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Editorial

Science-in-brief: Report on the Havemeyer Foundation workshop on acute colitis of the adult horse

The first Dorothy Russell Havemeyer Foundation workshop on acute colitis in the adult horse was held in Niagara-on-the-Lake, Ontario, Canada, in July 2019. Bringing together 35 participants from around the world, the goal was to the review current understanding of acute colitis in the adult horse and to identify key areas for collaborative research into this neglected illness. The need for collaborative and multicentre studies to develop guidelines for best diagnostic standards and evidence-based treatment protocols was among the critical priorities identified.

Agents of colitis

Acute equine colitis is a disease associated with significant morbidity and mortality in individual horses, with the potential to impact herds. Given the enormous size and the function of the equine large intestine, acute colitis can quickly become life-threatening. Acute colitis is responsible for about 5% of admissions to equine veterinary hospitals, with the aetiology undetermined in usually more than 50% of cases and with mortality of about 40%. The cumulative impact is profound but the relatively low numbers presenting in individual settings suggest that the scale of the problem is not usually recognised.

If equine researchers are to collaborate to improve understanding, there is a need to develop better standardisation of the term colitis (e.g. mild, moderate, severe, acute, chronic), so that individual studies can be combined to obtain statistical validity. While acute colitis is typically defined clinically by the presence of diarrhoea, which usually does not occur in horses with small bowel inflammation, other potential signs include fever, neutropenia, inappetence, ultrasonographic evidence of increased colonic wall thickness and increased fluid within the caecum and/or colon. Case fatality increases with the presence of toxic neutrophils in peripheral blood. Horses presenting with two or more signs of systemic inflammatory response syndrome (SIRS) at admission have a high fatality rate. Development of a colitis scoring system, which would include agent determination, would enhance the possibility of collaborative research into many aspects of acute colitis.

Colitis is a broad term used to describe a common symptomatology due to multiple aetiologies. *Clostridioides difficile*, *Neorickettsia risticii* and *Salmonella* spp. are the best-known agents of infectious equine acute colitis but there are important geographical and epidemiological differences relating to causes. For example, retrospective data presented from Denmark revealed cyathostomiasis as the most common cause with *Salmonella* spp. never being detected. By contrast, in Australia, *C. difficile* and *Salmonella* spp. infection were common, although 57% of cases had no aetiology determined. Noninfectious causes of colitis (e.g. acorn and nonsteroidal anti-inflammatory drug intoxication) may occur.

Clostridioides difficile was agreed to be the most diagnosed cause of equine acute colitis, but this might be biased by the limitations of current diagnostic approaches. The importance of direct toxin rather than gene detection in faeces or colonic content was recognised as important in diagnosis, since polymerase chain reaction (PCR)-based gene detection may pick up a carrier state.

In North America, an increase in the number of cases of Potomac Horse Fever (PHF) has been noted in recent years, with identification of novel strains of *N. risticii*, likely representing novel *Neorickettsia* species, that are not identified by current PCR-based tests. The diagnosis of salmonellosis is problematic in settings where *Salmonella* spp. are common, such as veterinary hospitals, which may skew aetiological diagnoses when it is not contributing to the colitis.

Because of PCR-based diagnosis, and poor current understanding, *C. perfringens* is likely overdiagnosed in adult horse colitis. More scientific evidence is needed to support the role of *C. perfringens* in adult horse colitis including the role of type C or the newly described NetF-positive strains, and be based on direct toxin rather than gene testing. Earlier data suggesting the importance of the *C. perfringens* enterotoxin (CPE) in acute colitis have not been supported by recent studies. Data presented suggested that *C. perfringens* is at best relatively unimportant in acute equine colitis.

For the first time, *Clostridium sordellii* was suggested to be a presumptive cause of acute fatal colitis in adult horses, providing support for the suggestion that there are as yet unknown causes of equine colitis. Experimental infection studies may be required to confirm its role. Viral causes of acute colitis are uncommon, although in outbreaks, equine coronavirus may cause diarrhoea that can mimic salmonellosis.

The equine microbiome and the pathophysiology of colitis

There was considerable interest in how next-generation DNA sequencing (NGS) technology is helping to better understand the equine intestinal microbiome and the microbiological consequences in cases of colitis. There is now extensive information on the characterisation of the equine faecal microbiome relating to age, antibiotic use, colic, deworming, diet, exercise, immunity, pregnancy and shipping, but more study is needed to confirm the trends and understand strain-specific variability among different healthy and sick states. The recent increased interest in human microbiome research is resulting in increased interest in the role of the equine microbiome in health and disease. One workshop participant reported a higher propensity for aged, geriatric, horses to develop colitis; is this universal and why does this occur? More needs to be known about the relation of the microbiota to the anatomic and physiologic compartmentalisation of the large intestine, including how this may impact the development of colitis. There is typically a marked change in the reduction in diversity of the equine faecal microbiome during colitis. Are the microbiome changes in equine colitis a cause or effect, and is some colitis the result of a 'dysbiosis'? We need to understand whether 'dysbiosis' is a useful concept. Are there rations that predispose some horses to colitis through changes in the large bowel microbiome? Does each specific cause of diarrhoea have a characteristic change in the faecal microbiome, or is the change similar in each case? Are there baseline trends in the faecal microbiome of horses affected by *C. difficile*, *Neorickettsia* spp. and *Salmonella* spp.; that is, are there 'more susceptible' microbiomes and horses? Will individual microbiome characterisation ever be used pre-emptively to identify at-risk horses?

The Workshop attendees agreed that the systematic quest for other 'new' aetiological agents and understanding the pathogenesis of colitis should be the priority of a multinational interdisciplinary research programme. Microbiome analysis based on 16S rRNA analysis currently operates at a genus-based, and indeed somewhat crude level, but NGS studies may be a first step to identify individual pathogens. Recent and ongoing remarkable improvements in culture-based methods are now isolating the majority of intestinal bacteria and are being integrated with metagenomic analysis to identify novel agents. Isolation is revealing greater intestinal microbe diversity than deep sequencing approaches. These powerful techniques could be applied to equine colitis and the hunt for new or previously ignored pathogens. The Workshop agreed that there is a need to create a biobank of colitis samples from multiple locations as part of the search for new agents.

Therapeutic microbiota manipulation, such as faecal microbiota transplant (FMT), has been used with great success in humans and other animal species, although the basis of its efficacy is still not fully agreed. FMT is highly effective in treating chronic *C. difficile* infection in humans. Pilot studies are being undertaken in horses with diarrhoea, and were presented, but well controlled clinical studies are necessary before these interventions can be recommended as a therapeutic option for horses with colitis. We know that the microbiome is considerably affected in cases of

colitis but more research is needed on FMT in colitis. Are there super donors in horses, what is the best protocol for performing the FMT, how should we screen donors, what happens to the patient microbiome after transfaunation, is it of value, does it last?

Diagnosis of acute colitis

Despite significant improvement over the past few years, the aetiology of approximately 50% of cases of colitis worldwide remains undetermined even after extensive diagnostic work up, including post-mortem examination, histopathology, bacterial cultures, PCR, toxicology, etc. Aetiologic diagnosis may be being avoided since it is expensive, but also because some of the data (e.g. *C. perfringens cpa* gene detection) are of unknown but confused significance. In addition, treatment currently depends largely on symptomatic supportive approaches based on clinicopathological findings rather than on any proven targeted interventions. PHF is an exception. It may be judged as better to spend money on treatment than on the high costs of a sometimes uncertain or negative diagnosis. Nevertheless, the absence of reliable (and rapid) diagnosis has very important consequences in limiting understanding of colitis, and its specific prevention and treatment.

There was a clear consensus among workshop participants of the need for a standardised testing protocol, for all known agents, to improve our ability to compare different studies. Multiplex PCR of known pathogens in an 'adult colitis panel' is commonly used for diagnosis of equine enterocolitis. One study identified about a quarter of positive samples with a known pathogen but was further complicated by the identification of a second pathogen in a quarter of these cases, and even a third pathogen in a further small proportion. Some co-infections are likely genuine, especially if there are underlying common predisposing factors such as antimicrobial drug use. However, determining whether what is identified in the faeces is actually the agent of disease rather than an incidental finding is a challenge, especially with highly sensitive PCR-based diagnostics. As noted earlier, there are clearly agents not being assessed in the panels as well as genes, such as the *cpa* toxin gene, that should not be in such panels since *C. perfringens* is quite frequent in healthy adult horses. Aetiological diagnosis of *C. difficile* infections has improved dramatically with the introduction of ELISAs to detect preformed clostridial toxins in colonic contents and faeces and is better than PCR-based approaches that can pick up carriers. The diagnostic PCR panels and other diagnostic approaches should be changed in the light of changing understanding, and effort made to identify their sensitivity and specificity.

Standardisation of *Salmonella* spp. culturing is necessary to assist with comparing data from various geographic locations and studies. For example, the amount of faeces being sampled in *Salmonella* spp. infections is a determinant of the relative sensitivity of the test. Are we missing cases due to our sampling practices? Testing for *Salmonella* spp. is well-described but is also complicated by a lack of clear quantitative analyses in PCR-based diagnosis and the persistence of shedding in healthy horses.

Diagnosis is challenging due to the multiple aetiologies involved. When deciding on an 'ideal test' one must know the prevalence in the healthy horse, the sensitivity and specificity of the test and the clinical value of a positive result. Although these seem straightforward, clinical consequences have been ignored in the literature. Practitioners need to decide both the pretest probability of a positive result and whether that positive result will alter the course of treatment. If the pretest probability is low, it may be best to apply financial resources towards treatment. While understanding that applying all possible tests may not be the best route for diagnosis and treatment of acute colitis, since it may not be cost-effective, it is still important to know when to test. This important question is therefore up to the practitioner and may be as simple as providing the owner with peace of mind or detecting infection by potential zoonotic agents.

Most common causes of colitis produce large bowel lesions which are many times morphologically indistinguishable. Nevertheless, if understanding is to improve, standardised diagnostic protocols are necessary for horses that die or are subjected to euthanasia because of colitis, as well as for clinically ill animals. Currently, the lack of standard protocols for necropsies of horses dying of colitis impedes progress.

Treatment and prevention of acute colitis

The standard care for horses with acute colitis has not been well defined by prospective randomised controlled clinical trials (RCTs). In particular, although flunixin meglumine, polymyxin B, lidocaine and smectite (Biosponge^{TM}) are widely used in the treatment of colitis, their value has not been well established. Why are we still using nonsteroidal anti-inflammatory drugs if they can contribute to colitis? The use of antimicrobials without evidence of sepsis is also controversial, as it could affect commensal bacteria remaining in the gut, worsening intestinal dysbiosis. Should morphine be used to replace the expensive lidocaine for pain control? Is meloxicam less harmful than flunixin meglumine, since it seems to reduce lipopolysaccharide-induced fluid flux when compared to flunixin in vitro and since flunixin can delay tight junction closure? Most of the therapeutics used for horses with acute colitis are based on no studies, or on only a few studies that were either retrospective or used small sample sizes. Regardless of cause, however, treatment of colitis is aimed at improving cardiovascular perfusion, providing oncotic support, addressing endotoxaemia, restoration of the gut microbiome and the prevention of laminitis. Prevention of laminitis through icing feet ('cryotherapy') has been a very important advance in addressing this common complication of colitis.

In the Workshop, one study presented found that the administration of plasma to horses with colitis did not improve survival in a retrospective study, whereas another found that horses administered plasma were more likely to survive than those treated with hetastarch. More work is need in this area. A proposal for a multicentre RCT of the value of hyperimmune plasma was announced at the Workshop, a collaborative venture that the participants hoped would pilot what will become a collaborative standard approach to assembling the number of cases needed for other treatment studies and speeding progress.

Prevention will depend on improved understanding of the agents of colitis, improved understanding of the large intestinal microbiome and how it can be manipulated, as well as the cost-benefit risk and efficacy of immunisation against established pathogens.

The future of colitis research: collaboration

The Workshop revealed how much needs to be done to understand and address acute colitis in adult horses. There are many questions about causes, and about diagnostic and treatment approaches which, given the relatively low case load presenting to individual equine veterinary settings, can best be answered by prospective RCT studies by collaborating centres. Acute colitis is a 'last frontier' in equine medicine but needs systematic study. Expanding understanding of the equine intestinal microbiome in health and disease, with improved culture techniques combined with metagenomics to identify known as well as new pathogens, requires excellent collaborative science, as well as considerable research funding. A critical priority is consensus and consistency on guidelines for infectious agent testing. A common scoring system of colitis severity will allow collaborative prospective comparison of the value of existing or novel treatments between studies in different settings that provide the statistical power to make useful conclusions.

Readers interested in joining the International Equine Colitis Research Group should contact larroyo@uoguelph.ca. We are grateful to the Dorothy Russell Havemeyer Foundation, and to its President Gene Pranzo, for support of this Workshop. A second Havemeyer Workshop on Equine Colitis will be organised for 2022.

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Supporting Information

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Supplementary item 1: International Equine Colitis Research Group Authors.