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# Small Things

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# Free Faecal Water: What do we know and can equine faecal microbiota transplantation be used to manage this issue?

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## Introduction

Free Faecal Water (FFW) is a condition recognised in horses. Horses with this condition defaecate normal faeces but before, after, or during defaecation (or sometimes even independently of defaecation) faecal water runs out of the anus (Kienzle et al. 2016). This condition is also often referred to as Faecal Water Syndrome or Free Faecal Liquid. Cases are normally isolated, with only one horse from a group or stable yard being affected. In general, horses often seem to be in good general health and do not appear to be affected negatively by the condition. However, FFW can be a real management issue to the owner who is involved in the daily care of the horse. For example, daily cleaning of the tail and legs of the horse may be required depending on frequency and amount of the faecal water produced. In some cases, the faecal water can cause skin irritation and skin lesions, subsequently affecting the horse's welfare.

FFW is recognized in horses of all breeds, including warm bloods and painted horses. No official information is available on prevalence of FFW, but the condition is seen frequently by equine veterinarians in Western Europe. Unfortunately, very little information on FFW is published (Ertelt and Gehlen 2015; Kienzle et al. 2016; Valle et al. 2013).

Many different causes have been implicated in the development of FFW (see below), and several treatment options for FFW have been suggested or applied by equine practitioners. The limited published information on FFW prevents evidence-based conclusions with respect to its aetiology and the objective evaluation of treatment. This paper provides an overview of the current state of knowledge regarding the aetiology and potential management and treatment options associated with FFW. This paper was written as a

starting point for a multidisciplinary discussion at the 9th European Equine Health & Nutrition Congress. The paper includes empirical information from the authors in terms of dealing with FFW cases, as well as some preliminary results of their clinical studies.

## Aetiology

*Diet*

Anecdotally, it has been suggested that FFW may be caused by nutritional factors. For example, increased amounts of alfalfa hay or feeding wrapped forages (such as haylage or silage) have been associated with an increased risk for developing FFW. In a study describing the effects of an abrupt change from hay to grass silage and haylage, the dry matter content of faeces was reported to decrease (Muhonen et al. 2009). However, the authors did not mention if this was seen as FFW or as softer faeces in general. Recently, an abstract was presented at the European Workshop on Equine Nutrition sharing the results of a survey on feeding routines and feedstuffs in horses with FFW (Lindroth et al. 2018). The majority of the horses included in the survey (57%) showed signs of FFW while fed wrapped forages, but not when fed hay only. Nevertheless, 20% of horses in the survey did show signs of FFW when fed hay only, demonstrating that forage type fed is not the only cause of FFW. The authors of the survey also stated that 48% of horses with FFW improved when turned out on pasture (Lindroth et al. 2018). Interestingly, this is in contrast to the findings of an earlier study in which horses kept on pasture full-time suffered from FFW more often (Ertelt and Gehlen 2015). Drinking cold water was also suggested to play a role in this increased risk of FFW (Ertelt and Gehlen 2015).

*Season*

Horse owners questioned in a survey indicated that in 47.5% of cases episodes of FFW were not related to the season of the year (Kienzle et al. 2016). Thirty-one per cent of cases occurred only in winter months, and 9.5% of horses only showed signs of FFW when grazing spring pastures. About 5% of horses showed episodes of FFW when the weather changed suddenly.

*General health*

Poor dental care and parasitic infections have been suggested to be involved in the development of FFW (Ertelt and Gehlen 2015). However, in a study performed in Germany, 90% of the horses suffering from FFW did not show any improvement after they had their teeth examined by a professional (Kienzle et al. 2016). In the same study, no differences were detected in results from faecal parasitological examinations of horses suffering from FFW compared to healthy horses (Kienzle, Zehnder et al. 2016). FFW occurs more frequently in horses that have experienced an episode of colitis (Ertelt and Gehlen 2015).

*General patient characteristics & social stress*

The sex of the horse was reported in one study to be a risk factor, with a higher risk for FFW associated with geldings (Odds ratio (OR) 3.40: 95% confidence interval (CI): 1.34-8.82)) (Kienzle et al. 2016). In the same study Painted Horses also had a higher risk for FFW (OR 4.11; 95% CI 1.34-12.99). It was speculated that these two risk factors might influence a horse's position in the social hierarchy. Many of the horses suffering from FFW are at the lower end of the social hierarchy and do not defend their feed against other

horses, potentially suffering from social stress (Kienzle et al. 2016). As it is well known that anxiety and stress can lead to an increase in intestinal motility, it is speculated that also gut motility might be involved in the development of FFW.

*The gut*
Water is absorbed mainly in the large intestines of the horse. Changes in the intestinal mucosa, such as a (low grade) chronic inflammation, might reduce the capacity to absorb water from the intestinal contents leading to increased moisture in the faeces. Alternatively, inflammatory changes in the gut may compromise the function of the enteric nervous system thereby affecting motility. Gut motility itself can also potentially affect the amount of fluid in the intestines. Abnormal strong phasic contractions in the large intestines might press out water from the digesta and thereby contribute to FFW. This theory is supported by the finding that the dry matter content of the normal faeces in horses with FFW is increased compared to horses without FFW (Ertelt and Gehlen 2015).

*The gut microbiome*
The gut microbiome plays an important role in equine health and may be altered during disease conditions. For example, the gut microbiome in horses with colitis differs from that observed in healthy horses (Costa et al. 2012). No studies have been published that addressed the gut microbiome of horses with FFW. Recently, some of the authors of this paper compared the faecal microbiome of horses with FFW to a healthy cohort with no FFW (Laustsen et al., unpublished results). No difference in alpha or beta diversity of the faecal bacterial/archaeal microbiota was seen between horses with and without FFW. However, it is unclear if faecal sampling is optimal for studying FFW associated changes within the hindgut and/or whether the microbiota of free faecal liquid itself should be analysed. Furthermore, FFW could be a result of a microbial dysbiosis at the mucosal surface, which would not necessarily be reflected in changes of the microbiota in digesta or faeces. As such, studies investigating the mucosal microbiome and gut tissue pathology of FFW horses may prove insightful. Another important issue potentially affecting the gut microbiome is the use of antimicrobial drugs in horses. This could potentially also contribute or trigger FFW and, therefore, also warrants further investigation.

*Conclusions on aetiology*
The aetiology of FFW seems to be multifactorial and is most likely not the same in all horses affected by FFW. From available scientific sources three general risk factors for FFW may be identified: 1) being a gelding 2) being a Painted Horse and 3) being low in the social hierarchy (Kienzle et al. 2016). As these factors may all contribute to social stress, this may be a key factor in the aetiology of FFW. Studies that address this phenomenon (including accurate objective assessment of stress) are needed to confirm if (social) stress is indeed a key causative factor in many FFW cases.

## Potential management & treatment options

*Nutritional strategies*
It is often speculated that FFW might be caused by nutrition related issues. Therefore, special attention should be paid to proper ration formulation, the feeding regimen and management and adequate provision of forage. Forages and other fibrous feeds may affect the water-holding capacity of the ration. Grass hay for example has the highest water-

holding capacity when compared to alfalfa hay and beet pulp (Parsons et al. 2011). In general, feeding hay results in a higher faecal dry matter content compared to feeding haylage or grass haylage (Muhonen et al. 2009). The effect of pasture access on clinical signs of FFW remains controversial (Lindroth et al. 218; Ertelt and Gehlen 2015). Currently, little evidence based scientific information is available regarding successful nutritional strategies that can be applied to horses with FFW. Nevertheless, making sure horses with FFW are fed a diet high in forage (preferably hay) may be beneficial.

*Dietary supplements*

Several supplements are currently marketed as potentially beneficial in resolving FFW. Only one of these supplements has been studied scientifically (Gerstner and Liesegang 2018). In this study EmendoMOL Plus (a montmorillonite-bentonite, whey and hop/absinthium extract containing product), was administered to eight healthy horses and several faecal parameters (including dry matter) were assessed. No effect of this supplement was observed in the tested horses. However, as the supplement was tested only in healthy horses no conclusions can be made regarding the value of this product for horses with FFW. Several studies have been performed assessing the effect of different probiotic and prebiotic supplements (Schoster et al. 2014). Unfortunately, none have been evaluated for effectiveness specifically in horses with FFW.

*Faecal Microbiota Transplantation (FMT)*

FMT is a procedure by which a faecal suspension is transferred from a healthy donor into the gut of the recipient (the patient). FMT has been used in horses for decades but procedures have been poorly described and studied (Schoster et al. 2014; Mullen et al. 2018; Mullen et al. 2014). In human medicine, FMT has recently gained a lot of attention as a treatment for Clostridium difficile infections and other disorders associated with alterations in the gut microbiota (Cammarota et al. 2017). Changes in the gut microbiota after FMT could be seen for 24 weeks in human patients (Grehan et al. 2010). A recent consensus statement on the use of FMT in human medicine describes treatment protocols for use in human hospitals (Cammarota et al. 2017). To date, no evidence-based protocol for FMT in horses exists. However, a recently published review paper included an expert opinion on equine FMT, and a protocol for FMT in horses was proposed (Mullen et al. 2018). The FMT protocols applied by the authors of this paper are included (See textbox).

Donor selection is likely to be an important aspect for the success of FMT. The donor should be free of disease (especially gastro-intestinal disease) and ideally not have been treated at all with antimicrobials (or at least not in the last 6 months). Also, in order to avoid iatrogenic infections, the donor should test negative for Salmonella, Equine Infectious Anaemia, Equine Corona Virus and be parasite free. No information is available on compatibility of donor and recipient horse, although one can anticipate that this might be crucial. For example, horses lacking a certain class or order of bacteria might benefit more when FMT is performed with faeces from a horse that has a high abundance of that particular class or order of bacteria. However, it is worth noting that the use of two different donors did not significantly affect the clinical outcome of the FMT treatment when applied to horses suffering from FFW (Laustsen et al. 2018), although it should be noted the number of horses included in this study was limited (10 horses with FFW, two donors). More FMT research involving horses is needed in order to provide better insight into this topic.

Several controversies exist in the application of FMT as part of a treatment plan. Many horses in which FMT might be considered are suffering from profuse diarrhoea. Although antimicrobial treatment is not indicated in this type of cases as standard treatment, quite often these horses also show signs of systemic sepsis, which does require antimicrobial therapy. The question is how does this affect FMT? Should antimicrobial therapy be discontinued when FMT is applied? Or should FMT be postponed until antimicrobial therapy can be stopped? There are also some researchers who argue in favour of the use of antimicrobials prior to FMT, to diminish resident gut microbiota and increase the chances for microbiota from the transplant to colonize. Concurrent use of gastric acid inhibitors seems useful to minimize microbial inactivation by the gastric acid, and thereby increase the amount of transplanted viable microbes reaching the hindgut. However, some veterinarians consider it important to keep this gastric acid barrier intact, preventing pathogenic bacteria from reaching the already compromised gut.

No scientific studies have been performed to evaluate safety and side effects of FMT in horses. To date, the authors of this paper have not seen any negative side effects in patients treated by FMT. Although transmission of disease may be a potential risk factor that should be discussed with the owner prior to treatment.

The efficacy of FMT as treatment for horses with FFW has been investigated recently (Laustsen et al. 2018). Faecal consistency of horses with FFW significantly improved after 14 days post FMT, and remained significantly better for the entire study period (164 days). However, it should be noted that not all patients responded similarly in terms of the extent and duration of improvement. Preliminary results from an on-going study at Utrecht University also show a positive effect of FMT in horses with FFW (Theelen et al., unpublished results). All horses with FFW that were treated with FMT (n=14) showed improvement of faecal consistency at day 3 of treatment. Long-term positive effects (> 2months) were seen in 50% of patients (n=6/12) for which follow up information was available. These findings support the use of FMT in treatment of horses with FFW.

**FMT protocol Utrecht University - The Netherlands**
At Utrecht University, 500 grams of fresh faeces (collected rectally) from the donor horse (which is Salmonella free, parasite free, EIA free and not treated with antimicrobials for a minimum of 6 months) is used for FMT. The fresh faeces is mixed into 2 litres of lukewarm water (ideally 37°C) and then filtered by use of a sieve to remove large particles that may potentially clog the nasogastric tube during the administration of the transplant. After this, 100 grams of grass pellets are added to the remaining fluid as a substrate for the microbes. When the grass pellets are completely in suspension, the transplant is ready to be administered to the patient by nasogastric intubation. After administration of the transplant, the horse also receives extra lukewarm water (1 litre per 100kg of BW) by nasogastric intubation. This is done to decrease gastric transit time and thereby increase the viability of microbes in the transplant that progress to the intestines. The use of omeprazole (to decrease gastric acid and minimize killing of transplanted microbes in the stomach) is optional, depending on the individual patient's health status and preference of the treating clinician. This procedure is repeated 3 times with 24-hour intervals.

**FMT protocol Hestedoktoren - Denmark**
This protocol is used at Hestedoktoren in Denmark and was also used in a recent study on the effect of FMT in horses with FFW (Laustsen et al. 2018). All horses that undergo FMT are treated with omeprazole (4mg/kg SID PO) 5 days prior to the first FMT, and continuing until the last FMT has been performed. All horses receive FMT for five consecutive days. Fresh faeces (500 grams) is collected rectally from the donor horse and mixed with 5 litres of saline. The transplant is then passed through a sieve to separate out the largest particles. The transplant is than administered to the patient by nasogastric intubation, followed by 0,5-1 g/kg BW of psyllium diluted in 3-5 litres of tap water. If required, horses are sedated with detomidine (0.01mg/kg) prior to nasogastric intubation.

## Practical approach to manage/ treat horses with FFW

When confronted with a horse with FFW it is important to take an extensive history to identify potential underlying problems. Special attention should be paid to feeding regimen, feeding management and potential (social) stress factors. Furthermore, the horse should undergo a thorough clinical exam. Underlying gastro-intestinal disorders should be excluded. Therefore, it is advisable to perform a parasitological examination of the faeces and also perform X-rays of the abdomen to exclude sand accumulation in horses with FFW. Performing a gastroscopy to rule out gastric ulcers is recommended. Special attention should be paid to excluding (low grade) inflammation of (part of) the intestines.

Intestinal inflammation can be quite complicated to diagnose. Hypoproteinemia and/or hypoalbuminaemia (usually mild), thickened intestinal walls on rectal examination and/or abdominal ultrasound (>3mm), an abnormal result of an oral glucose absorption test (decreased glucose uptake) and the presence of inflammatory cells in duodenal and/or rectal biopsies are all findings compatible with intestinal inflammatory conditions (Boshuizen et al. 2018). Bacteriological evaluation of a faecal sample might also be useful to rule out well-known pathogens, such as Salmonella and Clostridium perfringens/difficile. Currently, DNA sequencing-based determination of equine faecal

microbiota composition is only done for research purposes. This might, however, change in the near future, with commercial laboratories already offering this service to the general public for the analysis of human faecal samples.

When no underlying diseases are diagnosed and no feeding or management issues can be detected, or correction of these issues does not lead to resolving the FFW problem, empirical treatment can be considered. Based on author experience and supported by the results of a recent survey (Lindroth et al. 2018), suggesting a dietary adjustment to the owner (e.g. hay instead of silage or haylage, increasing the amount of forage or limiting pasture access) and re-evaluation of the clinical situation several weeks later can be helpful. In horses that are (suspected to be) suffering from (mild) chronic enteritis/colitis, prednisolone (1mg/kg SID PO) or dexamethasone (0,06mg/kg SID IM/IV) can sometimes be successful in resolving FFW. Sulfalazine (30mg/kg TID PO) may also be another potential treatment option if these horses are unresponsive to corticosteroid therapy. Finally, FMT can be considered as a safe option for treatment, albeit to date with varying success.

## Future directions for research

Studies that address potential factors involved in the aetiology of FFW and evaluate the effectiveness of different nutritional strategies, supplements and potential other treatment options are indicated. Studies evaluating the efficacy and safety of FMT in horses (with FFW or other conditions) would also be of interest to clinicians treating horses with different (gastro-intestinal) disorders. Until the aetiology and pathophysiology of FFW is fully understood, we are more likely managing symptoms rather than curing the actual condition. With the current rapid development of molecular techniques, it is likely that studies addressing the (faecal) microbiota composition and associated functional capacity of FFW horses may help to advance understanding of the aetiology and pathophysiology of this condition. Potentially, this may also facilitate the development of diagnostic or screening tools that can be applied to horses with FFW.

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