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Review Article

Equine faecal microbiota transplant: Current knowledge, proposed guidelines and future directions

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Summary

While certainly not a novel concept, faecal microbiota transplant (FMT) has recently garnered renewed interest in veterinary medicine due to its remarkable success in treating recurrent *Clostridium difficile* infection (CDI) in man. There is a dearth of information on indications and efficacy of FMT for the treatment of gastrointestinal disorders in the horse; however, based on evidence in man and other veterinary species, and anecdotal reports in horses, FMT may be a useful treatment for selected cases of acute and chronic diarrhoea and inflammatory bowel disease (IBD) in the horse. In the absence of evidence, expert opinion is offered on case selection and FMT procedure. More research is needed to explore the efficacy, indications and optimal preparation, storage and delivery of FMT to horses.

Introduction

Microbes are crucial to the life of the horse; as a hindgut fermenter, the horse is largely dependent on microbial production of volatile short-chain fatty acids for energy. The microbiota also plays an important role in the development of the mammalian immune system and maintenance of intestinal health by enhancing the intestinal epithelial barrier (Van den Abbeele *et al.* 2011; Kamada *et al.* 2013). New sequencing technologies have advanced our understanding of the complexity, diversity and richness of the equine intestinal and faecal microbiota (Shepherd *et al.* 2012; Dougal *et al.* 2013; Schoster *et al.* 2013; Costa *et al.* 2015a).

The intestinal microbiota of horses has recently been described and is dominated by a few main phyla, particularly Firmicutes. This phylum includes the vast Clostridia class that contains, in addition to a small number of pathogens, various genera associated with gut health (e.g. Lachnospiraceae, Rumincoccaceae and Faecalibacterium). Whilst there is likely a core of genera or species conserved among most or all horses, there are marked differences in composition, even at the class level among intestinal compartments and increasing diversity towards the distal gut (Costa *et al.* 2015a). Even among horses subjected to similar diet and husbandry practices, the intestinal microbiota shows a high degree of individual variation (Dougal *et al.* 2013; Schoster *et al.* 2013). Studies in horses have demonstrated alterations in the faecal microbiota, some even at the phylum level, in disease states including colitis (Costa *et al.* 2012), *post partum* colic (Weese *et al.* 2015), chronic laminitis (Steelman *et al.* 2012) and following antimicrobial administration in healthy horses (Harlow *et al.* 2013; Costa *et al.* 2015b). For example, in horses with undifferentiated colitis, Bacteroidetes was the most abundant phylum compared with Firmicutes in healthy control horses (Costa *et al.* 2012); meanwhile, orally-administered trimethoprim sulfadiazine (TMS) in healthy horses resulted in a drastic decrease in the members of the Verrucomicrobia phylum (Costa *et al.* 2015b). Whilst data implicating alterations of the microbiota as causes of disease are currently much stronger in other species, recent equine studies have provided support to the concept that 'dysbiosis' could be an important inciting cause of various types of disease. With advanced sequencing technologies emerging, one potentially important and yet underexplored area of study involves the therapeutic manipulation of the gastrointestinal (GI) microbiota in equine cases.

The concept of faecal microbiota transplant dates back to use of orally-administered faecal suspensions as a remedy for food poisoning in man during the Dong-jin dynasty in the 4th century in China (Zhang *et al.* 2012). The earliest written reference to FMT or 'transfaunation' in veterinary medicine is attributed to the 17th century Italian anatomist Fabricius Aquapendente who observed that cud taken directly from a healthy animal and placed in the mouth of an animal that had lost its capacity to ruminate would result in restoration of rumination and health (Borody *et al.* 2004). Rumen transfaunation remains a common treatment for a range of GI disorders in cattle (DePeters and George 2014). Whilst there are no peer-reviewed studies of FMT in horses, equine practitioners have a history of providing nasogastric (NG) administration of 'faecal tea' from healthy horses to horses with diarrhoea with anecdotal reports of success (Feary and Hassel 2006). Additionally, there is a multitude of commercial over-the-counter probiotic products for horses used clinically in the prevention and treatment of a variety of GI disorders, attempting to provide a more 'refined' approach to microbiota replacement. While generally regarded as safe, efficacy data are limited and a recent review of probiotic use in horses highlights the need for blinded, placebo-controlled efficacy trials to investigate their health benefits (Schoster *et al.* 2014). It was suggested that research emphasis should be placed on investigating the clinical outcomes related to administration of probiotic products that contain the bacterial species most abundant in the intestinal microbiota of healthy horses, species that tend to be different than those found in commercial probiotics (Schoster *et al.* 2014).

The surge in interest and research on FMT follows reports of notable clinical success in treating recurrent CDI in man (Gough *et al.* 2011; Kassam *et al.* 2013; Cammarota *et al.* 2014). The only randomised-controlled trial (RCT) evaluating FMT was terminated prematurely as the procedure proved to be considerably more effective in treating recurrent CDI than antibiotics alone (van Nood *et al.* 2013). A total of 15 of 16 patients who underwent FMT were cured; 13 patients after just one duodenal infusion of donor faeces compared with 4 of 13 patients who received the standard vancomycin treatment. Those successfully cured with FMT demonstrated increased microbiota diversity similar to that of the donor following treatment (van Nood *et al.* 2013). Although this trial included only a small number of patients, it generated tremendous interest in FMT in both the scientific community and popular press. With the success of FMT in treating recurrent CDI and emerging evidence of efficacy in other inflammatory GI conditions, such as severe CDI in immunocompromised patients (Kelly *et al.* 2014), severe CDI refractory to conventional medical therapy (Zainah *et al.* 2015) and ulcerative colitis (Colman and Rubin 2014), its utility in treating other GI and non-GI disorders, is being actively investigated (Aroniadis and Brandt 2013). However, treatment for CDI is currently the only approved use of FMT in man. The purpose of this paper is to review the current literature on FMT and transfaunation in man and animals, propose some initial guidelines for its application in equine medicine and outline areas for future study.

Potential mechanisms of action of faecal microbiota transplant to treat *Clostridium difficile* infection

Recent evidence indicates that the microorganisms that make up the intestinal microbiota are integrally involved in host homeostasis and alterations have been associated with a variety of disease processes in man, including many not previously associated with the gut or an infectious aetiology (e.g. chronic fatigue syndrome, autoimmune and neurological disorders, atherosclerosis and obesity) (Borody and Khortus 2012; Aroniadis and Brandt 2013; Smits *et al.* 2013). Much attention has been paid recently to the potential impacts of the microbiota on allergic and inflammatory conditions beyond the gut as mounting evidence clearly illustrates the role of the gut microbiota in systemic inflammation and immune tolerance (Van den Abbeele *et al.* 2011; Kamada *et al.* 2013). Studies in germ-free mice demonstrate that intestinal microbiota plays a vital role in physiological intestinal peristalsis, intestinal epithelial cell functions, development of the gut-associated immune system, systemic immunity, nutrition and metabolism (Frick and Autenrieth 2013). The GI microbiota produces antimicrobial products, competes directly for nutrients with pathogens, inhibits or inactivates bacterial toxins and produces bacteriocins and short-chain fatty acids that inhibit growth of pathogens and pathobionts (Kamada *et al.* 2013). The microbiota has also been shown to modify virulence factor expression of pathogens and facilitate host barrier function through upregulation of mucus production, antimicrobial molecules and secretion of IgA (Kamada *et al.* 2013).

Disruption of existing microbial communities has been implicated in the pathophysiology of CDI and may be an important factor in acute, undifferentiated and antibiotic-induced colitis in horses. *Clostridium difficile* is a normal inhabitant of the large intestine of a small percentage of healthy horses and man (Schoster *et al.* 2012; Petrof and Khortus 2014). In most colonised individuals, toxigenic strains of *C. difficile* do not produce disease, likely through inhibitory effects of the protective commensal microbiota. However, alteration of this complex balance can result in an environment where *C. difficile* can proliferate, produce toxins and cause disease. Antimicrobial administration has a profound and prolonged impact on the normal intestinal microbiota in horses and man (Jakobsson *et al.* 2010; Stevens *et al.* 2011; Harlow *et al.* 2013; Modi *et al.* 2014; Costa *et al.* 2015b) and has been suggested as a risk factor for CDI in both species (Båverud *et al.* 1997; Barr *et al.* 2013; Petrof and Khortus 2014). High throughput sequencing demonstrated that TMS administered per os to healthy horses had a greater effect than procaine penicillin intramuscularly (i.m.) or ceftiofur i.m. on the intestinal microbiota, with impacts on the faecal microbiota community structure persisting 25 days after the end of treatment (study endpoint) for all antibiotic-treated horses (Costa *et al.* 2015b). Other risk factors for CDI in horses include stressors such as transportation, hospitalisation, presurgical fasting, medical, or surgical treatment of GI or other disorders (Båverud *et al.* 1997). In man, a similar range of factors is associated with increased risk of CDI, including a recent focus on the potential role of proton pump inhibitors (PPIs), a class of medication also commonly used in horses, as a risk factor (Barletta and Sclar 2014).

Clostridium difficile causes infection by production of toxins that destroy the intestinal epithelium leading to severe inflammation and secretory diarrhoea (Rupnik *et al.* 2009). When conventional treatment (e.g. cessation of the inciting antimicrobial and use of antibiotics against *C. difficile*) fails, FMT may be an effective means of restoring the normal intestinal microbiota and treating CDI, particularly recurrent CDI. Potential mechanisms of action of FMT include competition for limited resources, direct elimination of *C. difficile*, interference with its pathogenicity by microbial products that neutralise toxins, restoration of secondary bile acid metabolism in colon and induction of immune-mediated resistance (Britton and Young 2014; Petrof and Khortus 2014). Some studies indicate that human patients receiving FMT for treatment of recurrent CDI commonly have clinical resolution of diarrhoea before they have evidence of faecal microbiota recovery (Khoruts *et al.* 2010; van Nood *et al.* 2013; Song *et al.* 2013) suggesting that the resolution of diarrhoea is related to factors other than full restoration of gut microbiota as represented by the faecal microbiota (a reasonable but incomplete proxy for the proximal intestinal tract). If stable engraftment of transplanted microbes occurs, it may take some time to achieve. Alternatively, the key for clinical resolution may not be development of an overall microbiota akin to the donor but restoration of key components (e.g. Lachnospiraceae). The potential mechanisms by which FMT improves outcome in other GI and non-GI disorders are being actively investigated, as is the determination of the direction of causality and reversibility of these conditions (Aroniadis and Brandt 2013; Smits *et al.* 2013; Khoruts and Weingarden 2014).

Faecal microbiota transplant in man

Faecal enemas were used infrequently starting in the 1950s for treatment of pseudomembranous enterocolitis, a condition now thought to be associated with CDI (Khoruts and Weingarden 2014). Earliest procedures were nonstandardised and delivered via enema, colonoscopy, nasoduodenal or nasogastric tube (Gough *et al.* 2011; Aroniadis and Brandt 2013). Standardisation and cryopreservation protocols have since been described (Hamilton *et al.* 2012; Petrof and Khortus 2014; Satokari *et al.* 2015). However, the need for cryopreservatives is unclear and a randomised clinical trial in man comparing fresh vs. frozen (without cryopreservative) stool showed lack of inferiority of frozen stool (S. Weese, unpublished data). Typically, each FMT dose is from a single donor to limit risk of disease transmission (Khoruts and Weingarden 2014). Donor screening tends to be intensive and expensive to reduce the risk of transmission of enteric or bloodborne pathogens or infusion of antigens (**Table 1**). These guidelines are not strictly evidence-based and optimal practices are unknown (Allen-Vercoe *et al.* 2012). In addition, research is needed to explore the potential transfer of antimicrobial resistance genes on plasmids or in the genome of donated bacteria (Dicks *et al.* 2014).

The impressive efficacy of FMT in achieving clinical cure of recurrent CDI in man has been systematically reviewed (Gough *et al.* 2011; Kassam *et al.* 2013; Cammarota *et al.* 2014). In 20 case series, 15 case reports and one RCT, 467 of 536 (87%) patients treated experienced resolution of diarrhoea (Cammarota *et al.* 2014). Resolution rates varied by site of infusion and were highest in caecum or ascending colon (93%). Interestingly, there are very few reports of complications although patients receiving FMT are often debilitated, elderly or immunocompromised with multiple systemic problems and disruption of the gut mucosal barrier (Khoruts and Weingarden 2014). Complications reported possibly associated with upper GI FMT delivery include upper GI bleeding, enteritis and peritonitis; no mortality associated with FMT was reported in 11 studies of FMT for CDI (Kassam *et al.* 2013). No severe adverse events directly attributed to FMT were reported in the studies in other reviews (Gough *et al.* 2011; Cammarota *et al.* 2014) and there was no difference in clinical outcomes in anonymous vs. patient selected donors (Kassam *et al.* 2013).

On the other hand, FMT for treatment of IBD has had more mixed results. In meta-analysis of 9 cohort studies, 8 case studies and one RCT, 54 of 119 (45%) patients achieved clinical cure and in the 6 studies where the microbiota was evaluated pre- and post FMT, there were variable associations with clinical response (Colman and Rubin 2014). Additional work is needed to define the case selection, timing and utility of FMT for treatment of IBD.

The American College of Gastroenterology recommends physicians consider FMT for a third recurrence of CDI nonresponsive to metronidazole or vancomycin (Surawicz *et al.* 2013). Nonprofit stool banks and universities have established donor screening and sample storage capabilities to serve physicians unable to perform donor selection and screening (Kelly 2014). A standardised microbiota suspension derived from fresh stool received United States Food and Drug Administration (FDA) fast-track status; phase 2 safety data showed no major adverse effects and an overall efficacy of 87% (Dubberke *et al.* 2014).

The FMT procedure is regulated by the FDA in the United States. In April 2013, the FDA moved to regulate FMT as an unapproved drug requiring all procedures and clinical trials to receive Investigational New Drug (IND) approval (Anon 2013a). Objection from the medical community resulted in the FDA adopting an interim policy of 'enforcement discretion' which allows clinicians to perform FMT for CDI not responsive to standard therapies without obtaining IND approval provided adequate informed consent is obtained (Anon 2013b). Faecal microbiota transplant for other indications including IBD, irritable bowel syndrome, metabolic syndrome, autoimmunity and autism requires IND approval. Transfaunation and FMT in animals are not currently regulated.

Rumen transfaunation

Rumen transfaunation has been recently reviewed (DePeters and George 2014). Early investigations of the effects of cud inoculation in calves revealed that their rumens contained bacteria and protozoa at 3 weeks of age compared with noninoculated calves whose rumens only contained bacteria (Pounden and Hibbs 1948). Rumen fluid from an alfalfa-fed steer transferred into a protozoa-free sheep-fed alfalfa resulted in establishment of all 24 species of protozoa within the sheep rumen (Dehority 1978), indicating that protozoa can successfully be transferred between host and recipient. While rumen fluid is known to contain viable microbes, volatile fatty acids, bicarbonate buffers and proteins, there are many yet unidentified components (DePeters and George 2014). The complexity of the rumen fluid milieu is now being explored via metabolomics analysis (Saleem *et al.* 2012; Zhao *et al.* 2014).

Rumen transfaunation remains a common treatment for indigestion in domestic ruminants to improve rumen function, intake and milk production following surgical correction of left displaced abomasum in dairy cattle, as well as to provide unique microorganisms capable of degrading plant toxins in exposed ruminants (Rager *et al.* 2004; Jasmin *et al.* 2011; DePeters and George 2014). Rumen transfaunation improved health and survival of calves in a herd experiencing bloody diarrhoea and death in preweaned calves (Pounden and Hibbs 1949). In our hospitals (T.J.D. and J.S.W.), we have for many years routinely used fresh rumen transfaunation as treatment for a large number of metabolic and infectious conditions in cattle exhibiting anorexia and those that have undergone abdominal surgery. Recently, Jing *et al.* (2014) reported that systemically administered endotoxin will alter the rumen microbiota. It is as yet unknown if transfaunation can restore the perturbations in rumen microbiota caused by systemic endotoxin; however, our clinical impression that transfaunation improves appetite and outcome in cows with toxic mastitis supports this premise.

Microbiota transplant in other veterinary species

In an early study on the effects of commensals on disease resistance in broilers, chicks inoculated with ingesta from adult roosters placed directly in the chicks' crops had fewer numbers of *Salmonella infantis* isolated and there were fewer carrier animals than noninoculated controls (Nurmi and Rantala 1972). With restrictions on use of antimicrobials in production animals, there is interest in alternative means of

TABLE 1: Donor exclusion criteria for faecal microbiota transplant donors in man

History and physical examination	
Risk of infectious agents	• Known HIV or viral hepatitis exposure • High risk sexual behaviours • History of incarceration • Use of illicit drugs • Tattoo or body piercing within 12 months • Travel history to endemic regions with a high risk of acquiring infectious pathogens • Current communicable disease or history of tropical disease • Other infectious disease risk factors including Creutzfeldt-Jakob disease
Gastrointestinal comorbidities	• History of irritable bowel syndrome or associated symptoms • History of inflammatory bowel disease including Crohn's disease, ulcerative colitis and lymphocytic colitis • Chronic diarrhoea • Chronic constipation or use of laxatives • History of GI malignancy or known colon polyposis • History of any abdominal surgery • Use of probiotics or other over-the-counter aids for regulating digestion • Family history of IBD, colon cancer
Systemic medical conditions	• Established metabolic syndrome or body mass index/waste:hip ratio suggestive of its emergence • Known systemic autoimmunity • Known atopic diseases • Chronic pain syndromes • Ongoing/intermittent use of any prescribed medications • Neurological, neurodevelopmental and neurodegenerative disorders • Psychiatric conditions • Surgeries or other medical conditions • Abnormal physical examination findings • Family history of disease
Additional factors known to affect intestinal microbiota	• Antibiotics for any indication within the preceding 6 months • Antivirals, antifungals, immunosuppressants
Laboratory screening	
Blood tests/serology	• HIV • Hepatitis A, B, C • Treponema pallidum • T lymphotropic virus in man • Complete blood count • Hepatic function panel • Serum triglycerides, HDL cholesterol, high sensitivity C reactive protein, fasting glucose • Fluorescent antinuclear antibody test
Stool testing	• Clostridium difficile toxin B • Culture for enteric pathogens (including *Salmonella, Shigella, Yersinia, Campylobacter, E. coli O157:H7, Vibrio*) • Ova and parasites including *Isospora* • *Helicobacter pylori*, *Giardia lamblia* and *Cryptosporidium* • Cyclospora, microsporidia • Norovirus, rotavirus and adenovirus • Vancomycin-resistant enterococcus

From Hamilton *et al.* (2012) and Anon (2015).

reducing food-borne pathogens. Piglets fed spent cider yeast had fewer *Salmonella* and *Escherichia* bacteria in faecal samples than control animals, which suggest that probiotics can potentially alter the gut microbiota and reduce pathogen loads (Upadrasta *et al.* 2013). There has also been recent interest in FMT in dogs and cats as a treatment for chronic diarrhoea. Anecdotal data currently dominate scientific study, although preliminary studies of efficacy and

safety have been previously reported (Weese *et al.* 2013; Murphy *et al.* 2014).

Equine faecal microbiota transplant

As in other neonates, foals' gut microbiota changes dramatically over time. Foals practice coprophagia from their dams and the environment which is thought to be a part of their normal development (Francis-Smith and Wood-Gush 1977). Foals pastured with their dams and other mares and foals were observed to ingest manure from their own dams and not from other mares or foals, immediately after defaecation, between the ages of 2–5 weeks and not before or after, suggesting an evolutionary adaptation for efficient inoculation of the GI tract (Francis-Smith and Wood-Gush 1977). Between Days 2 and 30 of age, increases in bacterial diversity and changes in relative abundance of the foals' faecal microbiota were noted (Bordin *et al.* 2013). By Day 30, foals had developed a faecal microbiota that remained stable for the remainder of the first year of life (Faubladier *et al.* 2014).

While this change in the microbiota over time is considered part of the normal developmental process, it is evident that certain disease processes, management practices and medications can alter the equine gut microbiota. For example, in adult horses, undifferentiated colitis, grass sickness, *post partum* colic, acute and chronic laminitis, simple obstruction colic, diet change and supplementation and as noted above antimicrobial administration, have also been associated with changes in the equine intestinal or faecal microbiota (Garrett *et al.* 2002; Willing *et al.* 2009; Grønvold *et al.* 2010; Costa *et al.* 2012, 2015b; Daly *et al.* 2012; Steelman *et al.* 2012; Harlow *et al.* 2013; Dougal *et al.* 2014; Fernandes *et al.* 2014; Moreau *et al.* 2014; Proudman *et al.* 2015; Weese *et al.* 2015).

Extrapolation of data from man and cattle is difficult because of the differences in targeted diseases and GI anatomy. As hindgut fermenters, horses have an intestinal tract profoundly different from man and ruminants. The types of chronic disease usually targeted by FMT in those species also have little relationship to important problems in horses. Due to the long small colon in the horse, FMT via enema may not be effective and to the authors' knowledge has not been investigated. Faecal microbiota transplant in horses has thus far been limited to administration via NG intubation. Efficacy of this route may be reduced due to bacterial inhibition and degradation by gastric acid, small intestinal enzymatic digestion and competition and degradation in the caecum due to fermentation. Therefore, while the clear impact of FMT in man and rumen transfaunation provides proof of concept, equine specific study is required. In a small case series, 3 out 4 horses with antibiotic-induced or undifferentiated colitis had an improvement in faecal consistency following treatment with FMT (Mullen *et al.* 2014). One author (T.J.D.) used fresh caecal transfaunation successfully in a horse with severe, subacute CDI, with normal manure consistency returning 12 h post treatment. Chronic diarrhoea, although uncommon in the horse, has anecdotally responded to treatment with FMT (Feary and Hassel 2006; McGovern 2013). Caecal contents collected from a recently deceased horse or faeces obtained from rectal evacuation of a healthy horse provided the transfaunate (Feary and Hassel 2006). These findings do provide initial support to the potential efficacy of FMT in horses; however, the variable and sometimes self-limiting nature of GI disease precludes a definitive assessment of efficacy from studies such as these.

Equine studies are needed to develop optimal practices, including transplant material type, donor characteristics, donor screening, transplantation methods and diseases to target. Antibiotic-induced and undifferentiated colitis and IBD, which has been associated with changes in the gut microbiota in man (Aroniadis and Brandt 2013; Smits *et al.* 2013) and dogs (Minamoto *et al.* 2015), may be potential candidates for additional FMT research in horses. Yasuda *et al.* (2015) demonstrated differences in the composition of the luminal and mucosal GI microbiota in rhesus macaques and preliminary results show a difference between the composition of the equine GI luminal and mucosal microbiota (K. Yasuda, unpublished data). As diseases such as IBD may be associated with perturbations in the mucosal microbiota, developing preparations to restore the mucosal microbiota health in horses with IBD might be helpful in ameliorating clinical signs. Based on findings in mice where intestinal microflora promotes peristalsis (Husebye *et al.* 1994), duodenitis/proximal jejunitis (DPJ) and ileus may be disorders that would benefit from FMT treatment, but further research in horses is needed.

There are no studies evaluating the use of FMT for treatment of diarrhoea in foals. Unlike in man, recurrent CDI is uncommon in the horse, with most equine CDI cases presenting as severe, acute enterocolitis. Moreover, acute, undifferentiated colitis is more common than CDI in horses. Regardless of the indication, administration of FMT may present a risk of donor-associated bacteraemia, although this treatment has been used in human patients with compromised mucosal barrier function and concurrent treatment with immunosuppressive medications without complications (Khoruts and Weingarden 2014) and infusion of a small volume of faeces relative to the amount of intestinal content already present would seem to be of limited additional risk. Additionally, restoration of normal commensals may actually improve IL-1β recruitment of neutrophils to the gut mucosa and protect against septic complications (Hasegawa *et al.* 2012).

A standard protocol for FMT in horses has not been developed. Preliminary guidelines based on limited data, extrapolation from the literature in man and expert opinions are provided (**Table 2**). Although adverse events associated with FMT in horses have not been reported, disease transmission from donor to recipient is possible (Naylor and Dunkel 2009) and proposed screening tests for donors are provided in **Table 2**. For foals undergoing FMT, the dam would make a suitable donor provided the screening criteria are met. Fresh faeces should be collected from the rectum of the screened donor, or caecal contents could be collected from horses that fit the inclusion criteria above and which are to be subjected to euthanasia because of an acute, noninfectious and non-GI disease event (e.g. acute, catastrophic musculoskeletal injury). Studies comparing the microbiota of the caecum and faeces have had variable results with some studies suggesting significant differences in population structure between the two (Dougal *et al.* 2012; Costa *et al.* 2015a) and others finding the highest similarity between the composition of the microbiota in the faeces and caecum vs. other intestinal compartments (Schoster *et al.* 2013). However, there are no data to suggest whether

TABLE 2: Equine faecal and caecal microbiota transplant guidelines

Guideline	Evidence
1. Informed consent from owner • Investigational therapy with potential risks including disease transfer, transfer of antimicrobial resistance and peritonitis	• FDA currently exercises enforcement discretion for FMT permitting physicians to treat human patients with recurrent CDI provided informed consent is obtained (Anon 2013b) • FMT in veterinary species is not currently regulated. • Complications are rare (Kassam *et al.* 2013; Dicks *et al.* 2014)
2. Patient selection criteria • Chronic diarrhoea • Antibiotic-induced colitis • Acute-severe colitis • IBD • Possibly duodenitis/proximal jejunitis	• Anecdotal for chronic diarrhoea in horses (Feary and Hassel 2006) • Minimal for acute-severe and antibiotic induced colitis in horses (Mullen *et al.* 2014) • Moderate efficacy for IBD in man (Colman and Rubin 2014) • No studies evaluating FMT for IBD or proximal duodenitis/proximal jejunitis in horses. Further study is needed before recommending treatments
3. Patient preparation • Discontinuation of antimicrobials • Pretreatment with PPIs	• Standard protocol for man (Hamilton *et al.* 2012) • No evidence-based information in horses
4. Donor selection criteria • Healthy • No antimicrobials or other medications in past 6 months • Screened for Equine infectious anaemia virus, *Salmonella* spp., GI parasites and equine coronavirus • Forage-based diet • Ideally housed in pasture environment and from same herd/facility as recipient	• Evidence for antimicrobials disrupting equine gut microbiota (Harlow *et al.* 2013; Costa *et al.* 2015b). • Six months withdrawal period is recommendation for donors in man (Hamilton *et al.* 2012) • Evidence for forage fed horses having different microbiome than concentrate fed horses (Dougal *et al.* 2014) • Evidence that Teaching Hospital resident horses have different microbiome than horses living on farms (Costa *et al.* 2012)
5. Preparation • Rectal evacuation of manure or harvest of caecal contents immediately post euthanasia • Mix with warm water or isotonic saline • Blend mixture to capture cellulolytic bacteria on long fibres • Strain mixture	• Authors' experience
6. Administration • Administer 2–3 l via nasogastric tube for average adult horse; 200 ml for foal • When possible, offer free choice long stem early cut hay following FMT • Repeat daily until improvement in faecal consistency or up to 3 days	• Authors' experience
7. Storage • Room temperature in an airtight container for short-term storage (hours or less while recipient is being prepared for the procedure) • At -20°C for long-term storage	• Authors' experience with noncryopreserved equine FMT • Frozen, cryopreserved human FMT had similar efficacy and safety to fresh FMT (Hamilton *et al.* 2012; Satokari *et al.* 2015) • Effect of freezing equine FMT has not been evaluated

caecal contents or faeces constitute a preferred transfaunate.

The processing and delivery of FMT is shown (**Fig 1**). In our hospitals (K.R.M., T.J.D., and J.S.W.), we have utilised frozen aliquots of FMT without cryopreservation. However, we simply do not know the impact of storage temperature and time on efficacy and in the absence of evidence fresh FMT is probably best. Discontinuation of antimicrobials before FMT as well as pretreatment with PPIs, similar to the human protocol, have been recommended to diminish antimicrobial and gastric acid-induced bacterial inhibition, but remains speculative (Feary and Hassel 2006). Following omeprazole 4 mg/kg bwt per os every 24 h, intragastric pH in clinically ill and clinically normal neonatal foals and healthy adult horses was significantly increased by 1, 2 and 48 h (first time point measured), respectively (Merritt *et al.* 2003; Sanchez *et al.*

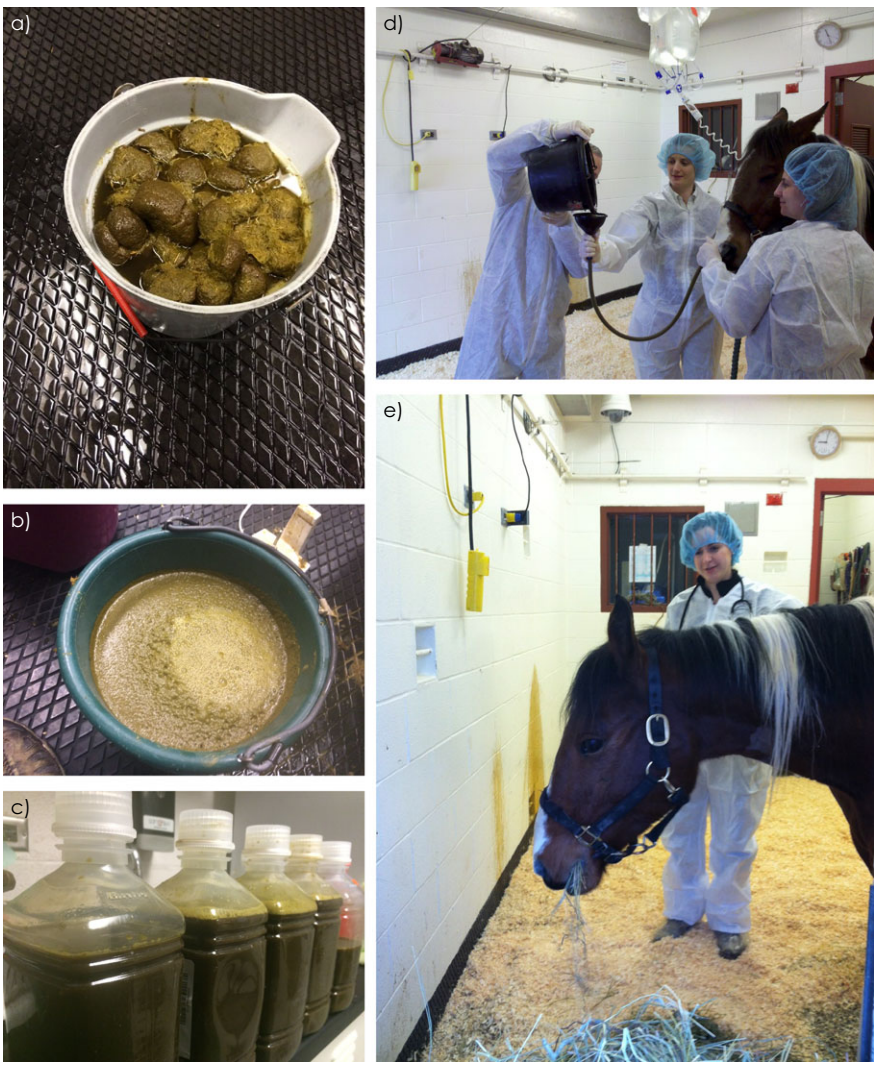

Fig 1: a) Faeces obtained by rectal evacuation or caecal contents obtained immediately post mortem from a healthy donor are combined with warm water or isotonic saline. b) Using an immersion blender, cellulolytic bacteria are released from long fibres. c) The mixture is passed through a wire mesh strainer and liquid collected in clean plastic bottles for fresh administration or frozen for administration later. Frozen preparation is thawed in warm water bath before administration. d) An average-sized adult recipient is administered 2–3 l FMT via nasogastric tube followed by 2 l warm water. e) Recipient is offered long stem first cutting grass hay.

2004; Javsicas and Sanchez 2008), suggesting that only a short pretreatment period with a PPI is needed to increase intragastric pH before FMT in horses. The optimal volume of FMT has not been determined. Typical dosage is provided in **Table 2.**

Future of FMT

Research is needed to determine the core microbiota required for equine GI health. It is likely that more important than achieving stable engraftment of the host microbiota in the recipient is establishing some key populations (e.g. Lachnospiraceae, Ruminococcaceae and perhaps other butyrate or acetate producers). The best way to deliver these bacteria (FMT, probiotic products or stool substitutes) needs to be evaluated. The use of shelf-stable stool substitutes made from purified isolates from a healthy donor (Petrof *et al.* 2013) would eliminate the need for donor screening and could improve accessibility and our ability to perform controlled efficacy studies. Research is needed to investigate the efficacy of FMT/stool substitutes for treatment of specific equine GI diseases including acute and chronic diarrhoea, IBD, DPJ and post foaling colic and for prophylactic use in, for instance, horses undergoing surgical procedures, horses being treated with antimicrobials and *post partum* mares. With the recent developments in understanding how the microbiota changes in various disease states, therapies aimed at microbiota restoration may represent the next frontier in equine gastroenterology.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

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Authorship

All authors contributed to the review article contents including the design, execution, interpretation and manuscript preparation. The original draft of this review manuscript was prepared by K. Mullen. All authors reviewed, commented and approved the final manuscript.

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