E. coli in those pigs.

**Materials and methods**

The research trial was approved by the animal care committee of the University of Guelph, in accordance with the guidelines of the Canadian Council of Animal Care.

**Pigs and treatment groups**

Forty-five pigs at 10 wk of age were obtained from a commercial farm in which Salmonella shedding in pigs was known to be prevalent based on previous diagnostic work. The pigs were transported to the Ponsonby General Animal Facility (University of Guelph) where they were randomly assigned to 1 of 9 pens (5 pigs/pen) and ear tagged. The treatment group consisted of 5 pigs (n = 25) and these pigs were provided with a diet containing 4 ppm of flavophospholipol (Flavomycin; Huvepharma, distributed by Bio Agri Mix, Mitchell, Ontario). The control group (n = 20 pigs) housed in 4 pens received an identical diet but without the medication. In order to accommodate the growing pigs, at the beginning of week 8 of the trial, 2 to 3 pigs were randomly removed from each pen and placed in the corresponding pen in a second, similar room in the research facility. Pigs that had been receiving the treatment ration continued to receive this feed and likewise pigs that were moved from control pens to the new room continued to receive the control ration. This movement of pigs resulted in a total of 18 pens (10 treatment, 8 control) from weeks 8 to 11 of the trial. Boot covers and gloves were changed to prevent transmission (10 treatment, 8 control) from weeks 8 to 11 of the trial. Rectal swabs were cultured in 9 mL TTB and incubated as homogenized fecal samples. Then, 100 µL of TTB culture was transferred to 9.9 mL of Rappaport-Vassiliadis broth (RV; Becton Dickinson) and incubated at 41°C for 18 to 24 h. Next, a loopful (~20 µL) of the RV broth was streaked onto xylose-lysine-tergitol 4 (XLT4) agar (Remel Thermo Fisher Scientific, Lenexa, Kansas, USA) and incubated at 37°C for 18 to 24 h. One colony was selected per Salmonella-positive sample. Salmonella isolates were confirmed by agglutination in Salmonella poly O antiserum (Becton Dickinson).

**Generic E. coli**

Pigs were tested for generic E. coli before treatment (week 1) and at the end of the trial (week 11). The fecal samples were added to buffered peptone water (Oxoid BPW; Thermo Scientific Oxoid, Thermo Fisher Scientific) at a 1 to 9 ratio, homogenized for 30 s with a Seward Stomacher 400 Circulator (Seward Laboratory Systems) and incubated at 37°C for 18 to 24 h. A loopful (~20 µL) of the BPW broth was streaked onto MacConkey agar (BBL; Becton Dickinson) and incubated at 37°C for 18 to 24 h. One colony was selected per E. coli-positive sample. Colonies were then streaked on to LB agar (Fisher BioReagents; Thermo Fisher Scientific) and incubated at 37°C for 18 to 24 h. Escherichia coli isolates were identified using the spot indole reagent (Remel Thermo Fisher Scientific) and Simmons citrate agar (BBL, Becton Dickinson) tests.

**Salmonella serotyping**

A subset of 24 Salmonella isolates consisting of the first (n = 11) and last positive Salmonella culture (n = 13) from 13 randomly selected pigs (7 treatment and 6 control) was serotyped. The first positive Salmonella cultures belonged to weeks 1 or 2, while the last positive Salmonella cultures belonged to pigs in weeks 3, 4, 7, 9, and 11. Isolates were submitted to Biovet (St-Hyacinthe, Quebec) for molecular serotyping by means of xMAP Salmonella Serotyping Assay Kit. The xMAP Salmonella serotyping assay is a microsphere-based, molecular serotyping method that detects genes which express serotype-specific O and H antigens.

**Antimicrobial susceptibility testing**

Salmonella isolates from pigs before treatment (week 1) and at week 3 and weeks 1 and 4 were included in antimicrobial susceptibility testing. Escherichia coli isolates from pigs at week 1 (before treatment) and week 11 (at the end of treatment) were tested. Antimicrobial susceptibility testing was performed by means of a micro-dilution broth using a Sensititre NARMS Gram-negative plate (TREK Diagnostic Systems, Thermo Fisher Scientific, Oakwood Village, Ohio, USA). The antimicrobials and MIC (minimum inhibitory concentrations in µg/mL) were: amoxicillin/clavulanic acid (Au; 32/16), ampicillin (A; 32), cefoxitin (Ce; 32), ceftriaxone (Cx; 64), chloramphenicol (C; 32), ciprofloxacin (Ci; 4), gentamicin (G; 16), nalidixic acid (Na; 16), tetracycline (Tc; 2), and cefotaxime (Cf; 2).
acid (N; 32), streptomycin (S; 64), sulfisoxazole (Su; 256), tetracycline (T; 32), trimethoprim/sulphamethoxazole (T/S; 4/76). Isolates that grew at concentrations of antimicrobials greater than the MIC threshold were classified as resistant. Isolates that grew only at concentrations below the threshold were identified as susceptible. Isolates with intermediate MIC breakpoints were classified as susceptible in order to prevent overestimation of resistance in isolates.

Briefly, isolates were streaked onto LB agar, incubated at 37°C for 24 h, then tested for antimicrobial susceptibility with the Trek Diagnostic System Sensititre equipment. The colonies on LB agar were added to sterile water, mixed, and adjusted to a 0.5 McFarland Standard. Then, 30 µL of the bacterial suspension were added to 11 mL of Müeller-Hinton broth with TES buffer and mixed thoroughly. The Sensititre AutoInoculator was then used to dispense 50 µL of the Müeller-Hinton broth suspension into the wells of the plate. Plates were covered with the adhesive seal and labeled. After incubation at 37°C for 18 h, plates were read using the Sensititre Automated Reading and Incubation System.

Statistical analysis

Data were entered into Microsoft Excel for Mac 2011 Version 14.5.5 (Microsoft, Redmond, Washington, USA) and after cleaning were imported into Stata (Stata/SE 14.1 for Mac; StataCorp, College Station, Texas, USA). A multilevel mixed-effects logistic regression model with pen (common environment) and pig (repeated measurements) as a random effect was used to compare the prevalence of Salmonella shedding among pigs in the treatment and control group. To control for the addition of a second room in weeks 8 to 11, room was held as a fixed effect.

To assess AMR, statistical analysis was only conducted for antimicrobials that had a change in resistance. Descriptive analysis of resistance patterns in Salmonella and E. coli isolates was assessed. A multilevel mixed-effects logistic regression model with pen as a random effect, room as a fixed effect, and resistance before treatment (week 1) as a covariate was used to separately assess each antimicrobial to compare AMR in Salmonella isolates recovered from pigs in the treatment and control groups. A similar model was built for E. coli isolates. Bonferroni adjustments were used to control for multiple antimicrobial comparisons.

Results

Salmonella shedding

Two pigs (1 from each group) were euthanized in week 3 for reasons unrelated to the trial. A total of 479 individual fecal samples and 135 pooled-fecal samples were collected. The prevalence of Salmonella shedding in individual pig samples from weeks 1 to 11 is shown in Figure 1. Salmonella was cultured from 36 (80%) pigs at arrival at the research facility (Week 1); over 11 weeks it was recovered from 27% and 28% of the individual fecal samples from the treatment and control groups, respectively. Over the 10-week period during which treatment pigs received feed containing 4 ppm of flavophospholipol, the presence of Salmonella in the fecal samples collected from pens or pigs was not significantly different between treatment and control pigs (P > 0.05).

Salmonella serotypes

Twenty-four isolates recovered at different times from 13 pigs were serotyped. In total, pigs shed 8 serotypes over the study period including S. Typhimurium (25%), S. Livingstone (25%), S. Senftenberg (21%), S. I:Rough-O (8%), S. Montevideo (8%), S. Benfica (4%), S. Amstredam (4%), and S. Infantis (4%). Of the 11 pigs with first and last isolates, 91% (n = 10) were reinfected with a different serotype. Pigs were initially colonized by S. Typhimurium (36%), S. Senftenberg (36%), S. Benfica (9%), S. Amsterdam (9%), and S. I:Rough-O (9%); however, reinfection with S. Livingstone (45%) was the most common followed by S. Typhimurium (18%), S. Montevideo (18%), S. Senftenberg (9%), and S. I:Rough-O (9%).

E. coli shedding

Using the remaining fecal samples from 18 and 14 pigs from the treatment and control groups, respectively, generic E. coli was cultured at 2 time points (at entry and at the end of the finisher period). Generic E. coli was found at week 1 and week 11 in all pigs tested.

Antimicrobial resistance

Overall, Salmonella isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline. After controlling for multiple antimicrobial comparisons using Bonferroni adjustments, the P-value required for significance was ≤ 0.01. No significant difference was found in antimicrobial resistance associated with Salmonella isolates recovered from pigs in the treatment and control groups (P > 0.01). The antimicrobial resistance profiles for Salmonella isolated from pigs in control and treatment groups at weeks 1, 3, and 4 are shown in Table 1. The most prevalent resistance pattern associated with Salmonella in week 1 was “S” in both the treatment (65%) and control (75%) groups. In weeks 3 and 4, “ASSuT” (67%) and “SSuT” (40%) were common in the treatment group while “ASSuT” (week 3 = 53%; week 4 = 43%) was the most prevalent in the controls (Table 1). Penta-resistance (ACSSuT) was also observed in Salmonella isolates (Table 1).
Escherichia coli isolates had resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulphamethoxazole (Figure 2). After controlling for multiple antimicrobial comparisons using Bonferroni adjustments, the P-value required for significance was \( \leq 0.0045 \). When assessing resistance for each antimicrobial for E. coli isolates, there was no difference in antimicrobial resistance in isolates recovered from the treatment group compared with the control group \( (P > 0.0045) \). Antimicrobial resistance patterns in E. coli isolates are shown in Table 2. The most prevalent resistance patterns in E. coli isolates in week 1 were “T” (28%) and “ST” (28%), while “AT” (17%) and “ACST” (17%) were most common in Week 11 in the treatment group. Resistance patterns “ACSSuT” (29%) and “SSuT” (14%) were most common in the control group in week 1, but during week 11 “ACSSuTT/S” (36%) and “ASSuTT/S” (14%) were commonly recovered.

### Discussion

Challenge studies have been primarily used to assess the impact of flavophospholipol on Salmonella shedding and antimicrobial resistance in pigs and poultry. However, the results are inconsistent. Some experimental challenge studies found flavophospholipol to be effective in reducing Salmonella shedding in swine and broilers (8,13). For example, Dealy and Moeller (13) found a significantly reduced duration and prevalence of Salmonella shedding in 4- and 5-week-old pigs challenged with S. Typhimurium and fed a ration containing 4.4 ppm of flavophospholipol compared to challenged pigs fed a non-medicated control diet over a 7-week period. Over the duration of the Dealy and Moeller (13) trial, fecal samples were collected on 11 occasions; Salmonella were cultured using tetraphionate brilliant green broth (TBGB) and plated onto brilliant green agar (BGA). Bolder et al (8) found 9 ppm of flavophospholipol to be effective in decreasing the rate and magnitude of shedding of S. Enteritidis in broilers challenged at 6 wk of age. Weekly fecal samples were cultured using incubation in buffered peptone water (BPW) followed by plating onto BGA (8).

In contrast, other experimental challenge studies conducted in chickens and pigs with S. Typhimurium found that flavophospholipol was not effective in reducing Salmonella (14,15). Letellier et al (15) found that 0.5 ppm of flavophospholipol in-feed given to pigs that were inoculated with S. Typhimurium 14 d after beginning treatment did not significantly reduce the presence of S. Typhimurium in feces by day 28 of the trial. This study used rectal swabs collected from pigs; Salmonella was cultured using TBGB and plating on to BGA (15). Similarly, Humbert et al (14) found 1-day-old chicks \((n = 46)\) treated with 5 ppm of flavophospholipol and challenged with S. Typhimurium at day 2 did not have a different Salmonella shedding pattern from that of untreated chicks \((n = 92)\) at days 3 and 6 (14). Cecal samples collected from chicks were cultured using BPW as pre-enrichment, tetraphionate Müller-Kaufmann broth and RV broth for enrichment, and then plating onto BGA and Wilson Blair agar (WBA).

Letellier et al (15) used a low dosage of flavophospholipol (0.5 ppm), while Humbert et al (14) conducted their trial over a very short time. In contrast, Dealy and Moeller (13) and Bolder et al (8), conducted trials over a longer duration with higher than the recommended dosage of flavophospholipol \((> 4 \text{ ppm})\). Furthermore, these studies (8,13) used fecal samples which have been found in previous studies to have a greater sensitivity than rectal swabs in detecting Salmonella (16,17). The discrepancies in the results from different studies might be due, in part or in whole, to different animal species (poultry versus pigs) used, study design (number of animals, number of replicates, duration of trial, treatment dosage, and length of treatment period), challenge model (challenge serotype and strain characteristics, challenge dosage), and methods used for Salmonella culturing.

Unlike the challenge studies in which animals were experimentally infected in a controlled environment with Salmonella and fed low dosages of flavophospholipol for a short period, the current study used naturally infected pigs and followed these pigs over 10 wk, which was more representative of the farm situation. The present study also had improved sensitivity compared with some of the challenge studies due to repeated weekly fecal sample collection. However, our findings indicate that naturally infected pigs fed flavophospholipol had similar Salmonella shedding to pigs fed a non-medicated diet. An important difference between controlled challenge studies and the present study was that this population of pigs was infected

### Table 1. Antimicrobial resistance patterns of Salmonella isolates from grower-finisher pigs fed a diet containing 4 ppm flavophospholipol (treatment) and pigs fed a similar but non-medicated diet (control). Pigs were naturally infected with Salmonella and entered the trial at 10 weeks of age (Week 1).

<table>
<thead>
<tr>
<th>AMR pattern</th>
<th>Treatment ((n = 17))</th>
<th>Control ((n = 16))</th>
<th>Treatment ((n = 15))</th>
<th>Control ((n = 15))</th>
<th>Treatment ((n = 5))</th>
<th>Control ((n = 7))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>65</td>
<td>75</td>
<td>7</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AT</td>
<td>6</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>AST</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>SSu</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>SSuT</td>
<td>12</td>
<td>6</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>ACST</td>
<td>12</td>
<td>6</td>
<td>67</td>
<td>53</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>ACSSuT</td>
<td>12</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

AMR = antimicrobial resistance; A = ampicillin; C = chloramphenicol; S = streptomycin; Su = sulfonamides; T = tetracycline.

\( n \) = number of isolates.
may have colonized the gut and possibly invaded tissues such as mesenteric lymph nodes before the start of the trial, making it difficult for the treatment to alter the microbiome or eliminate the pathogen. If a high degree of Salmonella colonization occurred in the microbiome before the pigs received treatment, this may have prevented flavophospholipol from significantly reducing Salmonella shedding. If these same pigs were provided with the treatment diet at an earlier stage (i.e., beginning at weaning) when the pigs were most susceptible to alterations in the microbiome, then a more favorable outcome might have occurred. Future studies to examine the benefits of administering flavophospholipol as a preventive measure, before pigs are infected with Salmonella need to be conducted in order to rule out the possibility that under certain conditions flavophospholipol can reduce Salmonella shedding.

While previous challenge studies found flavophospholipol to be capable of reducing antimicrobial resistance (13,18), the present study found no difference in antimicrobial resistance in Salmonella recovered from pigs fed a ration containing flavophospholipol and from pigs consuming a non-medicated feed. It is possible that 3 and 4 wk of treatment with flavophospholipol may not be adequate for resistance patterns to be altered. In an earlier study, Dorr and Gebreyes (18) challenged 60-day-old pigs with S. Typhimurium DT 104 or DT 193 then treated them with 4.85 ppm of flavophospholipol and isolated Salmonella weekly over 20 wk. This treatment resulted in less resistance to ampicillin and amoxicillin-clavulanic acid compared to control. Dealy and Moeller (13) compared isolates from pigs treated with 4.4 ppm of flavophospholipol to control pigs over 7 wk and found a reduction in the number of isolates resistant to ampicillin, streptomycin, triple sulf, and tetracycline. It is possible that the antimicrobial resistance curing properties of flavophospholipol may require longer than 3 or 4 wk to be effective.

No difference was found in antimicrobial resistance in E. coli isolated from flavophospholipol treated pigs compared with

**Table 2.** Antimicrobial resistance patterns of commensal E. coli isolates from grower-finisher pigs fed a diet containing 4 ppm flavophospholipol (treatment) and pigs fed a similar but non-medicated diet (control). Naturally Salmonella infected pigs entered the trial at 10 weeks of age (week 1) and were monitored for 10 weeks.

<table>
<thead>
<tr>
<th>AMR pattern</th>
<th>Week 1 (%)</th>
<th>Week 11 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>T</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>ST</td>
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</tr>
<tr>
<td>SSSuT</td>
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<td>7</td>
</tr>
<tr>
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<td>11</td>
<td>0</td>
</tr>
<tr>
<td>CSSuT</td>
<td>0</td>
<td>7</td>
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<tr>
<td>ACST</td>
<td>0</td>
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<tr>
<td>AGSSuT</td>
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</tr>
<tr>
<td>ACSuT</td>
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<td>AuASuT</td>
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<tr>
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</tr>
<tr>
<td>AuACeSSuT</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Au — amoxicillin/clavulanic; A — ampicillin; Ce — cefoxitin; C — chloramphenicol; Ci — ciprofloxacin; G — gentamicin; N — nalidix acid; S — streptomycin; Su — sulfonamides; T — tetracycline; T/S — trimethoprim/sulphamethoxazole.

* n = number of isolates.

with multiple serotypes. Although the pigs were fed the recommended dosage, the multiple serotypes present made it difficult to estimate the effectiveness of flavophospholipol on Salmonella shedding due to reinfec tions and renewed shedding in pigs.

Most challenge studies have been conducted in young pigs (shortly after weaning), while the pigs in the current study were older (grower-finisher pigs). It is likely that Salmonella may have colonized the gut and possibly invaded tissues such
isolates from control pigs by week 11. Earlier work by van den Bogaard et al (12) found that a dosage of 9 ppm flavophospholipol effectively suppressed multidrug resistant E. coli in challenged pigs. Researchers found a decrease in the mean degree of resistance against oxytetracycline (12). The standard dosage of 4 ppm flavophospholipol used herein may not have been sufficient to reduce antimicrobial resistance in generic E. coli isolates. Based on previous findings, a higher dosage or exposure for a longer time may be required to reduce antimicrobial resistance in Salmonella and E. coli isolates. It is also important to note that the use of Bonferroni adjustments, when assessing multiple antimicrobials for Salmonella and E. coli isolates, was a limitation for this type of study design. Future research with different study designs to better assess the impact of flavophospholipol on antimicrobial resistance may be beneficial.

Salmonella challenge studies that found flavophospholipol to significantly reduce antimicrobial resistance were conducted using 1 strain of Salmonella (13,18). There is a possibility that flavophospholipol was ineffective in the present study due to the presence of multiple serotypes and a situation of repeated infections with different serotypes. Antimicrobial susceptibility of isolates at the start of the trial was compared with data on isolates from weeks 3 and 4. These weeks were chosen compared to the end of the trial because of the high prevalence of Salmonella at this time. However, most pigs at weeks 3 and 4 were found to be shedding a different serotype than at week 1. Furthermore, the plasmid profiles of the E. coli and Salmonella isolates in this study are unknown. A previous study reported variations in plasmid profiles from a single Salmonella serotype found on Ontario swine farms (19). Given the presence of multiple serotypes in the present study, the possibility of variation in plasmid profiles exists that may not exist in the single strain used in challenge studies. Although the present study may have been representative of the farm situation, it was difficult to estimate the effectiveness of flavophospholipol on Salmonella shedding and antimicrobial resistance of Salmonella.

The multi-resistance in Salmonella (18,19) and E. coli (20–23) isolates found in this study has been previously reported in different pig populations. However, there is a lack of literature on resistance patterns found in Salmonella and E. coli isolates recovered from flavophospholipol treated pigs. Dorr and Gebreyes (18) found there were significantly fewer S. Typhimurium DT 104 isolates with resistance to just streptomycin over time. Although the present study conducted descriptive analysis, there were noticeably fewer Salmonella isolates resistant to just streptomycin from weeks 1 to weeks 3 and 4. However, over time there was a greater number of isolates that were multi-resistant to “SSuT,” “ASSuT,” and “ACSSuT” in both the treatment and control groups. In particular, in the flavophospholipol group a greater number of isolates were found to be resistant to “SSu” compared with the control group. Although there may be less resistance to just streptomycin in isolates, there is a growing number of isolates that are resistant to 1 or more antimicrobials, including streptomycin over time. It is also important to note that the resistance in isolates may be affected by the multiple serotypes that were present in the population. Unlike challenge studies in which pigs are experimentally infected with S. Typhimurium (18), in the present study assessing resistance patterns in this pig population over time was difficult due to the changes in serotypes amongst pigs over the trial. Similarly, in E. coli isolates, from weeks 1 to 11, there was increasing multi-resistance in isolates. However, there was no distinctive trend in resistance patterns between the treatment and control groups.

In conclusion, naturally infected pigs subsequently given flavophospholipol at 4 ppm in feed continued to shed Salmonella for a similar length of time compared to non-mediated pigs. Susceptibility to antibiotics in Salmonella (after 2 or 3 wk) and E. coli (after 10 wk) isolates was similar in the treatment and control groups.

Acknowledgments

We acknowledge the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) — Food Safety Research Program, Swine Innovation Porc, Huvepharma Animal Health and the University of Guelph-OMAFRA Research Partnership for financial support.

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Equine sarcoid tumor products now available to Canadian veterinarians

NovaVive Inc., an animal health immunobiology company, is pleased to announce that an equine-specific version of its Immunocidin® product for equine sarcoid tumors is now available to Canadian veterinarians. The new product, Immunocidin® Equine, is packaged in 5mL vials for the convenience of practitioners. The company believes that there is no other regulator-approved equine sarcoid therapy in North America.

Equine sarcoids are locally aggressive tumors that are considered to be the most common skin tumors of horses worldwide. They are often found around the eyes, head/face, neck, chest, and shoulder, and at the site of old scars. Young to middle-aged horses are most commonly affected, and it is estimated that sarcoids affect 1 in 100 horses in North America.

Current treatment options include surgery, ligation, cryotherapy, topical treatment, chemotherapy, radiation therapy, or laser removal. Their application results in limited efficacy and, occasionally, adverse reactions. In addition, some of these products do not have regulatory approval for use in sarcoid tumors. Immunotherapy is a safe and effective treatment option that is gaining interest in both human and veterinary oncology.

Immunocidin® Equine is administered by intratumoral injection, but the response is generalized and untreated sites frequently undergo regression as well.

“Immunocidin Equine has a high post-treatment tumor-free rate and is well-tolerated by horses, including older animals,” said Dr. Stan Alkemade, Chief Veterinary Scientific Officer at NovaVive Inc. “The product has minimal side effects and an excellent safety profile. It offers an economical treatment option to veterinarians.”

Here are before and after pictures of a horse treated with Immunocidin. The second picture was taken two weeks after four injections (administered at 14-day intervals).

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

Dechra Pharmaceuticals announce the launch of Zycortal.ca

Dechra Pharmaceuticals, a leader in animal health endocrinology, is committed to partnering with veterinarians in order to care for their clients’ and their pets. We are pleased to announce the launch of Zycortal.ca, a website created to support veterinarians and pet owners in the education and management of Addison’s disease. Also known as Hypoadrenocorticism, Addison’s disease is relatively rare with symptoms that are similar to more common diseases. With treatment and monitoring, your dog will be able to live a normal and active life. However, if left untreated Addison’s disease can be potentially fatal. Zycortal.ca includes extensive information about Addison’s disease, its diagnosis, treatment and ongoing management as well as a personalized logbook, where pet owners can keep and share with their veterinarian a record of their pet’s progress while they undergo treatment for Addison’s disease. Dechra is one of the fastest growing global brands in the veterinary market; our mission is to deliver the highest quality products and support to our partners to help keep their patients in good health.

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Comparison of hand-sewn and oversewn stapled jejunojejunal anastomoses in horses

José L. Bracamonte, Ian Devick, Keri L. Thomas, Steven Hendrick

Abstract — The objective of this study was to compare the biomechanical properties of hand-sewn jejunojejunal anastomoses to those of oversewn stapled jejunojejunal anastomoses. Jejunojejunal anastomoses were constructed from harvested jejunal segments using a single-layer Lembert technique (1HS), double-layer simple continuous/Cushing technique (2HS), stapled side-to-side technique oversewn with Cushing pattern (SS), and closed 1-stage stapled functional end-to-end technique oversewn with Cushing pattern (FEE). Anastomosed segments were distended with fluid until the point of biomechanical failure. The 2HS had the longest construction time of all anastomoses. Bursting pressures were significantly higher for hand-sewn jejunojejunal anastomoses than those for oversewn stapled jejunojejunal anastomoses. No significant differences were found in bursting pressures between 1HS and 2HS or between SS and FEE. Hand-sewn jejunojejunal anastomoses proved to be biomechanically stronger than oversewn stapled jejunojejunal anastomoses when initially constructed. However, all anastomotic types would be secure techniques to be used clinically based on the supraphysiological pressures they are capable of withstanding.

Résumé — Comparaison des anastomoses jéjuno-jéjunales cousues à la main et agrafées et cousues chez les chevaux. Cette étude avait pour objectif de comparer les propriétés biomécaniques des anastomoses jéjuno-jéjunales cousues à la main et celles des anastomoses jéjuno-jéjunales agrafées et cousues. Des anastomoses jéjuno-jéjunales ont été construites à partir de segments jéjunaux prélevés en utilisant la technique Lembert à couche unique (1HS), la technique Cushing à double couche simple continue (2HS), la technique agrafée côté à côté selon la méthode Cushing (SS) et la technique fonctionnelle de bout en bout fermée en 1 étape avec couture selon la méthode Cushing (FEE). Des segments anastomosés ont été dilatés avec du liquide jusqu’au point de défaillance biomécanique. La technique 2HS présentait le temps de construction le plus long de toutes les anastomoses. Les pressions de rupture étaient significativement supérieures pour les jéjuno-jéjunostomies cousues par rapport aux jéjuno-jéjunostomies agrafées et cousues. Aucune différence significative n’a été constatée au niveau des pressions de rupture entre 1HS et 2HS ou entre SS et FEE. Les jéjuno-jéjunostomies cousues à la main se sont avérées plus fortes sur le plan mécanique que les jéjuno-jéjunostomies agrafées et cousues lors de la construction initiale. Cependant, tous les types anastomotiques seraient des techniques sûres pour utilisation clinique en se basant sur les pressions supra-physiologiques qu’elles sont capables de supporter.

Introduction

Numerous surgical techniques have been reported for anastomosis of the small intestine in horses, including end-to-end, side-to-side and functional end-to-end using either a hand-sewn or stapling technique (1–6). Until recently, double-layer hand-sewn end-to-end anastomosis has been the most commonly used technique in horses. However, single-layer Lembert anastomosis has been emerging in popularity as a safe and effective alternative to be used in horses for end-to-end anastomoses (7). Nieto et al (8) demonstrated that a single layer Lembert anastomosis was faster to perform and created a larger lumen diameter, while having equivalent bursting strength compared to a double-layer anastomosis. Stapled anastomoses, on the other hand, are favored by others because of their reported reductions in surgery time, reduced contamination and improved anastomotic healing (5,9). Stapled small intestinal anastomoses are most commonly performed in horses using a side-to-side technique or a functional end-to-end...
technique (1). An experimental study reported no difference in bursting strength between stapled side-to-side and closed 1-stage, stapled functional end-to-end anastomoses; however, the latter was significantly faster to construct (10). Several experimental studies have been performed determining the bursting strength and characteristics of hand-sewn and stapled anastomotic techniques in the equine jejunum (8,10–12). Bursting strength measures the resistance of the intestinal wall to increasing intraluminal pressure until leakage or disruption occurs, and can be expressed as bursting pressure or bursting wall tension (13). Nieto et al (8) reported mean bursting pressures for single and double-layer jejunal anastomoses of 208 mmHg and 221 mmHg, respectively. Bickers et al (10) compared the bursting strength of stapled side-to-side and closed 1-stage stapled functional end-to-end jejunal anastomoses and reported mean bursting pressures of 31.27 mmHg and 48.64 mmHg, respectively, for these 2 stapled anastomotic techniques. The bursting pressures reported for hand-sewn jejunojejunostomes were approximately 4 to 5 times higher than the bursting pressures reported for stapled jejunojejunostomes using a similar experimental set-up (constant infusion rate of 1 L/min). The significantly lower bursting pressures for stapled jejunojejunostomes compared to those of hand-sewn jejunojejunostomes may be concerning especially in the immediate postoperative period. The holding strength of intestinal anastomoses has been reported to be crucial during the first 3 d after surgery (14). Furthermore, one may question that the biomechanical strength of stapled anastomoses may be even lower if stapled devices are used in excessively thick or edematous tissue, which can increase the risk of failure of the anastomosis. Therefore, reinforcement of stapled anastomoses would seem appropriate in order to provide the required security to prevent potential complications, such as leakage or hemorrhage, which may occur at the site of the anastomosis. Reinforcement of stapled anastomoses has been performed by either placing interrupted sutures at both ends of the staple line or by oversewing the entire staple line (15). There is a clinical perception by surgeons who favor using stapling devices, that oversewing stapled anastomoses will biomechanically strengthen the anastomotic constructs to the point of making them as strong or stronger than hand-sewn anastomoses. However, to the authors’ knowledge, there are no studies that have compared the biomechanical strength of oversewn stapled jejunojejunostomes specifically to hand-sewn jejunojejunostomes in horses.

The purpose of this study was to determine if oversewn stapled anastomoses were biomechanically as strong as hand-sewn anastomoses. Our objectives were to compare construction times, bursting strength, and mode of failure of the following jejunojejunostomes: single-layer continuous Lembert (1HS), double-layer simple continuous/Cushing (2HS), oversewn stapled side-to-side (SS), and oversewn closed 1-stage stapled functional end-to-end (FEE). We hypothesized that oversewn stapled jejunojejunostomes would have comparable bursting strengths and construction times to those of hand-sewn jejunal anastomoses.

**Materials and methods**

Seven adult horses (4 geldings and 3 mares; 2 Thoroughbreds, 3 American Quarter horses, 1 Paint, 1 Canadian Warmblood) euthanized for reasons unrelated to gastrointestinal tract disease were used in this study. Horses ranged from 3 to 16 y of age [mean: 8.3 y ± standard deviation (SD) 4.5 y] and weighed between 450 and 735 kg (mean: 548 kg ± 109 kg). Immediately following euthanasia, 5 mid-jejunal segments, 60-cm long were collected from each horse. Segments were rinsed with saline (0.9% NaCl) solution and the intestinal lumen was evacuated of ingesta.

A randomized block design was used to assign each segment of intestine to 1 of the following groups: control (C); single-layer continuous Lembert (1HS); double-layer simple continuous/Cushing (2HS); oversewn stapled side-to-side (SS); or oversewn closed 1-stage stapled functional end-to-end (FEE). All anastomoses were preserved in lactated Ringer’s solution at 4°C and tested within 4 h of the horse being euthanized.

**Construction of jejunojejunostomes**

A board-certified surgeon (JLB) performed all jejunal anastomoses. For all hand-sewn anastomoses, the intestinal segments were sharply transected at their midpoints at approximately 60° to the mesenteric attachment. The transected ends were apposed with the use of stay sutures placed at the mesenteric and antimesenteric borders of both intestinal segments. Mosquito forceps were placed at the end of each stay suture to apply constant tension in order to keep the ends of the intestinal segment stretched and in apposition while the surgeon performed the hand-sewn anastomoses.

**Single-layer Lembert jejunojejunostomy (1HS)**

Single-layer anastomoses were performed as previously described (16). Polydioxanone (2-0 PDS II, Johnson & Johnson Medical Products, Peterborough, Ontario) was used to suture the seromuscular and submucosal layers using a continuous Lembert pattern that was interrupted at the mesenteric and antimesenteric borders. Suture bites were taken such that the needle entered the tissue ~1 mm from the incised edge and exited the tissue ~5 mm from the incised edge. Suture bites were taken 3 mm apart. When performing the single-layer anastomoses, care was taken to engage the submucosa with each suture bite.

**Double-layer jejunojejunostomy (2HS)**

Double-layer anastomoses were performed as previously described (16). For the first layer, 2-0 PDS was used in a full-thickness simple continuous pattern to appose all layers of the intestinal wall. Suture bites were taken approximately 5 mm apart and 3 mm from the incised edge. A second layer was then performed to oversew the seromuscular layer using a Cushing pattern with 2-0 PDS. Suture bites were placed approximately 3 mm from the incision and were 5 mm long. The suture pattern was interrupted at the mesenteric and antimesenteric borders to prevent a purse-string effect during construction of the anastomosis.
Oversewn stapled side-to-side jejunoojejunostomy (SS)

An 80-mm, linear stapling device (ILA-80; Covidien, Norwalk, Connecticut, USA), loaded with a 4.8-mm staple cartridge was placed across the midpoint of a 60-cm intestinal segment. The stapling device was then fired to create 2 blind ends, and obtain two 30-cm segments. The blind ends were oversewn using a Cushing suture pattern with 2-0 PDS. Thereafter, the 2 blind ends were placed in an isoperistaltic fashion at the antimesenteric borders, overlapping approximately 10 cm. Stay sutures were then placed at each end of the overlapping segments of jejunum. Two adjacent 1-cm stab incisions were made on the antimesenteric border at 1 end of the overlapping segments. A 100-mm linear stapling device (ILA-100; Covidien) with a 4.8-mm staple cartridge was then used, with the arms of the stapling device inserted into each of the created stab incisions. After the jaws of the stapler were properly positioned, it was held closed for 30 s (precompression time) and then fired once to create the stoma. Stab incisions were closed with 2-0 PDS in a single layer using a continuous Cushing suture pattern and the suture pattern was continued to oversew the staple line to the end of the anastomotic construct. Thereafter, the suture pattern was interrupted; the anastomotic construct was rotated 180° and a Cushing suture pattern was used to complete oversewing the entire staple line.

Oversewn closed 1-stage stapled functional end-to-end jejunoojejunostomy (FEE)

Closed 1-stage stapled functional end-to-end anastomoses were constructed as previously described (17) with slight variation due to the ex vivo nature of the construction. For this study, the intestinal segment was positioned in an antiperistaltic fashion such that the intestine was folded back on itself. Two stay sutures were placed to secure the segments of jejunum relative to each other before creating the anastomosis. The first stay suture was placed ~10 cm from the apex created in the folded segment of jejunum. The second stay suture was placed approximately 15 cm relative to the first stay suture. Thereafter, an adjacent 1-cm stab incision was made in the antimesenteric border of each jejunal segment, ~2 cm away from the first stay suture. The arms of the 100-mm linear stapler (ILA-100; Covidien) were then inserted into each of the stab incisions. Special care was taken to ensure that the antimesenteric borders of each jejunal segment were correctly apposed, before coupling and locking the stapling device. The ILA-100 stapler was then held closed for 30 s (precompression time) and fired once to create the stoma. The ILA-100 stapler was removed and a 90-mm linear stapler (TA-90; Covidien) was positioned diagonally across the anastomosis to close the common stab incisions. The TA-90 stapler was fired such that the staple line overlapped a portion of the anastomotic staple lines on the antimesenteric borders of the anastomotic construct. With the stapling device still in place, sharp transection was then made close to the TA-90 stapler in order to remove the intestinal apex. A continuous Cushing suture pattern with 2-0 PDS was used to oversew the staple line created to close the stab incisions. The entire anastomotic staple line was then oversewn using a continuous Cushing suture pattern with 2-0 PDS. The staple line was oversewn starting on the antimesenteric side and interrupted at the crotch of the “V” where the 2 intestinal segments merged. The anastomotic construct was then rotated 180° and the mesenteric side was similarly oversewn.

Anastomosis construction time

For the 1HS and 2HS groups, the time recorded for the construction of the anastomosis was the interval between the placement of the first stay suture and the completion of the anastomotic construct. For the SS group, time was recorded started with the placement of the 80-mm linear stapling device across the midpoint of the intestinal segment to create the 2 blind ends and ended with completion of the oversewing of the staple line. For the FEE group, time for construction began with the placement of the first stay suture and ended when oversewing of the staple line was completed.

Biomechanical testing

For this study, bursting pressure (BP) was used as a biomechanical indicator of the bursting strength for all anastomotic constructs. Biomechanical testing was performed as previously described (16). Briefly, each intestinal segment was submerged in 30 L of 0.9% NaCl solution in a custom-made bath. The open ends of the intestinal segments were attached to 2 barbed connectors, one of which was coupled to an end cap with a bleeder valve. The other barbed connector was attached to a T-connector attached to a pressure transducer (P55 Compact Differential Pressure Transducer; Validyne Engineering, Northridge, California, USA) and a peristaltic roller pump (Karl Storz-Endoskope SCB Hamou Endomat 263310 20; Karl Storz GmbH & Co., Mississauga, Ontario) for delivery of fluid. Intra-luminal pressures were continuously monitored and recorded with a strain gauge amplifier (Interface Model SGA AC/DC Powered Signal Conditioner; Interface, Scottsdale, Arizona, USA), an analog to digital data acquisition card (National Instruments, NI USB-6210; Austin, Texas, USA) and a personal computer with data acquisition software (LabView; National Instruments, Austin, Texas, USA). The volume of fluid infused into each intestinal segment during the bursting trial was measured using a load cell (Interface, Model SSM Sealed S-Type Load Cell, Interface) and associated instrumentation consisting of the same strain gauge amplifier, analog-to-digital data acquisition card, and computer with data acquisition software used to determine the increase in sample pressure. For each measurement, two 3-L 0.9% NaCl solution bags stained with new methylene blue dye were used for fluid delivery into the intestinal segment.

Bursting strength (bursting pressure) was determined by increasing the intra-luminal pressure of the intestinal segment until mechanical failure occurred. Fluid was infused into the intestinal segment by the peristaltic roller pump at a constant rate of 1 L/min until failure, signaled by leakage of blue dye into the fluid bath. Bursting strength was the maximum pressure, or bursting pressure, that was recorded before a sudden decrease in the slope of the pressure versus time curve or, alternatively, the pressure that was recorded when the methylene blue solution...
Table 1. Mean ± standard error values of anastomosis construction time and bursting pressure from single-layer continuous Lembert (1HS), double-layer simple continuous/Cushing (2HS), oversewn stapled side-to-side (SS), and oversewn closed 1-stage stapled functional end-to-end (FEE) anastomoses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Construction time (min)</th>
<th>Bursting pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N/A</td>
<td>257.63 ± 45.41</td>
</tr>
<tr>
<td>1HS</td>
<td>19.52 ± 1.40</td>
<td>197.31 ± 32.27</td>
</tr>
<tr>
<td>2HS</td>
<td>26.34 ± 1.42</td>
<td>224.84 ± 48.94</td>
</tr>
<tr>
<td>SS</td>
<td>22.07 ± 2.33</td>
<td>108.65 ± 30.04</td>
</tr>
<tr>
<td>FEE</td>
<td>16.41 ± 1.44</td>
<td>134.18 ± 8.47</td>
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</tbody>
</table>

*Values marked with the same superscript letters were not significantly different from each other. N/A — Not available.*

was observed to leak onto the outer surface of the intestinal segment. The bursting pressure in mmHg at the time of biomechanical failure, the mode of failure (leaking or bursting), and the location of the failure were recorded.

Statistical analyses were performed using software package Stata 12 (StataCorp, College Station, Texas, USA) and for all statistical tests, *P* < 0.05 was considered significant. Descriptive statistics were reported as mean ± standard error (SE). A Shapiro-Wilk test was used to determine whether the data were normally distributed. Mixed linear regression models were used to compare bursting pressure and construction times by surgical technique. Variation among horses was controlled as a random effect in the above models. In each of the models, breed and age were evaluated as potential confounding variables. The residuals for each model were tested for normality and homoscedasticity.

Results

Time for construction of anastomosis

The FEE was the jejunojejunostomy anastomotic technique with the shortest construction time (Table 1). Mean ± SE construction time for the FEE was significantly shorter than for SS (*P* < 0.0001), 1HS (*P* = 0.0012), and 2HS (*P* < 0.0001). The 1HS had a shorter construction time than did SS (*P* < 0.001). The 2HS group had the longest construction time compared with 1HS (*P* < 0.001), FEE (*P* < 0.0001), and SS (*P* < 0.0001).

Bursting strength

Mean ± SE BP for the control jejunal segments was significantly higher than for 1HS (*P* < 0.001), SS (*P* < 0.0001), and FEE (*P* < 0.0001) groups (Table 1). No differences were found between control segments and 2HS (*P* = 0.0503). There were no significant differences in BP between 1HS and 2HS (*P* = 0.0963) or between SS and FEE (*P* = 0.1216). Both hand-sewn jejunojejunostomies (1HS and 2HS) had significantly higher BP than did oversewn stapled jejunojejunostomies (SS: *P* < 0.001, *P* < 0.001) and (FEE: *P* < 0.001, *P* < 0.001), respectively.

Mode and location of failure

All control jejunal segments failed by bursting along the mesenteric border. All 2HS anastomotic constructs failed by bursting. Failure of 5 of the 2HS constructs occurred 3 to 5 cm away from the anastomosis while the anastomosis remained intact. In 2 of the 2HS constructs, failure occurred at the intestinal wall adjacent to the anastomosis with the suture remaining intact (Figure 1). In the 1HS group, 5 anastomotic constructs failed by bursting 1 to 3 cm away from the anastomosis, while 2 constructs failed by leakage at the anastomosis where the first suture bite was taken to appose the mesenteric border (Figure 2). In all hand-sewn anastomoses the sutures remained intact. In the FEE group, 5 constructs failed by bursting and 2 by leakage. Failure of all FEE constructs occurred consistently at the crotch of the “V” where the 2 intestinal segments merged (Figure 3). For the SS group, 4 constructs failed by leakage and 3 by bursting. Failure occurred consistently at the staple end of the stoma adjacent to one of the blind stumps of the jejunum (Figure 4). In 4 constructs failure occurred at the end of the staple line where the stab incisions were made and in 3 constructs failure occurred at the end of the staple line away from the stab incisions.

Discussion

This study compared the bursting strength between hand-sewn and oversewn jejunal anastomoses. The results indicate that oversewing the staple line of stapled side-to-side and closed 1-stage stapled functional end-to-end jejunojejunostomies does not provide the additional reinforcement to make these anastomotic constructs biomechanically as strong as hand-sewn anastomoses when initially created.
Use of stapling devices has become more prevalent in equine abdominal surgery because of the reduction in surgery time and minimized risk of abdominal contamination when these instruments are used appropriately (5,18,19). The greatest advantage claimed for the use of stapling devices has been speed in construction of anastomoses (5). There is evidence that stapling devices can decrease the surgical time required to perform an intestinal resection in horses by over 50% (20).

However, an experimental study comparing stapled side-to-side jejunojejunal anastomosis to traditional double-layer hand-sewn jejunojejunal anastomosis found no difference in their construction times (18). Additionally, another study found no difference in duration of surgery when hand-sewn end-to-end, stapled functional end-to-end, or stapled side-to-side anastomoses were performed in horses undergoing jejunal resection and anastomosis (21). In our study, although we found a statistical difference in construction times between oversewn stapled techniques and hand-sewn techniques, we believe a 10-minute difference in time (found between FEE and 2HS) may not be clinically significant. Nevertheless, surgeons should make every effort to shorten surgery times when undertaking exploratory celiotomies because of the reported negative association between duration of surgery and survival rates (22).

Evaluation of the bursting strength has been the preferred method to investigate anastomotic integrity and intestinal healing in horses (8,10–12,16,23,24). Several studies have compared the bursting strength of jejunojejunal anastomoses in horses using different suture patterns or stapling techniques (8,10–12). A previous experimental study reported single and double layer hand-sewn jejunojejunal anastomoses to have bursting pressures significantly higher (8) than values reported for stapled side-to-side and closed 1-stage stapled functional end-to-end jejunojejunal anastomoses (10) using similar methodology. Moreover, these findings have also been reported for jejunoileal anastomoses in which bursting pressure for single- and double-layer end-to-end jejunoileal anastomoses were 4 to 5 times higher than those for stapled side-to-side and stapled functional end-to-end jejunoileal anastomoses (16). In horses, this may be relevant in the immediate postoperative period where excessively thickened or edematous tissue may make the anastomosis more susceptible to failure. Bickers et al (10) reported mean bursting pressures of 48.64 mmHg for closed 1-stage stapled functional end-to-end jejunojejunal anastomosis and 31.27 mmHg for stapled side-to-side jejunojejunal anastomosis. Experimentally, intraluminal pressures ranging from 8 to 24 mmHg have been reported during extraluminal intestinal obstruction of the jejunum in conscious ponies (25). Interestingly, in that study, cyclic pressure peaks up to 60 mmHg were frequently measured during the obstructive trials, which are higher than bursting pressures reported by Bickers et al (10) for stapled side-to-side and closed 1-stage stapled functional end-to-end jejunojejunal anastomoses. Therefore, one could argue that oversewing stapled jejunojejunal anastomosis is warranted for anastomotic security. In our study, we found that oversewing the staple line of stapled jejunojejunal anastomoses (SS and FEE) did not strengthen the construct to obtain bursting pressures comparable to those obtained in hand-sewn jejunojejunal anastomoses and we therefore failed to prove our hypothesis. The bursting pressures we report for oversewn stapled jejunojejunal anastomoses were 1.5 to 2 times lower than those for single and double hand-sewn anastomoses. Nevertheless, all tested oversewn stapled anastomotic constructs failed at pressures from 96 to 166 mmHg. These pressures are significantly higher than the intraluminal pressures reported in non-surviving horses with obstructive small intestinal disease, which reached pressures up to 21 cm H₂O (15.44 mmHg) (26) and higher than the cyclic pressure peaks (60 mmHg) measured during the obstruction trials (25). Although single and double-layer hand-sewn jejunojejunal anastomoses proved to be biomechanically stronger anastomotic constructs, we believe that oversewn stapled side-to-side and closed 1-stage stapled functional end-to-end anastomoses will be unlikely to fail in clinical cases because of the supraphysiological pressures they are capable of withstanding.

A limitation of this study is that a “stapled only” group was not used to compare the bursting strength of anastomoses with staples only to those of oversewn stapled anastomoses and hand-sewn anastomoses. We chose not to include a stapled only group as the bursting strengths of stapled side-to-side and closed...
1-stage stapled functional end-to-end jejunojejunal anastomosis have been previously reported experimentally (10) and therefore we were aware of their low bursting pressures. In addition, the purpose of our study was to specifically compare oversewn stapled anastomoses to those of hand-sewn anastomoses. The mean bursting pressures we report in this study (134.18 mmHg for oversewn closed 1-stage stapled functional end-to-end jejunojejunal anastomosis and 108.65 mmHg for oversewn stapled side-to-side jejunojejunal anastomosis) are 2.5 to 3 times higher, respectively, than those reported by Bickers et al (10) using a similar experimental set-up. Therefore, although a stapled only group was not included in this study, our results show that oversewing stapled jejunojejunal anastomoses would considerably strengthen the anastomotic construct. Nevertheless, further research is warranted to compare the bursting strength between stapled and oversewn stapled anastomoses.

The submucosa is the strongest layer (holding layer) in the small intestine and therefore, its inclusion in an anastomosis is crucial to provide biomechanical strength and resistance (27,28). A Cushing suture pattern is an inverting pattern that penetrates the submucosal layer but not the lumen of hollow viscera (29). By oversewing stapled anastomoses using an inverting suture pattern, the staple line primarily serves to reduce contamination during resection, while the inverting suture pattern serves as the primary method of closure (19). We initially believed that oversewing stapled anastomoses with a Cushing suture pattern would create a stronger anastomosis of similar biomechanical strength to single and double-layer hand-sewn anastomoses. However, the results of this study clearly demonstrate that single and double-layer hand-sewn anastomoses are stronger constructs than oversewn stapled anastomoses when initially created. We attribute the difference in bursting strength between hand-sewn and oversewn stapled anastomoses to i) the disparity in anastomotic geometry, and ii) location of stress raisers in the stapled anastomotic techniques. The hand-sewn anastomoses resemble the geometry of a cylinder; therefore, when forces are applied to hand-sewn anastomotic constructs they will be evenly distributed along the intestinal walls (30). Biomechanically, a construct is strongest when force is evenly distributed over its area (31), as would occur in hand-sewn single and double layer anastomoses. The 2 stapled techniques reported in this study, however, are shaped in a complex manner, resulting in unevenly distributed forces along the wall of the intestinal segment and largely different actual stress distributions making them prone to fail at lower pressures (28). Oversewn closed 1-stage stapled functional end-to-end anastomoses consistently failed at the crotch of the “V” where the 2 intestinal segments merged, while oversewn stapled side-to-side anastomoses consistently failed at the staple end of the stoma. These locations have been previously reported as sites of failure in stapled jejunojejunal anastomoses (10) and stapled jejunoileal anastomoses (16). Although not specifically tested, the crotch of the “V” and the end of the staple line most likely create stress raisers where high local stresses concentrate, ultimately resulting in rapid failure at relatively low bursting pressures. Further research is warranted to investigate these areas of high stress concentration in stapled anastomoses in order to provide more secure anastomotic constructs.

Anastomotic leakage is a feared complication following resection and anastomosis that can lead to devastating consequences in horses such as septic peritonitis (1). In our study, 2 single-layer Lembert constructs failed by leakage. Failure in these constructs consistently occurred where the first suture bite was taken to appose the mesenteric border. The bursting pressures at the time of leakage in these constructs were 176.12 and 186.70 mmHg, which are significantly higher than the intraluminal pressures in non-surviving horses with obstructive small intestinal disease (26). It has been reported that failure to gain a substantial purchase through the seromuscular layer at this area makes anastomotic leakage inevitable (1). In clinical cases, edema forms rapidly in the strangulated intestinal wall making it difficult to distinguish between the mesenteric attachment and the seromuscular layer. Therefore, when performing a single-layer Lembert jejunojejunal anastomosis, surgeons should ensure that optimal seromuscular layer purchase is obtained to appose the mesenteric borders to prevent anastomotic leakage at this area. Nevertheless, a recent clinical study (7) has shown single-layer continuous Lembert anastomosis to be a safe and effective option for small intestinal anastomosis as horses had comparable short- and long-term survival rates to those reported previously for double-layer anastomoses (21,32).

We found no difference in the bursting strength between single-layer and double-layer anastomoses (power = 0.23) or between oversewn stapled side-to-side and oversewn closed 1-stage stapled functional end-to-end anastomoses (power = 0.58). A larger sample size would have been needed to determine differences between these hand-sewn and oversewn anastomoses. Although hand-sewn anastomoses proved to be biomechanically stronger than oversewn stapled anastomoses when initially created, all reported anastomotic types would be secure techniques to be used clinically as they will provide the biomechanical strength to withstand the forces and pressures that a newly created anastomosis may experience in the immediate postoperative period. Nonetheless, further research is needed to evaluate the healing characteristics and biomechanical properties of oversewn stapled jejunojejunal anastomoses during the early postoperative period, to provide more conclusive evidence as to whether oversewn stapled anastomoses (SS and FEE) continue to have lower bursting strength than hand-sewn anastomoses after the immediate postoperative period.

References
This highly recommended reference book provides veterinary students, veterinarians, and scientific editors with valuable information pertaining to veterinary technical and scientific terminology.

Reviewed by Stella Wheatley, Hon BSc, Assistant Managing Editor, Journals, Canadian Veterinary Medical Association, Ottawa, Ontario.

Saunders Comprehensive Veterinary Dictionary, 4th edition


The first notable feature of this edition is its size. The previous edition, which measured 7 × 9.5 × 2.3/4 inches, has now grown to 8.5 × 10 3/4 × 2 inches. It is not easily thrown into a backpack. The font size, while still quite readable, has been decreased to accommodate more information and allow for the addition of new figures and photographs.

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Brucella canis: An update on research and clinical management

Kevin L. Cosford

Abstract — In Canada, Brucella canis remains a potentially devastating infectious agent that is still considered uncommon, despite the increasing international movement of dogs. There may be a growing risk to the Canadian canine population due to a reliance on outdated seroprevalence studies and the lack of federal regulation. With the complex diagnostic and management challenges associated with Brucella canis, a One Health approach is necessary to address the need for ongoing research, including updating canine and human seroprevalence rates in Canada, elucidating the pathogenesis, and determining the most appropriate treatment and prevention strategies. Clinical management decisions are often complicated by currently available treatment protocols, and health risks to both canine and human populations. This article integrates recent research focusing on the pathogenesis, diagnosis, and treatment of Brucella canis, and outlines current clinical management approaches.

Résumé — Brucella canis : mise à jour sur la recherche et la gestion clinique. La brucellose canine, causée par un agent infectieux important et potentiellement dévastateur, est toujours considérée rare au Canada malgré l’arrivée croissante de chiens provenant de régions ayant une prévalence supérieure de l’infection par Brucella canis. Il y a un risque grandissant pour la population canine canadienne parce que l’on se fie à des études de séroprévalence désuètes et qu’il existe une absence de règlements fédéraux. En raison des défis complexes liés au diagnostic et à la gestion de Brucella canis, l’approche Une seule santé est nécessaire afin d’aborder le besoin de poursuivre la recherche, y compris la mise à jour des taux de séroprévalence canine et humaine au Canada, la clarification de la pathogénèse, la définition de l’éventail potentiel de manifestations cliniques et la détermination du traitement et des stratégies de prévention les plus appropriés. Les décisions de gestion clinique sont souvent compliquées par les protocoles de traitement actuellement disponibles et les risques pour la santé des populations canine et humaine. Cet article intègre de la recherche récente portant sur la pathogénèse, le diagnostic et le traitement de Brucella canis et présente les approches de gestion clinique actuelles.

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Introduction

Although endemic to Canada, Brucella canis is an elusive infectious agent of unknown significance to most practitioners. Clinical disease attributed to Brucella canis infection occurs sporadically, reinforcing the perception that the disease is uncommon in Canada compared with other regions of North America such as Mexico and the southeastern USA. Seroprevalence rates in the southeastern USA are estimated to be 7% to 8% (1).

Earlier reports from Quebec (1970’s) and southwestern Ontario (1980) still serve as the main Canadian seroprevalence data with rates of 1.6% and 0.3%, respectively (2,3). There is currently a paucity of seroprevalence studies in western Canada, but outbreaks have been observed in a Saskatchewan kennel and the Calgary, Alberta area (4,5).

With the unprecedented rates of animals moving across international borders and the lack of federal regulation, canine brucellosis may be changing its geographical distribution. In 1988, a Canadian Veterinary Journal article documented the identification of 2 strains, an American type strain RM66 and a Mexican strain Mex 51, in 11 Brucella canis isolates from Canadian dogs (6). Characterization of the current circulating strains is warranted. Until this information is available, Canadian veterinarians should be aware of the agent and consider it as a reasonable differential diagnosis in appropriate cases, regardless of historical information, or neuter status.

Establishing a diagnosis can be challenging due to the wide spectrum of clinical manifestations reported and the limitations of available diagnostic tests. The intent of this article is
to familiarize clinical, public health, and research veterinarians with the etiology, transmission, pathogenesis, course of infection, clinical manifestations, diagnosis, treatment, prevention, and public health aspects of the disease.

**Etiology**

Bacteria in the genus *Brucella* are nonmotile, nonencapsulated, non-spore-forming, facultatively intracellular Gram-negative coccobacilli or short rods (7,8). Four of the six classical *Brucella* species are known to cause disease in dogs and humans: *Brucella canis* (natural reservoir animal is the dog), *Brucella melitensis* (sheep, goats), *Brucella suis* (pigs), and *Brucella abortus* (cattle, bison, buffalo) (7,8). The remaining 2 of the 6 classical *Brucella* species [*Brucella neotomae* (rodents, desert rats) and *Brucella ovis* (sheep)] are not associated with disease in dogs. Additional *Brucella* species including both terrestrial forms (*B. microti, B. inopinata*) and marine forms (*B. maris, B. pinnipediae, B. ceti*) are of uncertain pathogenicity to dogs.

*Brucella canis* was discovered in 1966–1967 during an investigation of abortion in beagles, in which the organism was isolated from aborted tissues and vaginal discharge (9–12). *Brucella canis* was initially thought to be a biotype of *Brucella suis* based on genotypic and phenotypic similarities (13). The significance of this distinction is paramount to the Canadian swine industry and the Canadian Food Inspection Agency (CFIA) as Canada is considered free of *Brucella suis* biovar 3 (4). Differentiation between *Brucella canis* and *Brucella suis* biovar 3 can be challenging (4). A multiplex conventional polymerase chain reaction (PCR) has been optimized to differentiate between these *Brucella* species (14).

The host range for *Brucella canis* is predominantly domestic dogs, but other species have been investigated. Serologic studies of wild canids have documented positive antibody titers in foxes and coyotes (7). Experimental studies involving conjunctival and oral inoculation of cattle, swine, and sheep with *B. canis* showed that these host species were highly resistant to *B. canis* infection, despite 2 field reports of *B. canis* in cattle (7). Similarly, oral experimental infection of cats documented transient bacteremia in 3/14 but none developed agglutinating antibody titers (7).

**Transmission**

Major routes of transmission for this venerally transmitted agent are genital, conjunctival, and oronasal mucosae, as occurs during normal reproductive, social, and grooming activities in dogs (7,8,15,16). The primary sources of transmission are reproductive fluids: vaginal discharges and semen. Tissues and fluids associated with the fetus, the placenta, and the vagina after abortion or stillbirth have approximately 10⁶ organisms/mL (7). Minor fluids associated with the fetus, the placenta, and the vagina after reproductive fluids: vaginal discharges and semen. Tissues and fluids associated with the fetus, the placenta, and the vagina after abortion or stillbirth have approximately 10⁶ organisms/mL (7). Minor

routes of transmission include *in utero*, broken skin, blood transfusions, feces, milk, and fomites such as contaminated syringes, vaginoscopes, and artificial insemination equipment (7,8,15,16).

**Pathogenesis**

**Current paradigm**

*Brucella* bacteria attach to mucous membranes, penetrate the epithelial barrier, and are taken up by the mononuclear phagocytic system, where they reside intracellularly. This is accomplished by utilizing virulence factors presumably via the type IV secretory system, and inhibiting the bactericidal myeloperoxidase-peroxide-halide system through the release of 5-guanosine and adenine (8,17,18). The intracellular organisms then travel through the reticuloendothelial system to local lymph nodes (retropharyngeal, inguinal, superficial iliac), liver, spleen, and possibly bone marrow. After 7 to 30 d, the bacteria move into the blood stream to cause intermittent bacteremia. The organism targets “steroid-dependent” reproductive tissues, including the prostate, testicles, epididymides, gravid uterus, and placenta (8). Evaluation of a Saskatchewan kennel outbreak of brucellosis found that the gestational, non-gravid uterus was also a reservoir for the bacterium (4). A mixed inflammatory response consisting of lymphocytes, plasmacytes, and histiocytes has been observed in these reproductive tissues (4,7,8). Focal coagulative necrosis of the chorionic villi, necrotizing arthritis, and numerous bacteria in trophoblastic epithelial cells can be found in the aborted placenta (4,8).

Non-reproductive body systems become affected as the bacteremia spreads organisms and antibody-antigen complexes to the end-arterial circulation of the intervertebral disk (discospondylitis), or the eye (anterior uveitis or endophthalmitis) (7,8). Interestingly, in experimentally infected dogs, immunosuppression with glucocorticoids or anti-lymphocyte serum may increase susceptibility to initial infection, but does not appear to alter the severity of disease or the course of infection (7). Elucidation of this organism’s role in idiopathic inflammatory conditions such as meningocencephalitis, panniculitis, lymphadenitis, hepatitis, and splenitis should be given due consideration by future research initiatives.

**Recent research**

A murine study has confirmed the pathogenic strategy of *B. canis* as an intracellular bacterium, with an intracellular trafficking route indistinguishable from that of *B. abortus* (17). The study documented a less robust response in mice infected with *B. canis* compared with *B. abortus* in terms of proinflammatory cytokines (TNF-alpha, IL-6, IL-12), IFN-gamma levels, splenic inflammation, and hepatic granulomas (17). It appears that *B. canis* may be less pathogenic than other *Brucella* species in this murine model, which supports clinical observations.

Another study in mice and dogs support a Th1 immune response as essential for protection from *B. abortus* infection (19). VirB proteins are virulence factors that are part of the type IV secretory system. VirB proteins are presumably on the outer surface of the *Brucella* bacterium and are believed to promote intracellular survival. Anti-VirB antibodies promote
Complement-dependent bacteriolysis (19). Immunization of mice with VirB proteins resulted in increased IFN-gamma and undetectable IL-4 in VirB-vaccinated individuals compared to the placebo, which is a pattern consistent with a Th-1 response (19). In addition, VirB-vaccinated mice challenged intraperitoneally with live B. abortus had a splenic bacterial load of 1 log lower than the placebo (19). Similarly, peripheral blood mononuclear cells of VirB-vaccinated dogs produced significantly higher levels of IFN-gamma than in the placebo; and in vitro complement-dependent bacteriolysis was significant in VirB-vaccinated dogs versus the placebo (19). Further studies evaluating vaccination against the virulence factor, VirB, are warranted.

A new perspective on rough and smooth colony morphologies has recently been proposed. Colony morphologies have been classified into smooth and rough forms, based on the respective presence or absence of the most external antigen, O-polysaccharide, within the lipopolysaccharide (LPS) of the cell wall. Traditionally, smooth (B. melitensis, B. abortus, and B. suis) and rough (B. canis, and B. ovis) forms were believed to represent laboratory artifacts that occur with Gram-negative bacterial colonies in culture (7,20). Recent research suggests that the loss of O-polysaccharide results from the spontaneous excision of the wbkA glycosyltransferase gene (21). This phenomenon is referred to as smooth to rough dissociation (20,21). The significance of colony morphology and Brucella LPS genetics remains controversial, but a potential link to virulence might exist (20,21).

Different strains or isolates of B. canis have also been reported. A less mucoid (M-) laboratory strain is maintained in the laboratory as the antigen source for serology assays (22). Interestingly, this M-strain is believed to be avirulent in dogs, but has been reported to infect a laboratory worker in a similar fashion to wild-type Brucella canis (22). A Swedish outbreak investigation documented differences within prophage gene content of American, African, and European isolates compared to Asian strains (23). The significance of these strains in terms of their relative pathogenicity remains unclear.

Course of infection
Bacteremic episodes can last for years as experimentally infected dogs can have positive blood cultures for 5.5 y (8). The animal seroconverts as early as 2 to 4 wk but this can be as long as 8 to 12 wk after infection (1,7,8). After 3 to 4 mo the degree of bacteremia declines, but the organism remains persistently in the blood or sequestered in tissues. The current paradigm with respect to the outcome of B. canis infection is that cell-mediated immune responses typically result in self-elimination within 2 to 3 y on average (7). Alternatively, humoral immune responses do not eliminate the organism resulting in persistently infected dogs (7).

Experimentally infected dogs allowed to recover naturally were immune to subsequent oral or intravenous rechallenge for up to 4 y (7). In contrast, infected dogs that did not self-eliminate the organism were susceptible to oronasal challenge 12 wk after completion of antimicrobial therapy (7). Antibiotic therapy is widely believed to be unsuccessful at eliminating persistent infection in dogs.

Clinical manifestations
Brucella canis is typically associated with reproductive abnormalities but a wide range of non-reproductive signs can occur (1). The organism has been given the nickname "the Great Imposter" to illustrate this point (1). It is important to remember that most infected dogs do not appear seriously ill. Deaths are rare except in utero, in newborns, and in animals with severe illness (7).

Female dogs infected venereally experience early embryonic death 2 to 3 wk after transmission, which looks like failure to conceive or infertility (7). If the pregnancy progresses spontaneous abortion occurs most commonly between 7 and 9 wk of gestation (45 to 55 d), which is referred to as a late stage spontaneous abortion (7). Normal canine gestation is 57 to 72 d (24). Mucoid, serosanguinous, or gray-green vaginal discharge persists for 1 to 6 wk after abortion (7). Endometritis has also been observed (7). Some B. canis infected bitches can give birth to litters that appear clinically normal. These puppies are born infected and can manifest disease later in life (7).

During the acute stage, venereally infected male dogs may initially experience epididymitis and scrotal edema, while orchitis occurs less frequently. Scrotal dermatitis also occurs due to self-induced irritation from licking. The disease can then progress to a chronic stage characterized by testicular atrophy (unilateral or bilateral) and infertility. Affected males develop chronic epididymitis and, ultimately, infertility due to anti-sperm agglutinating antibodies and delayed-type hypersensitivity reactions against the spermatozoa, leading to spermatogenic arrest (6). In male dogs that develop chronic epididymitis, 90% of sperm are abnormal at 20 wk after infection (1). Some male dogs do not develop spermatic abnormalities and infertility, but still spread the organism most likely through prostatic fluid. Prostatic disease manifestations, such as prostatitis, have also been observed (7).

Non-reproductive manifestations of B. canis infection most commonly include chronic uveitis, endophthalmitis, and discospondylitis. Infected dogs with ocular involvement can present with blepharospasm, aqueous flare, constricted pupils, synechiae, hypopyon, and hyphema. Dogs with discospondylitis can present with stiffness, back pain, lameness, exercise intolerance, paresis, and possibly paralysis due to spinal compression. Other manifestations of B. canis infection include lymphadenitis (common), pyogranulomatous dermatitis (rare), endocarditis (rare), appendicular osteomyelitis (rare), and meningoencephalitis (unknown frequency) (7). Various nonspecific signs have been associated with B. canis infection, including fever (rare), lethargy/fatigue, exercise intolerance, decreased appetite, weight loss, and behavioral anomalies such as loss of alertness and poor performance of tasks (7).

Diagnostics
Routine diagnostics such as complete blood (cell) count (CBC), serum biochemistry profile, and urinalysis are often normal. Occasionally, nonspecific findings supportive of inflammatory disease are identified, such as leukocytosis, neutrophilia, hyperglobulinemia, and hypoalbuminemia (18). In cases with suspected discospondylitis, imaging with plain radiography or computed tomography is indicated to identify end vertebral...
Table 1. Comparison of traditional serologic assays for the diagnosis of brucellosis in dogs.

<table>
<thead>
<tr>
<th>Test</th>
<th>Antigen</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>How to use test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Slide Agglutination Test (RSAT)</td>
<td><em>B. suis</em> (27,28) (M-) strain <em>B. canis</em> (29)</td>
<td>Moderate to high sensitivity — older studies suggest high (30,31) — newer studies suggest 70.58% (32)</td>
<td>Low to moderate specificity — older studies suggest 40%–50% (27,30) — newer studies suggest 83.34% (32)</td>
<td>Screening test (1,7,8)</td>
</tr>
<tr>
<td>2-MercaptoEthanol Rapid Slide Agglutination Test (2ME-RSAT)</td>
<td>(M-) strain <em>B. canis</em> (8)</td>
<td>Lower sensitivity than RSAT 31.76% versus 70.58% (32)</td>
<td>Higher specificity than RSAT 100% versus 83.34% (32)</td>
<td>Confirmatory test (1,7,8)</td>
</tr>
<tr>
<td>Tube Agglutination Test (TAT)</td>
<td><em>B. canis</em> (8)</td>
<td>High sensitivity (1,8)</td>
<td>Low specificity (1,8)</td>
<td>Screening test (1,7,8)</td>
</tr>
<tr>
<td>Indirect Fluorescent Assay (IFA)</td>
<td>Anti-canine immunoglobulin (IgG) directed against antibodies to <em>B. canis</em></td>
<td>Unknown sensitivity (7)</td>
<td>Unknown specificity</td>
<td>Screening test (1,8)</td>
</tr>
<tr>
<td>Agar Gel Immunodiffusion Assay using Cell Wall Antigen (AGIDcpa)</td>
<td>Lipopolysaccharide antigen from the cell wall of <em>B. canis</em> (8,36)</td>
<td>High sensitivity (1,7,8)</td>
<td>Lower specificity than AGIDcp (37)</td>
<td>Screening test (1,8)</td>
</tr>
<tr>
<td>Agar Gel Immunodiffusion Assay using Cytoplasmic Antigen (AGIDcp)</td>
<td>LPS-free, soluble, internal cytoplasmic proteins extracted from <em>B. canis</em> or <em>B. abortus</em> (8,36)</td>
<td>Low sensitivity — 52.94% sensitive (32) — 47.06% false negatives (32)</td>
<td>High specificity 100% (32,37)</td>
<td>Confirmatory test (1,8)</td>
</tr>
</tbody>
</table>

*False positives — 10% using *B. canis* antigen versus 50% using *B. suis* antigen.
*Results are semiquantitative (8,30,33–35) with a titer of >1:200 — has a good correlation with the organism being recovered from blood culture; 1:200 — presumptive of active infection; 1:25, 1:50 — recovery or chronic infection.
*False positives occur due to nonspecific cross reactions with cell wall antigenic complexes.
*Reacts with antibodies against *Brucella* spp. (*B. canis*, *B. abortus*, *B. suis*); therefore, specific to the *Brucella* genus but not individual species.

body osteomyelitis. Similarly, magnetic resonance imaging (MRI) along with cerebrospinal fluid (CSF) analysis and bacterial culture are performed in cases of suspected meningococcal arthritis. Uveitis or panophthalmitis may warrant taking aqueous or vitreous humor aspirates for cytology and culture, under an ophthalmologist’s care.

History, clinical signs, and ancillary diagnostics may prompt more definitive testing for *B. canis*. A positive culture can be definitive but low sensitivity leads practitioners and researchers to serology and PCR. Definitive testing for *B. canis* has been plagued by many pitfalls including sensitivity, specificity, quality control, and availability.

Blood culture

The traditional gold standard diagnostic test for *B. canis* has been culture of blood, urine, vaginal discharge, semen, or aborted fluids/tissues (1,7,8,25,26). Samples should be collected sterilely in a standard aerobic culture vial or a green top (heparinized) tube, stored on ice (not frozen) and shipped within 24 h to the laboratory, where Farrell’s medium or Thayer-Martin’s modified medium can be used for culture (7,25,26). Unfortunately, our ability to detect this organism is limited due to low levels of bacteria; intermittent shedding; poor sample choice for submission; inappropriate handling of sample; slow growing, fastidious forms; and incorrect culture media (7). A negative culture should not rule out infection, as the low sensitivity corresponds to an unacceptable number of false negatives. Although culture is an inappropriate screening test, it is the ideal confirmatory test.

**Traditional serologic assays**

Traditional serologic assays for *B. canis* are summarized in Table 1. Rapid slide agglutination tests (RSAT), tube agglutination tests (TAT) and immunofluorescent antibody tests (IFA) are typically used as initial screening tools to rule out infection (7,8,32). False negatives can occur as a result of testing prior to seroconversion, and low circulating antibody titers in some chronically infected dogs (7). False positives are the predominant concern with these serology assays due to both nonspecific and specific cross reactions with shared surface antigens on *Pseudomonas aeruginosa*, *Bordetella bronchiseptica*, *Actinobacillus equuli*, *Streptococcus*, *Staphylococcus*, *Monaxella*-type organisms and Gram-negative bacteria (7,8,25,38). A screening test must be followed with a confirmatory test such as 2-mercaptoethanol RSAT (2ME-RSAT) or agar gel immunodiffusion assay using an internal cytoplasmic antigen (AGIDcp) (7,8,32). The more specific confirmatory test addresses the higher rate of false positives associated with the screening tests.

**ELISAs and PCR**

Research into new diagnostics for *B. canis* is focused on enzyme-linked immunosorbent assays (ELISAs) and PCR, which are summarized for researchers in Tables 2 and 3, respectively. Quality control and assurance are paramount with these assays, especially PCR, to ensure accuracy of the test result, given the potential impact of a positive or negative result on an individual dog, the canine population, an individual client or a kennel operator. Assuming accurate test results, the benefits of PCR are species and sometimes biovar identification; improvements
in sensitivity and specificity; minimal biological containment requirements; relatively short turnover time for results; and genetic fingerprinting to facilitate epidemiological studies and disease control (38).

Most PCR assays reported in the literature for detection of *B. canis* are genus-based not species-specific. In the past, multiplex PCRs have been used to differentiate between some *Brucella* species (14). In more recent years, *Brucella canis* — specific PCRs have been developed (23,50,51). These assays have yet to undergo extensive evaluation in canine populations to evaluate sensitivity and specificity. Until then, PCR should be used in conjunction with clinical information and serology.

### Treatment

The generally accepted recommendation is that treatment should be discouraged, and truly infected animals should be euthanized due to the risk to canine and human populations (1,7,8,25,26). Disease due to *B. canis* is not currently reportable in Canada, which leaves the decision-making process to the client and the veterinarian. Euthanasia serves as a strict approach, but if this is not possible due to client opinion, then isolation can be considered after appropriate client education and medical record documentation. Patients with no clinical manifestations of the disease should be isolated and allowed to self-eliminate the organism if possible. If an adequate Th1 response occurs, the patient might spontaneously recover in 2 to 3 y on average (7,8).

Although significant illness is rare, those patients experiencing clinical signs that warrant intervention will have to be either euthanized or treated. Treatment is notoriously unsuccessful as dogs experiencing morbidity have had a Th2 response leading to persistent infection. It is important for clinicians to remember that it is not only the antimicrobial therapy, but also the individual’s immune response that works in concert to determine the outcome of infection. Antibiotic therapy does not guarantee elimination of the organism, with relapse or re-infection believed to be common (1,7,8,25,26).

Original studies have demonstrated the superiority of combination antibiotic therapy over a single agent protocol. Traditionally, a tetracycline-based antibiotic (tetracycline hydrochloride, doxycycline, minocycline) is administered orally with daily or divided standard dosing for a minimum of 1 to 2 mo. The second antibiotic is an aminoglycoside (dihydrostreptomycin, streptomycin, or gentamicin) administered parenterally with daily standard dosing for either the initial 7 to 14 d of treatment, or a 7-day period every 3 to 4 wk (7,8,18,25,30,52). Aminoglycosides have significant limitations: nephrotoxicity monitoring and possible hospitalization with intravenous fluids; parenteral administration; streptomycin availability; and inadequate ocular and central nervous system penetration (18).

A recent report documents the successful treatment of 3 dogs with chronic or recurrent uveitis using combination antimicrobial therapy (doxycycline, enrofloxacin, and streptomycin, with or without rifampin) (54). All 3 dogs in this report responded in terms of clinical factors like resolution of ocular inflammation and conversion to seronegativity. Negative serology was attained after a median of 96 wk (range: 36 to 112 wk) of therapy (54).

Another article documents the response of 12 dogs in a breeding facility that was experiencing infertility and spontaneous abortions. A novel single agent enrofloxacin treatment protocol consisted of 5 mg/kg body weight (BW) orally every 12 h for 30 d with additional courses administered to females during all subsequent estrual and luteal cycles (range: 0 to 2 cycles) (55). Fourteen months later, the dogs in this study did not have any further abortions, transmission to offspring was not observed, vaginal secretions were culture negative after subsequent births, fertility was maintained, and titers declined (55). Veterinarians will appreciate the significant antimicrobial resistance concerns that might arise with respect to long-term, intermittent fluoroquinolone use.

In these studies, clinical improvement and declining antibody titers were observed with antibiotic therapy, but definitive elimination of the organism was not demonstrated (53,54). The intermittent nature of clinical disease manifestations, particularly those involving reproductive performance abnormalities, makes definitive comments about treatment efficacy impossible without an untreated group. Ethical concerns make a negative control group in clinical patients unlikely in future research endeavors.

Unfortunately, the treatment and monitoring protocols are often lengthy and time consuming, leading to escalating expense and declining client compliance (8). To the author’s knowledge, there is no universally accepted treatment protocol especially in terms of treatment duration, which has involved 1 to 2 mo of therapy, 90-day treatment cycles separated by 1 to 2 mo, or indefinite antimicrobial use (8,18). Monitoring the AGID, every 2 to 6 mo can potentially help guide both the recognition of relapse, and the duration of antibiotic therapy with 2 consecutive negative results suggesting adequate therapy (8). Monitoring is indefinite and relapse necessitating retreatment is considered likely (8).
Prevention

Although not universally standardized, detailed prevention strategies in breeding facilities have been proposed by the USDA and the Georgia Department of Agriculture websites (1,26). Dogs should test negative on serial screening tests performed 8 wk apart prior to admission to a kennel or a breeding program. Dogs testing positive should be isolated and decisions made about euthanasia, or treatment and monitoring. An important preventative measure will involve sterilization.

Prevention also involves rigid attention to biosecurity. Principles of infection control will include: one-time-use protective equipment (gloves, goggles, masks, gowns, boots); thorough hand washing; appropriate sample handling; routine disinfection (i.e., 2.5% sodium hypochlorite, quaternary ammonium compounds or 70% ethanol with a minimum of 10 min contact time); biofilm prevention (minimize organic material); drying and exposure to sunlight; education of staff and clients; and notification of laboratory personnel receiving specimens as to the suspected diagnosis (1,7,8,25,55).

Public health

Approximately 100 to 200 cases of human brucellosis (all Brucella species) are diagnosed annually in the USA (55). One case report that has received a lot of attention is that of a 3-year-old female toddler who is believed to have acquired B. canis infection from a Yorkshire terrier puppy in New York City (56). In addition, HIV-positive patients with appropriate CD4 counts and negative viral loads have also been diagnosed and successfully treated for B. canis infections (55,57).

The pathogenicity of Brucella canis is considered relatively low, making it less of a perceived public health concern than other Brucella species, in particular Brucella melitensis, and biotypes 1 and 3 of Brucella suis (23,25,55). It is important to remember that B. canis is not reportable in Canadian provinces or territories, and Brucella is not routinely tracked beyond the genus level at the Center for Disease Control (CDC). The absence of a structured regulatory program for B. canis means that we do not know if human infection is underdiagnosed, especially when considering the nonspecific clinical signs, and the variable incubation period of 2 wk to 3 mo (25,55). Diagnostic limitations are also a complicating factor, necessitating research into ELISA and PCR technologies.

Currently reported clinical signs associated with human brucellosis include fever (often periodic and nocturnal), fatigue, headache, weakness, malaise, chills, sweats, weight loss, hepatomegaly, splenomegaly, and lymphadenopathy (7,8,25,55). Serious complications from infection in humans include epidural abscess, pleural effusion, oral lesions, lower extremity aneurysms, and culture negative endocarditis (7,8,25,55). Deaths are rare except with serious underlying sites of infection or delayed treatment. Unlike in dogs, treatment of human B. canis infections has been associated with elimination of the organism (7,8,25,55).

In conclusion, Brucella canis should be considered as a potential differential diagnosis for small animal and public health practitioners in Canada. A One Health approach is essential to update our understanding of canine and human seroprevalence rates, pathogenesis, and management options.

References


Table 3. PCR assays for the detection of Brucella antigen in dogs.

<table>
<thead>
<tr>
<th>Primers directed to</th>
<th>Detection: Genus or Species</th>
<th>Sample</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S-23S rDNA Interspace region (44)</td>
<td>Brucella genus</td>
<td>Whole blood</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>16S-23S rDNA Interspace region (45)</td>
<td>Brucella genus</td>
<td>Vaginal swabs</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>16S-23S rDNA Interspace region (46)</td>
<td>Brucella genus</td>
<td>Semen</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>16S rRNA sequence (47)</td>
<td>Brucella genus</td>
<td>Whole blood</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>16S-23S rRNA Interspace region (48)</td>
<td>Brucella genus</td>
<td>Inguinal lymph node</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>16S-23S rRNA Interspace region (49)</td>
<td>Brucella genus</td>
<td>Whole blood Serum</td>
<td>Whole blood: 97.14% Serum: 25.71%</td>
<td>Whole blood: 100% Serum: 100%</td>
</tr>
<tr>
<td>Intergenic spacer IS711 fragment (23)</td>
<td>Brucella genus</td>
<td>Males: preputial swab, semen, or urine Female: vaginal swab</td>
<td>Not available</td>
<td>Not available</td>
</tr>
</tbody>
</table>

- PCR on vaginal swabs and semen in these studies correlated with blood PCR, and blood culture as assessed by a Kappa co-efficient and the McNemar test.
- Both Brucella genus-based and Brucella canis specific PCRs used in Swedish outbreak investigation.
- Brucella canis inoculated samples; PCR onuffy coats separated from whole blood was approximately 100 times more sensitive than from whole blood.


1. C) Cattle have a limited bone marrow reserve and have a limited degree of neutrophilia in response to inflammation.

2. D) Candida albicans is a yeast which infects intact mucous membranes, most commonly the tongue and the esophagus. The infection is most common in young ungulates, and is usually associated with an underlying primary debilitating condition (i.e., thrush is a secondary disease). Streptococcus pyogenes and Corynebacterium pyogenes are bacteria which trigger a suppurative inflammatory response (i.e., pus formation or abscess formation); these agents most commonly affect the upper respiratory tract. Actinobacillus lignieresii is the cause of “wooden tongue,” a granulomatous condition resulting from opportunistic invasion of damaged lingual tissue by the causative bacterium. Histophilus somni is associated with respiratory, neurologic, and reproductive infections in the cattle.

3. A) Serum IgM titer is the best test for diagnosis of an active infection.

4. E) Valvular endocardiosis is an age-related, degenerative change in which there is accumulation of a myxomatous connective tissue matrix within the valve leaflets, causing nodular thickening. The suffix “-osis” implies a degenerative condition; bacterial infection of the heart valves would lead to valvular endocarditis, or inflammation of the valves. Since endocardiosis most commonly affects the atriocventricular valves (and the mitral valve more commonly than the tricuspid), the condition may be associated with a systolic heart murmur.

5. D) The flehmen response occurs in stallions following exposure to mares in estrus. The stallion will curl its upper lip and drop its penis.

Answers to Quiz Corner
Les réponses du test éclair

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Acute sternal subluxation in an indoor cat

Louise Lam

Abstract — A 1-year-old spayed female cat was presented with tachypnea and a protrusion on the ventral thorax. Radiography revealed ventral sternal subluxation between the 6th and 7th sternebrae. There was no evidence of respiratory distress, even after follow-up, and a conservative management approach was successful in this healthy, young, indoor cat.

Discussion

In carnivores, the sternum consists of 8 sternebrae, including the manubrium (1st sternebra), body (2nd to 7th sternebrae), and xiphoid process (8th sternebra) with xiphoid cartilage (1,2). In most species, the intersternal joints are comprised of impermanent synchondroses, with the manubriosternal joint being a synovial joint in some (3). In the cat, the manubrium is slightly keeled, the sternal body consists of 6 articulated sternebrae, and the last sternebra is the xiphisternum with a distal xiphoid process (4). Sternal luxations or subluxations may involve partial or full dislocation(s) at any of the articulations along the sternum.

Sternal luxations or subluxations are a potentially painful condition that may or may not affect the integrity of the thoracic wall. If the thoracic wall is punctured by dislocated segments, the thoracic cavity's ability to maintain negative transthoracic pressure for inspiration is compromised, potentially leading to...
impairment of ventilation. It may even result in paradoxical movement of the thoracic wall due to instability of the wall (1).

The possible causes of sternal luxations or subluxations include trauma (blunt force or animal attacks), idiopathic, and, anecdotally, secondary to chronic respiratory disease. This condition is distinct from *pectus excavatum* or *pectus carinatum*, which are structural deformities in the thoracic wall.

There is little documentation in the literature about sternal luxation or subluxation, its occurrence, or the options for management. Several small animal surgery textbooks briefly mention sternal fractures and luxations and that their occurrence is relatively rare (1,5–7). The first reported successful management of traumatic sternal luxation was published in 2014, revealing no complications 18 mo after surgery (8). The dog in that case had an acute history of falling down the stairs followed by clinical signs of dyspnea, tachycardia, and ventral thorax discomfort. Radiographs revealed luxation at the 3rd and 4th sternebrae with dorsal displacement. Surgical reduction of the sternum using a dynamic compression plate resulted in rapid recovery and minor complications (8). Despite some reports of success, sternal reduction involves open chest surgery, which carries surgical risks and the potential for complications. Moreover, traumatic sternal dislocations often include intrathoracic injuries. For instance, a case report in 2015 described a cat with manubrio-sternal luxation and tracheal stenosis after being hit by a car (9). Although surgery was performed, the owners opted for intra-operative euthanasia following suture dehiscence involving the trachea (9).

The infrequent number of reports may be due to several possible reasons. Anecdotal evidence suggests that the most common reason for sternal dislocation is trauma, which often includes other life-threatening complications and possible euthanasia or death that is not reported. Moreover, it may be underreported or missed due to the possible perception that it is a rare condition (5). Despite the lack of peer-reviewed reports, there is additional information about the management of sternal luxations and subluxations on veterinary forum discussions, such as Veterinary Information Network (VIN). Anecdotal findings indicate that sternal luxations and subluxations are usually managed conservatively, due to risks associated with surgical repair of the thoracic cavity. Therefore, regardless of thoracic wall integrity, most of these cases are managed symptomatically without documentation of surgical options, or in many cases these patients are euthanized. If the patient does not exhibit labored breathing, the best option is medical management since surgery of the thoracic cavity carries many risks, including compromise to the thoracic cavity and its ability to maintain normal negative intrathoracic pressure, failure of surgical closure, anesthetic risks, and contamination risks. This was the approach used in this case report; analgesia and an anti-inflammatory drug were used to manage the patient’s symptoms, and the owner was instructed to monitor for respiratory distress. This strategy is supported by an emergency surgery textbook, which describes the case of a cat with subluxation at the 5th and 6th sternebrae (10). The patient was medically resuscitated with intravenous fluids, oxygen, pain therapy, and antimicrobial drugs. Hospitalization was continued for several days with supportive care as needed. The patient showed initial improvement, had septic complications due to a limb fracture, but was eventually discharged from hospital (10).

In the case of respiratory distress involving penetration of the thoracic wall, medical management alone would not be sufficient to ensure quality of life in the long term. Either surgery or euthanasia would need to be considered without further delay. Guidelines on surgical approach are described in surgical emergency textbooks, which mostly describe general open chest surgery instead of specific instructions on sternal reduction, but the fundamental principles should not be different (1,6,10). Surgical approaches for *pectus carinatum* and *pectus excavatum* may be considered based on similar open chest surgical principles (11). Note that penetrating thoracic wall injury does not always warrant surgical exploration unless there is evidence of ongoing hemorrhage, pneumothorax, or sepsis (6). However, suspected full-thickness thoracic wall defects resulting in air movement in the pleural space require immediate attention to maintain intrathoracic and pleural space integrity (6).

In all cases, pre-surgical management of the patient includes cleaning and bandaging external wounds, pain control, and preoperative antimicrobial therapy (10). Penetration of the thoracic wall by dislocated segments should be treated as an open wound continuous with the pleural cavity and requires immediate debridement and sealing with suture closure, petroleum-based ointments and gauze packs, pleural evacuation, and stabilization (1,6). Soft tissue penetration, rupture, or herniation should be considered. Rib fracture and intercostal vessel lacerations related to the type of sternal luxations or subluxations should also be taken into account. Internal fixation of the sternum with small intramedullary pins is a possible approach, as described with rib fracture fixations (1). Chest bandages should be used with caution due to concern with impairment of ventilation (1). Potential complications include failure of closure leading to loss of negative intrathoracic pressure required for ventilation, persistent ventilation impairment, hypoxemia, and sepsis.

In conclusion, sternal luxations and subluxations are rarely reported and there is little documentation about their successful management. Absence of breathing impairment indicates medical management with oxygen, intravenous fluids, and pain control is appropriate. Due to risks involved with open thoracic wall exploration, surgery is not indicated unless there...
is breathing impairment due to penetration of the chest wall. Further documentation of similar cases may help guide the proper management of sternal luxation and subluxation.

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References


New Products

Nouveaux produits

New class of probiotics, with a unique mode of action, will now be available across Canada

MicroSintesis Inc. is delighted to announce they have signed an exclusive distribution agreement with VHS to distribute its first product, YGIA, which contains Proteobiotics, newly discovered molecules produced by Probiotics.

MicroSintesis, a new animal health company, has, for the past five years, been researching this novel probiotic technology which has the potential to significantly impact the way animals are treated for gut health and intestinal distress. The MicroSintesis technology platform builds on the discovery that probiotics produce bioactive substances that are responsible for a significant portion of a probiotic’s activity. Termed “Proteobiotics,” these substances directly interfere with a pathogen’s genome and down-regulate genes involved in infection, promoting a healthy digestive tract as a result. Proteobiotics, therefore, supports a more selective use of antibiotics, which may reduce the incidence of antibiotic resistance development, long-term. YGIA-14 is the first product of its kind to contain both a highly effective probiotic and this new Proteobiotic technology.

“The majority of our veterinary customers to date been using our YGIA-14 product with huge success,” says Hannah McIver, CEO of MicroSintesis. “Both pet owners and veterinarians alike want to treat their animals in ways that don’t negatively impact their natural body’s flora. YGIA-14 provides a stronger and faster response than regular probiotics, without the negative impact of antibiotics.”

VHS Veterinary Healthcare Solutions (VHS) is an independent Canadian veterinary marketing and sales company dedicated to delivering innovative products to the veterinary market. With seven sales representatives across Canada, the partnership now gives MicroSintesis the opportunity to sell their products nationally while providing VHS a platform product on which to expand their offering in key clinics.

“VHS is very excited to add the YGIA-14 product to our portfolio,” said Dr. Tom Branton, President of VHS. “The science behind this product coupled with the professionalism and knowledge of the MicroSintesis team are exceptional. More than ever, animal health practitioners and pet owners are seeking natural treatments for their pet ailments. YGIA-14 is a great new treatment modality to address gastrointestinal health. The VHS Sales Team is looking forward to introducing YGIA-14 to clinics across Canada.”

YGIA-14 will be available for sale through veterinary hospitals across Canada in June 2017.

Contact: Veterinary Healthcare Solutions, 2516 Binder Crescent, Oldcastle, ON N0R 1L0; website: www.vhs.vets.com or www.microsintesis.com
Veterinary Dermatology
Dermatologie vétérinaire

Developments in small animal veterinary dermatology
Kinga Gortel

I recently received a visit from a pair of unrelated 8-year-old housemate West Highland White terriers referred for chronic pruritic skin disease (Figure 1). Both Westies, a spayed female and a castrated male dog, had exhibited skin and ear disease starting before they were 1 year of age. Their histories were long and convoluted but in short, both dogs suffered from seasonal atopic dermatitis, recurrent pyoderma, and recurrent otitis externa. Previous therapies had included oral corticosteroids and cyclosporine. To my surprise, while the clinical appearance of the 2 dogs was similar, the female dog had generalized demodicosis: abundant Demodex mites (predominantly D. canis with fewer D. injai) were found on all deep skin scrapings.

The idea for this article came about when I thought about these dogs, and realized how dramatically veterinary dermatology had changed in their lifetime. The treatments I considered were ones I could not have imagined when they were 1 year old and just starting to show clinical signs. Today, I could be optimistic about helping them be comfortable quickly, without disturbing the bottle of prednisone gathering dust on my pharmacy shelf. This article describes some of the key therapeutic developments in small animal veterinary dermatology in the last decade. Most have had a very favorable effect on the landscape of dermatology in Canada, but others have provided unfortunate therapeutic challenges.

This article and the upcoming features in the Veterinary Dermatology column are a collaboration of The Canadian Veterinary Journal and the Canadian Academy of Veterinary Dermatology (CAVD). The CAVD is a federally incorporated not-for-profit organization that has promoted the advancement of veterinary dermatology in Canada for over 30 years. The initiatives of the CAVD include:
• providing continuing education such as lectures and webinars to veterinarians, animal health technicians/technologists, and veterinary students;
• keeping members up-to-date with developments in the form of a member publication, electronic newsletters, and other communications;
• developing clinical tools such as the Dog and Cat Itch Scale and the Cytology Scale for Ears and Skin (see www.cavd.ca); and
• funding research

The CAVD invites all veterinarians, veterinary technicians and technologists, and veterinary students with an interest in veterinary dermatology to join the academy to stay current with the advances and challenges in this dynamic field. Memberships ($25 annually, free for students) are available at www.cavd.ca

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Conflicts of interest: In the last 5 years, Kinga Gortel has received honoraria, consulting fees, and/or has collaborated with Royal Canin, Purina, Zoetis, and Novartis/Elanco.

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THE DERMATOLOGY FEATURE IS GENEROUSLY SPONSORED BY ROYAL CANIN.
LA RUBRIQUE SUR LA DERMATOLOGIE EST GÉNÉREUSEMENT COMMANDITÉE PAR ROYAL CANIN.
The most recent advances in veterinary dermatology have affected the way we treat our most common patients: allergic dogs. The spring of 2016 marked the arrival of a much-anticipated product in the Canadian veterinary market. Apoquel (oclacinib; Zoetis Canada, Kirkland, Quebec) was approved in Canada for the control of pruritus associated with allergic dermatitis and the control of atopic dermatitis in dogs at least 12 months of age. Apoquel inhibits the activity of various pro-inflammatory and pruritogenic cytokines including Interleukin (IL)-31, one of the key mediators of itch in dogs. Since its launch, Apoquel has provided veterinarians with a novel, effective, and well-tolerated treatment for these very common ailments (1-3). A key benefit of the drug is its impressively rapid effect (1), comparable to that of oral corticosteroids. I chose Apoquel for treating the male Westie patient. I hoped that in addition to rapidly reducing his severe pruritus, it would provide an effective long-term therapy for his atopic dermatitis.

For the female Westie with atopic dermatitis and generalized demodicosis, I preferred to avoid drugs with the potential to inhibit normal immune function. This would have been difficult in the past, but with the recent availability of a novel therapy I had another option for controlling her pruritus. Cytopoint (lokivetmab; Zoetis Canada), a caninized anti-IL-31 monoclonal antibody, was launched in June 2017 to aid in the reduction of clinical signs associated with canine atopic dermatitis. It has been shown to be a safe (4) and effective (5,6) treatment for this disease. Despite the overlapping indication and activity on the same key cytokine, Cytopoint works very differently from Apoquel. Remarkably, it can be effective even in some dogs whose signs are not adequately controlled with the latter. Cytopoint differs from other treatments in a number of ways. It does not affect normal immune function, and can be given to dogs of any age (but should never be used in other species). It can be used in dogs with diseases that could preclude using Apoquel, Atopica (cyclosporine; Elanco Canada, Guelph, Ontario), or corticosteroids. These include patients with malignancies, severe infections, and — like my Westie patient — generalized demodicosis.

It would be difficult to overstate the positive impact that Atopica has made on veterinary dermatology. For a decade, Atopica was the only highly effective alternative to corticosteroids available in Canada for the control of clinical signs of atopic dermatitis in dogs (7). It remains the most effective licensed treatment for feline allergic dermatitis. The use of Atopica for dermatologic indications was a game-changer that allowed, for the first time, relief for many allergic patients either without corticosteroids, or with a significant steroid-sparing effect. Compared to the therapies just described, Atopica has a slower onset of action (1). After several weeks of administration, however, its efficacy in reducing pruritus in dogs is similar to that of Apoquel (1) and the dosing frequency for Atopica can often be decreased. Cyclosporine has also received extensive extra-label use for various inflammatory dermatologic conditions. These include proliferative otitis externa, seborrheic adenitis, perianal fistulac, chronic pododermatitis, pemphigus foliaceus, reactive histiocytosis, and many other immune-mediated and autoimmune conditions (8).

Systemic corticosteroids are no longer the mainstay in treating allergic dogs, but one newer topical product deserves mention. A potent dermal corticosteroid spray with a low rate of percutaneous absorption, Hydrocortisone Aceponate (Cortavance Topical Spray Solution; Virbac Canada, Cambrige, Ontario), is licensed for use in dogs for a period of 7 consecutive days. Several studies confirm the efficacy and safety of this product for longer periods if needed, (7,9) though patients treated in this way should be carefully monitored for cutaneous atrophy (10).

The role of the skin barrier has received ample attention in human and veterinary allergy, and skin barrier defects have been demonstrated in canine atopic dermatitis (11). Several topical therapies and veterinary diets have been shown to enhance this barrier, and can be a useful part of the multi-modal treatment of allergic pets.

Even with the extraordinary advances in symptom-relieving treatments, addressing the cause of allergy remains a key part in managing the disease. Allergen-specific immunotherapy can change the course of atopic dermatitis or possibly effect a cure, rather than simply achieving symptom control. The development of sublingual immunotherapy products has made this treatment possible for clients reluctant to give injections. Early investigations of this treatment are promising (12), although larger studies are needed. The investigation of cutaneous adverse food reactions (food allergies) can still be difficult. A reliable...
serologic test for food allergens does not exist (13), and an elimination diet trial with subsequent provocation testing remains the diagnostic procedure of choice. Although client compliance remains a major hurdle in diet trials, the selection of veterinary diets available has greatly increased. Novel protein diets now include exotic offerings such as rabbit, kangaroo, and alligator-based foods. The hydrolyzed protein diet selection has also expanded to include extensively hydrolyzed feather protein foods for dogs and cats (Allerrogen; Royal Canin, Guelph, Ontario).

Although it is overshadowed by the developments in allergy treatment, there has also been a revolution in the treatment of a typically stubborn parasitic disease. For dogs with generalized demodicosis, like my female Westie patient, slow improvement has typically meant many months of administering drugs with significant toxic potential. This all changed after the arrival of the first oral isoxazolines in Canada (fluralaner, Bravecto; Merck Animal Health, Kirkland, Quebec) in 2014. By 2015, a study showed unprecedentedly rapid efficacy after 1 dose of fluralaner in 8 dogs (14). A larger unpublished case study subsequently confirmed an overall response rate of 100%, with most (87.1%) of the 163 dogs achieving negative skin scrapings in 1 month, with the remainder by 2 months (15). Afoxolaner (NexGard; Mérial Canada, Baie D’Urfé, Quebec) and sarolaner (Simparica; Zoetis Canada) have also subsequently shown rapid efficacy (16,17). The treatment of demodicosis with isoxazolines intended for flea and tick control is extra-label use. Select isoxazolines have also been successfully used in the extra-label treatment of ectoparasites including Demodex catti (18), Otodectes cynotis (17), and Sarcoptes scabiei (19,20).

Along with these remarkable additions to our therapeutic arsenal have come some unwelcome challenges. The increase in methicillin-resistant staphylococci, particularly Staphylococcus pseudintermedius, S. schleiferi, and S. aureus, has been of particular concern in companion animals. These infections are especially troublesome in dogs because of this species’ predisposition to developing recurrent pyoderma. These bacteria are resistant not only to all of the beta-lactam drugs so commonly used to treat skin infections, but frequently to other antimicrobial drugs as well. Empirical systemic drug selection is contraindicated when one of these infections is suspected based on a treatment failure (21). Unfortunately, systemic treatment of methicillin-resistant staphylococcal infections can require the use of potentially toxic drugs such as rifampin and amikacin. Resistant staphylococci are now endemic in many areas. A Canadian study examining canine pyoderma cases found them in 12.1% of staphylococcal skin cultures in primary care practices (22).

If there is any good news, it is that these resistant bacteria have made it apparent that systemic therapy is often unnecessary for pyoderma. For the very common superficial infections, topical therapy — such as chlorhexidine shampoos and sprays — can be just as effective as antibiotics, even when caused by resistant staphylococci (23). Topical therapy is now recommended as the sole treatment for surface and superficial methicillin-resistant staphylococcal infections whenever pet and owner compliance can be expected (21). Although the selection of topical products is limited in Canada compared to other markets, it is improving, with 2% to 4% chlorhexidine shampoos, sprays, wipes, and solutions now available for companion animals.

The catalog of developments is much longer, with just a few more listed here. Various new formulations of antibiotics allowing for easier compliance are available, with a once-daily cephalosporin (cefpodoxime proxetil, Simplipic; Zoetis Canada), an injectable cephalosporin with a long half-life (cefovecin sodium, Convenia; Zoetis Canada), and various palatable tablet formulations of cephalaxin and amoxicillin-clavulanate now marketed. Gel preparations for otitis externa requiring infrequent application (e.g., Osurnia; Elanco Canada) are also simpler to administer. The selection of topical therapies in Canada is expanding, though the selection of products containing chlorhexidine combined with an azole antifungal for mixed bacterial and Malassezia infections remains limited (e.g., Douxo Pyo products; Ceva Animal Health, Cambridge, Ontario). Veterinary dermatology is an exceptionally active area of research and publication. Open-access clinical guidelines have been published by the World Association for Veterinary Dermatology (21,24), and by the International Committee on Allergic Diseases of Animals (25,26) to help veterinarians with several of the most common skin diseases.

It is an exciting time to be practicing veterinary dermatology, even if the expanding therapeutic arsenal can make treatment selection more complicated than before. Some of the developments mentioned in this article will be profiled in future Veterinary Dermatology columns. As for the Westies: they are reportedly doing very well, and I look forward to seeing them for follow-up examinations soon. Like most pets with chronic skin disease, they have a long road ahead of them. But thanks to the changing landscape of veterinary dermatology, I have more hope than ever that the road ahead for these dogs will be smoother than the one they have traveled for their first 8 years.

References


Veterinary Practice Management
Gestion d’une clinique vétérinaire

Managing your purchases? Get out your calculator
Vous gérez vos achats? Sortez votre calculatrice!

Darren Osborne, MA

Cost of goods is the highest expense in mixed animal practices, and the second highest expense in companion animal hospitals. Yet, it’s one of the most ambiguous areas of veterinary practice management. Responsible veterinarians who try to manage their cost of goods are often advised to lower their spending. They’re given advice such as, “Your purchases should be 20% of sales every month,” or simply, “You’re buying too much.” However, this advice is almost always given without any veterinary industry data or knowledge of how much is being sold. For a veterinarian who is selling twice the average amount of goods, their cost of goods sold will be twice the average.

What is cost of goods sold?
The term “cost of goods sold” (COGS) is used to describe the amount a veterinarian will spend to purchase anything that is sold to a client, such as pet food, medication, over-the-counter products and toys. In veterinary medicine, cost of goods also incorporates the cost of products that are used to deliver services, but are not sold directly to the client, such as gauze, antiseptic, suture material, and vaccines. Separating the amount used in the hospital from the amount sold to clients is ideal for managing

Le coût des biens est la dépense la plus élevée dans les pratiques vétérinaires mixtes et la deuxième dépense en importance dans les cliniques pour animaux de compagnie. Pourtant, c’est l’un des domaines les plus ambigus de la gestion d’une pratique vétérinaire. On conseille souvent aux vétérinaires responsables qui tentent de gérer le coût des biens de réduire leurs dépenses. On leur offre souvent des conseils comme : «Votre achats devraient représenter 20 % de votre chiffre d’affaires tous les mois» ou simplement «Vous achetez trop». Cependant, ces conseils sont presque toujours communiqués sans posséder de données de l’industrie vétérinaire ou de connaissances sur le volume des ventes. Pour un vétérinaire qui vend deux fois la quantité moyenne des produits, le coût des produits vendus s’établira à deux fois la moyenne.

Quel est le coût des produits vendus?
Le terme «coût des produits vendus» (CPV) est utilisé pour décrire le montant dépensé par un vétérinaire pour acheter les biens vendus à un client, comme les aliments pour animaux, les médicaments, les produits en vente libre et les jouets. En médecine vétérinaire, le coût des produits inclut aussi le coût

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purchasing, but this rarely happens. So, remember that when accounting for cost of goods sold, this is actually “cost of goods used in the practice” (or COGUIT).

**Myth: One size fits all**

The most common myth is that everyone can use the same number to manage their cost of goods. This must come from other industries in which product sales are more consistent. If sale of products always represents 50% of revenue, then one figure would work in all situations. In veterinary medicine though, there is variability in pet food and pharmacy sales. A companion animal veterinarian with a keen interest in nutrition may sell twice the amount of food and will therefore have twice the COGs. The average revenue from product sales may be 40% of gross revenue (15% pet food and 25% pharmacy), but the range for most practices is 32% to 48%.

The markup on products sold through the pharmacy will affect the contribution to revenue and resulting COGS. If one hospital sells a product for 25% less than a second hospital, the first hospital will have lower revenue and cost of goods will be higher. For example, suppose a product costs $10 to stock. If one hospital marks it up 100% and sells it for $20, their total revenue is $20, and their cost of goods is $10 or 50%. If a second hospital marks up the same product 50% and sells it for $15, total revenue is 15% and cost of goods is 67%. The two hospitals sold the same product, but the cost of goods varies because the markup varies.

Another factor that affects the cost of goods is professional fees. If two hospitals charge the same markup on products, but one charges twice as much for professional services, the COGS will differ greatly (Table 1).

Hospital 1 should have no problem keeping purchases below 20% of gross revenue, but it would be impossible to lower cost of goods to below 25%.

**Myth: Hospitals need to decrease purchases**

When veterinarians ask for assistance managing their COGS, many consultants assume there’s a problem. Their first reaction is to suggest a decrease in spending. If the cost of goods is lowered, and all things are equal, expenses will be lower and there will be an increase in profit. But many hospitals are already purchasing responsibly. If purchases are lowered, the hospitals will start to run out of inventory. Most hospitals have a great relationship with their distributors and order products as they need them. They sell a bag of dog food to a client on Tuesday and then order more on Wednesday, but they won’t order on Tuesday if there isn’t enough dog food in the stock.

**Table/Tableau 1. Factors affecting the cost of goods sold./Facteurs affectant le coût des produits vendus.**

<table>
<thead>
<tr>
<th></th>
<th>Hospital 1</th>
<th>Hospital 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sale of professional services</td>
<td>$100</td>
<td>$200</td>
</tr>
<tr>
<td>Vente de services professionnels</td>
<td>$100</td>
<td>$100</td>
</tr>
<tr>
<td>Sale of products/Vente de produits</td>
<td>$200</td>
<td>$300</td>
</tr>
<tr>
<td>Total revenue/Revenu total</td>
<td>$50</td>
<td>$50</td>
</tr>
<tr>
<td>Cost of products/Coût des produits</td>
<td>25%</td>
<td>16%</td>
</tr>
<tr>
<td>COGS percentage/Pourcentage du CPV</td>
<td>20%</td>
<td>20%</td>
</tr>
</tbody>
</table>

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and then order a replacement bag on Wednesday. The hospital’s purchases match client purchases.

Other problems with decreasing inventory are, for example, higher sales of parasite medication due to increased prevalence of ticks and increased pet food sales through veterinary webstores. The introduction of tick prevention medication in the last few years has resulted in a new source of revenue for veterinary practices but it has also resulted in higher purchases. Similarly, many hospitals are finding an increase in pet food sales from offering online purchases through their webstore. Increased sales associated with automatic reorder is increasing both diet revenue and diet purchases.

**Reality: Revenue from sales varies and so should cost of goods**

Data from the 2016 Practice Owners Economic Survey provides direct evidence on the variability of purchases. It explains the trend associated with revenue from products and the resulting cost of goods. Many veterinarians know that increased product sales will lead to increased purchases, but many believe that they can keep purchases as a percent of revenue stable regardless of product sales. They think that keeping purchases at 20% of gross revenue is possible as product sales increase. The data show that as the contribution to revenue increases, so does the cost of purchases as a percent of revenue. As product sales as a percentage of revenue increase, so do product purchases as a percent of revenue. If product sales as a percent of gross revenue increase, so does product purchases as a percent of revenue (Figure 1).

For companion animal practices, the cost of goods rises a 1/4% with each percent increase in sales. For example, if a hospital increases sale of products from 40% to 44% of revenue, COGS will go up 1%.

In mixed and large animal practices, in which the sale of medications is a bigger part of the practice, the cost of goods goes up 0.38% for each percentage contribution from product sales (Figure 2). If a dairy veterinarian adds a large herd to the practice, medication sales, as a percent of gross revenue, may go from 40% to 50% and the cost of goods would go up 3.8%.

La plupart des cliniques possèdent une excellente relation avec leurs distributeurs et commandent des produits en fonction de leurs besoins. Elles vendent un sac de nourriture pour chiens à un client le mardi, puis commandent un sac de remplacement le mercredi. Les achats de la clinique correspondent aux achats des clients.


Les données provenant du Sondage économique 2016 auprès des propriétaires de pratique fournissent des preuves directes à l’égard de la variabilité des achats. Elles expliquent la tendance associée au revenu provenant des produits et au coût des produits qui en découle. Beaucoup de vétérinaires savent que l’augmentation des ventes de produits entraînera une hausse des achats, mais bon nombre croient aussi qu’ils peuvent maintenir la stabilité des achats par rapport au revenu sans égard à la vente de produits. Ils croient que le maintien des achats à 20 % du revenu brut est possible avec l’augmentation de la vente de produits. Or, les données montrent que, au fur et à mesure que la contribution au revenu augmente, le coût des achats en tant que pourcentage du revenu progressera aussi. Lorsqu’il se produit une hausse de la vente des produits en tant que pourcentage
Product sales can be calculated as a percentage of gross revenue and entered into the following formulae:

<table>
<thead>
<tr>
<th>Companion animal</th>
<th>COGS % of gross = Product sales % of gross * 0.25 + 0.17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed and large animal</td>
<td>COGS % of gross = Product sales % of gross * 0.38 + 0.19</td>
</tr>
</tbody>
</table>

The range in markups and professional fees means the equation may not fit all hospitals. Since the cost of goods varies, be sure to use numbers that reflect your hospital’s sales practices.

On peut calculer les ventes de produits en tant que pourcentage du revenu brut et les inclure dans les formules suivantes :

<table>
<thead>
<tr>
<th>Animaux de compagnie</th>
<th>% du CPV du revenu brut = % des ventes de produits du revenu brut * 0.25 + 0.17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cliniques mixtes et pour grands animaux</td>
<td>% du CPV du revenu brut = % des ventes de produits du revenu brut * 0.38 + 0.19</td>
</tr>
</tbody>
</table>

La fourchette de majoration du prix et des honoraires professionnels signifie que l’équation pourra ne pas convenir à toutes les cliniques. Vu que le coût varie, il faudra vous assurer d’utiliser les chiffres qui reflètent les pratiques de vente de votre clinique.

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In today's world, in some cases for better and in others for worse, scientific information can be rapidly disseminated and made available for use, interpretation, and extrapolation by the non-scientific public (1). To address recent speculation in a PubMed citation that dogs may serve as a reservoir host for Zika virus (ZIKV), it should be made abundantly clear that to date there is no evidence to indicate that dogs a) are capable of transmitting ZIKV to humans, or b) are a reservoir host for ZIKV (2).

If extrapolation regarding zoonosis is going to be built on a single seroconverted dog from a group of dogs who may also show seroconversion to the cross-reactive human dengue virus, it should not be ignored that dogs are not a competent reservoir for several long-established arboviral flaviviruses including dengue, yellow fever, Japanese encephalitis, West Nile, and St. Louis encephalitis viruses (3). Humans and domestic animals are exposed to a wide range of infectious agents for which we and the animals are not susceptible host species. Seroconversion of an individual does not equate to fulfillment of the myriad biologic requirements that are necessary to establish competency as a reservoir host (4). Although there are always idiosyncratic cases in which an infectious agent is able to gain foothold in an aberrant host species (often associated with individual immune compromise), no such case of canine ZIKV infection exists (5).

To this day the course of human history does show abundant evidence — whether recorded from statesmen (6–8), scientists (9), or Nobel laureates (10), or whispered in homes — for the nobility, affection, and loyalty of man's best friend (11). Yet in the United States alone, over half a million dogs are euthanized annually (12) for no additional reason than overpopulation. Until meaningful empirical evidence emerges that immunologically unmanipulated dogs can be susceptible to ZIKV, this kinder and already burdened species should be spared undue speculation.

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