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NATURALLY OCCURRING LARGE COLON SAND ACCUMULATIONS AND THEIR MEDICAL TREATMENT IN HORSES

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ACADEMIC DISSERTATION

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To my Mother

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ABSTRACT

Problems related to equine large colon sand accumulations have been known for at least a century, but evidence-based medicine on the subject remains scarce. The current medical treatments are largely based on case reports and small studies on research horses with artificial sand accumulation or without confirmed accumulation. Therefore, clinical studies on naturally acquired sand accumulation are needed. The main objectives of the present studies were to define clinical signs related to the presence and size of large colon sand accumulations and to determine the optimal way to medically treat horses with these accumulations.

A questionnaire study was performed to determine whether certain clinical signs are linked to the amount of sand in the colon and to clarify associations between equine large colon sand accumulations and individual traits and management-related factors. Owners or caretakers of 447 horses that had been radiographed for suspected large colon sand accumulation, responded to an internet based survey on clinical signs and management of their horses. The most common clinical signs of large colon sand accumulation noted by the owner were colic, poor performance and changes in faecal consistency (diarrhoea, increased faecal water). Colic (odds ratio (OR) 2.03, confidence interval (CI) 1.42-2.91) and poor performance (OR 1.89, CI 1.33-2.68) alone were associated with the size of sand accumulation. Horses with combinations of clinical signs, such as diarrhoea and poor performance or colic and diarrhoea also had a higher probability of a large sand accumulation; OR 2.12, CI 1.42-3.44 for diarrhoea and poor performance, and OR 2.64, CI 1.69-4.13 for colic and diarrhoea. Dominant position in the herd was protective for large colon sand accumulation (Spearman rho 0.170, P=0.002), while greediness predisposed the horse to the condition (Spearman rho 0.184, P<0.001). No significant association was detected between the studied management-related factors and large colon sand accumulation.

A retrospective clinical study compared the effectiveness of medical treatment of large colon sand accumulation with nasogastric intubation of psyllium and/or magnesium sulphate ($MgSO_4$) (both 1 g/kg bodyweight (BW) once a day for 4 to 7 days, N=170 horses) with feeding psyllium (1 g/kg BW for 2 to 5 weeks, N=57 horses). The daily administration of psyllium and $MgSO_4$ in hospital via a nasogastric tube removed the sand accumulation more efficiently than multiple weeks of feeding with psyllium alone *($P<0.05$)*. This finding raised the need to examine the effect of psyllium and $MgSO_4$ further, using a comparative approach in a randomised prospective study.

Based on the results of the aforementioned retrospective study, it was hypothesized that the combination treatment with psyllium and $MgSO_4$ would remove more sand than either treatment alone, or no treatment. The effect of psyllium (1 g/kg BW, N=12) or $MgSO_4$ (1 g/kg BW, N=10) alone or combined (1 g/kg BW each, N=12) was evaluated prospectively in hospitalized horses with naturally acquired sand accumulation treated utilising nasogastric intubation once a day for four days. There were significant differences between treatments. The combination of psyllium and $MgSO_4$ removed accumulations over a four-day period more effectively than either medication alone (number of resolved horses: P=0.012 for psyllium, and P=0.03 for $MgSO_4$, respectively). Of 12 horses treated with a combination of psyllium and $MgSO_4$, nine fulfilled the pre-determined criteria for clearance of sand from the ventral colon within four days. In contrast, only 3/12 horses treated with psyllium and 2/10 horses treated with $MgSO_4$ cleared the sand accumulation. Furthermore, the effects of combination of 1 g/kg BW psyllium and 1 g/kg BW $MgSO_4$ in 20 horses were compared with 20 untreated, hospitalized control horses that were solely removed from the sand source. After four days, the treated horses had cleared their accumulation (median area of sand decreased from 250 cm^2 to 0 cm^2) significantly more ($P<0.001$) than the control horses (median area of sand decreased from 285 cm^2 to 176 cm^2).

In conclusion, large colon sand accumulations should be considered a differential diagnosis not only when colic, poor performance or changes in faecal consistency are seen, but also when the horse presents with combinations of various clinical signs. The most effective treatment to remove sand accumulations that had developed naturally in the population studied was repeated nasogastric intubation of psyllium and $MgSO_4$.

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Contents

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

I Niinistö, K.E., Määttä, M.A., Ruohoniemi, M.O., Paulaniemi, M., Raekallio, M.R. 2019. Owner-reported clinical signs and management-related factors in horses radiographed for intestinal sand accumulation. Journal of Equine Veterinary Science 80:10—15.

II Kaikkonen, R., Niinistö, K., Lindholm, T., Raekallio, M. 2016. Comparison of psyllium feeding at home and nasogastric intubation of psyllium and magnesium sulfate in the hospital as a treatment for naturally occurring colonic sand (geosediment) accumulations in horses: a retrospective study. Acta Veterinaria Scandinavica 58:73.

III Niinistö, K., Hewetson, M., Kaikkonen, R., Sykes, B.W., Raekallio, M. 2014. Comparison of the effects of enteral psyllium, magnesium sulphate and their combination for removal of sand from the large colon of horses. The Veterinary Journal 202:608—611.

IV Niinistö, K., Ruohoniemi, M.O., Freccero, F., Raekallio, M. 2018. Investigation of the treatment of sand accumulations in the equine large colon with psyllium and magnesium sulphate. The Veterinary Journal 238:22—26.

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ABBREVIATIONS

BCS	Body condition score (Henneke et al. 1983)
BW	Body weight
CI	Confidence interval
DSS	Dioctyl sodium succinate
FLASH	Fast localized abdominal sonography of horses
GI	Gastrointestinal
ICC	Interstitial cells of Cajal
i.v.	Intravenous
$MgSO_4$	Magnesium sulphate
NGT	Nasogastric tube
OR	Odds ratio
q12h	Every 12 hours (or other frequency)
5-HT	5-hydroxytryptamine (serotonin receptors)

DEFINITIONS OF COMMONLY USED TERMS

Colic		Refers to the clinical signs shown by a horse when it has abdominal pain.
	Acute	Colic signs observed daily for less than 3 days.
	Chronic	Colic signs observed daily for 3 days or longer, except when masked by analgesics (Mair & Hillyer 1997).
	Recurrent	At least two episodes of colic requiring veterinary attention during the preceding 12 months (Cohen & Peloso 1996).
	Sand	Horse has acute or chronic/recurrent signs of colic that can be related to large colon sand accumulation either during or after active signs.
Faecal sand excretion		The amount of sand excreted with feces. Is related to amount of sand eaten, but not to large colon sand accumulations or sand colic.
Geophagia		Deliberate geosediment or soil ingestion.
Inreased fecal water		Horse defecates normal feces, but before, after, or during defecation or even independently of defecation, fecal water is seen running out of the anus (Kienzle et al. 2016).

Pica	Disorder characterized by craving or appetite for non-edible substances not limited to soil, but also other materials such as hair or wood.
Sand accumulation	Any type of soil or geosediment that has accumulated in the equine large colon. In radiography, sand accumulation has mineral opacity.
Sand enteropathy	Intestinal pathology, particularly colitis caused by large colon sand accumulation.

1 INTRODUCTION

Problems related to equine large colon sand accumulation have been known for at least 100 years (McIntyre 1917), but evidence-based medicine on the subject remains scarce. Horses can ingest sand accidentally when mixed with food or eat it deliberately. While many horses ingest sand, only some get problems and others can safely pass it through the entire gastrointestinal (GI) system. Besides colic, previous reports mention various clinical signs, most often diarrhoea and weight loss, in relation to large colon sand accumulations (Bertone et al. 1988, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017).

The diagnosis of sand accumulation has been refined from finding it only in surgery or necropsy or during accidental enterocentesis to determining the degree of accumulation using ultrasonography or, more accurately, using abdominal radiography (Udenberg 1979, Ragle 1989a, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002). The accuracy of the earlier recommended faecal sand test has been questioned (Hardy 2017), while auscultation of the ventral abdomen has been shown to be of some use in diagnosis of large colon sand accumulation (Ragle et al. 1989b).

Treatment has often focused on management of acute sand colic either medically or surgically; the prognosis in both treatments has improved over the years (Ford & Lokai 1979, Udenberg 1979, Ragle et al. 1989a, Hart et al. 2013, Kilcoyne et al. 2017). The criteria for colic surgery (regardless, of knowledge about the sand accumulation status) are intractable pain, abdominal distention, absent borborygmi, absent or decreasing fecal output, findings on rectal palpation, and/or ultrasonographic findings indicative of the need for surgery such as large colon displacement (Colahan 1987, Specht & Colahan 1988, Rakestraw & Hardy 2012, Hart et al. 2013, Hardy 2017, Kilcoyne et al. 2017) (Table 1).

Table 1. Case reports and studies regarding equine large colon sand accumulation.

No. of horses	Type of sand accumulation	Diagnosis of sand in the GI tract	Design	Reference	Year
	Natural	Necropsy	Observation	McIntyre	1917
	Natural	Rectal	Observation	Ferraro	1973
1	Natural	Rectal	Case report	Ford & Lokai	1979
324*	Natural	Rectal/ auscultation	Case series	Rollins & Clement	1979
1	Natural	Surgery	Case report	Udenberg	1979
1	Natural	Radiography	Case report	Ramey & Reinertson	1984
4	Natural	Radiography	Case series	Bertone et al.	1988
48	Natural	Surgery	Case series	Specht & Colahan	1988
40	Natural	Surgery	Case series	Ragle et al. (a)	1989
15	Induced	Auscultation	Prospective	Ragle et al. (b)	1989
2	Natural	Surgery	Case series	Ragle et al.	1992
4	Induced	Faecal sand	Prospective	Lieb	1997
1	Natural	Surgery	Case report	Gilroy & Bellamy	1998
12	Induced	Radiography/ necropsy	Prospective	Hammock et al.	1998
4	Induced	Faecal sand	Prospective	Lieb & Weise	1999
52	Natural	Radiography	Case series	Kaikkonen et al.	2000
14	Natural	Radiography	Case series	Ruohoniemi et al.	2001
8	Natural	Intake of sand	Prospective	Weise & Lieb	2001
32	Natural	Radiography/ ultrasongraphy	Prospective	Korolainen & Ruohoniemi	2002
211	Natural	Faecal sand	Prospective	Husted et al.	2005
41	Natural	Surgery	Case series	Granot et al.	2008
67	Natural	Radiography	Prospective	Kendall et al.	2008
51	Natural	Radiography	Case series	Keppie et al.	2008
8	Unknown	Faecal sand	Prospective	Landes et al.	2008
83	Natural	Surgery	Case-Control	Packer et al.	2008
12	Induced	Faecal sand	Prospective	Hotwagner & Iben	2009
6	Unknown	Faecal sand	Prospective	Cieśla et al.	2011
30	Unknown	Observed to eat	Prospective	Aytekin et al.	2011
62	Natural	Radiography	Case series	Hart et al.	2013
35	Natural	Radiography/ surgery	Case series	Graubner et al.	2017
153	Natural	Radiography/ surgery	Case series	Kilcoyne et al.	2017
21	Natural	Fecal/ ultrasonograpy	Prospective	Siwinska et al.	2019

**Includes horses with and without sand accumulation*

Currently, medical treatment of sand colic uniformly consists of pain medication and laxatives; the efficacy of various laxatives has been debated. The purported benefits of various laxatives, mainly mineral oil and psyllium, on sand clearance are based on clinical experience, case reports and studies with small groups of horses, sometimes with conflicting results and without radiographic confirmation of sand clearance. Previous studies include retrospective case series with variable treatments (Bertone et al. 1988, Hammock et al. 1998, Ruohoniemi et al. 2001, Hogwagner & Iben 2008, Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017) and small experimental studies with induced sand accumulation or only considering faecal excretion of sand without confirmed diagnosis of colonic sand accumulation (Lieb 1997, Hammock et al. 1998, Lieb & Weise 1999, Landes et al. 2008, Cieśla et al. 2011) (Table 1).

The reason for equine soil ingestion is still unclear, although some links to trace minerals or soil composition have been suggested (McGreevy et al. 2001, Aytekin et al. 2011). Individual horses have differing ability to transit feed and sand through the GI tract (Fintl et al. 2004). Some of the possible mechanisms are presented in a causal diagram (Figure 1). While multiple clinical signs have been connected to large colon sand accumulation, there has been no systematic evaluation of their association with sand accumulation. Despite multiple reports and studies of sand removal, there are no controlled studies of horses having naturally acquired large colon sand accumulation, either prospectively or retrospectively. The efficacy of medical treatments relative to no treatment has been questioned since Hammock et al. (1998) studied research ponies with surgically placed sand accumulation and could not detect any benefit from psyllium treatment. The present studies were designed to fill some of the aforementioned gaps in current knowledge.

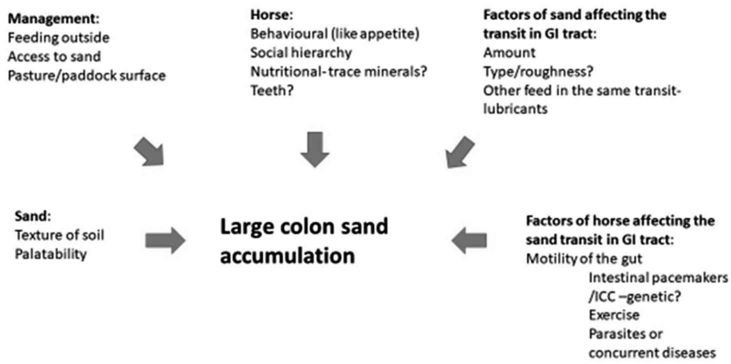


Figure 1. Causal diagram of mechanisms related to large colon sand accumulation.

2 REVIEW OF THE LITERATURE

2.1 GEOPHAGIA

Geophagia is not unique to horses, but has been reported in many species, e.g. humans, various ruminants, monkeys and dogs (Rigg & Askew 1934, Healy 1967, Melendez et al. 2007, Moles et al. 2009, see review by Abrahams 2012). Within species, many factors, such as age, gender or sex and time of year, affect soil consumption (Kreulen & Jager 1984, Abrahams 2012). Young dogs are more prone to pica, a phenomenon observed in other species as well (Houpt 1982, Abutarbush & Petrie 2006). Children, mainly toddlers up to the age of four years, most commonly exhibit incidental geophagia (Shivoga & Moturi 2009). In humans, female gender is more associated with geophagia and pica (Vermeer & Frate 1979).

Geophagia can be useful, or even necessary. Sheep have been shown to obtain up to 50% of their daily intakes of copper, iron, iodine, manganese, sodium and selenium from involuntary soil ingestion (Abrahams 2012). Less than 20 g of soil a week prevented copper deficiency in sheep, although they have been shown to eat substantially more during the peak of winter (Rigg & Askew 1934, Healy 1967). Involuntary soil consumption with vegetables or cereals, especially in developing countries, has been shown to give a dietary source of minerals to people (Hallberg & Björn-Rasmussen 1989). Similarly, on a calcium-deficient diet, a deliberate consumption of calcium-rich soil by pregnant and lactating women as well as growing children has been reported (Vermeer 1966). In birds, geophagia is a method of calcium ingestion (Abrahams 2012).

Contrary to many other species, horses do not regulate the intake of minerals other than sodium (Ralston 1987). Ponies with calcium deficiency did not develop a preference for calcium supplementation (Schryver et al. 1978). In a study of 30 horses, 15 geophagic horses showed no difference in haematological or biochemical values, but they had lower serum iron concentration and lower serum copper/zinc ratio than control horses without geophagia (Aytekin et al. 2011). Both wild and domesticated horses choose certain sites (“licks”) to ingest soil (McGreevy et al. 2001, Abrahams 2012). It is sometimes assumed that geophagic horses would prefer coarse soil, but in one study it happened rarely, and for most of the soil the textures were similar to control samples that the horses were not prone to lick (McGreevy et al. 2001). No significant difference existed in zinc or phosphorus concentrations of the soil (McGreevy et al. 2001). Even though the concentrations of iron and copper were higher in the soil of geophagic sites than in adjacent control samples, their level was not markedly greater than the general background levels measured (McGreevy et al. 2001). Whether geophagia in horses could result from lack of minerals, is not yet proven, and therefore research on this topic would be important.

The greatest accidental sand intake with food is expected to occur when horses eat grain directly on sand or retrieve grain dropped onto a sandy surface (Ferraro 1973, Gilroy & Bellamy 1998, Weise & Lieb 2001, Moles et al. 2010). Some horses eat sand deliberately, while others ingest it accidentally when trying to reach all of their food (McIntyre 1917). Pasture quality has been identified as a risk factor for increased faecal sand excretion. Both short and long grass in combination with sandy soil increased the risk, while clay soil had the lowest risk for faecal sand excretion (Husted et al. 2005). In the same study, feeding directly on the ground was shown to be a risk factor when combined with short (1–5 cm) or no grass. Lack of feeding outdoors when horses were kept on pastures with short grass increased the risk of faecal sand excretion, while it had no effect in paddocks with no grass (Husted et al. 2005). Underfeeding or not getting enough roughage has been suggested to predispose horses to eating sand (Ferraro 1973, Rollins & Clement 1979, Hanson 2002).

While geophagia can be beneficial, eating of soil has led to excessive incisor tooth wear in sheep, possibly also causing abrasions along the GI tract (Healy

1967). Other clinical problems, most commonly sand accumulation, impaction or intoxication, have been reported in various species (Raoofi et al. 1996, Melendez et al. 2007, Moles et al. 2010, Abrahams 2012). In certain mineral-dense areas, lead toxicity and even potassium and fluoride overdose have been described following geophagia (Abrahams 2012). Intestinal sand accumulation similar to that in horses is not common, but has occasionally been reported in other species, such as mules (Raoofi et al. 1996), alpacas (Aburtabush & Petrie 2006), cattle (Melendez et al. 2007, Erickson & Hendrick 2011, Simsek et al. 2015), dogs (Moles et al. 2010), and wild animals (Jegede et al. 2016). Bovine abomasal sand impactions due to sand tainted silage, other food, or water have been reported (Erickson & Hendrick 2011, Simsek et al. 2015).

Geophagia and sand accumulations may be associated with another concurrent medical condition. Equine gastric sand accumulation has been described concurrent with gastroduodenal and oesophageal ulceration and pyloric stenosis (Heidman et al. 2004, Bezdekova 2009). An Alsatian puppy was diagnosed with colonic (transverse and descending colon) sand accumulation some weeks after treatment of babesiosis and anaemia, but their causal relationship was unknown (Muhammad et al. 2014). An outbreak of sand ingestion and impaction has been reported in post-partum dairy cows, possibly due to acid-base imbalance (Melendez et al. 2007). In humans, there is debate about whether low blood iron concentration is the reason for geophagia or a consequence of it (Gardner & Tevetoglu 1957, Hooda et al. 2002, Abrahams 2012).

Currently we know that horses consume sand both deliberately and accidentally, like other species. Pasture quality and feeding practices have been connected to geophagia in horses. Thus far, no clear benefits of geophagia in horses have been shown. Though sand accumulations concurrent to other medical conditions are seen, their relationship is unknown. To be able to efficiently prevent large colon sand accumulations, the etiologies should be further clarified.

2.2 EQUINE LARGE COLON SAND ACCUMULATION

2.2.1 GEOGRAPHICAL VARIATION

Horses commonly excrete sand in their faeces without any signs of problems related to large colon sand accumulation (Landes et al. 2009, Macedo Filgueiras et al. 2009, Cieśla et al. 2011). For example, when horses were kept on a sandy pasture in a city, all of them (112/112) excreted sand in their faeces, although none had observed clinical signs related to the GI tract (Macedo Filgueiras et al. 2009).

Geophagia in horses and problems associated with large colon sand accumulation have been reported worldwide, but the situation seems to be concentrated in certain areas. The first report concerned military horses in Egypt (McIntyre 1917), but thereafter the written sand-related discussion is missing until the 1970s (Ferraro 1973, Ford & Lokai 1979, Rollins & Clement 1979). Most of the earlier reports came from USA, where GI problems related to sand tend to cluster and have been reported at least in Arizona (Rollins & Clement 1979), Colorado (Bertone et al. 1988), Michigan (Kaneene et al. 1997, Keppie et al. 2008), Utah (Ramey & Reinertson 1984, Rood 2011), California (Ferraro 1973, Ragle et al. 1989a) and Florida (Ford & Lokai 1979, Specht & Colahan 1988, Lieb 1997, Hart et al. 2013). From Canada, the earliest report came from British Columbia (Udenberg 1979), but there is also a report of gravel impaction from eastern Canada (Gilroy & Bellamy 1998).

Publications regarding equine large colon sand accumulations in Northern Europe have mainly come from Finland (Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Korolainen et al. 2003). Sand accumulation is also a common condition in Denmark (Hustedt et al. 2005) and Sweden (Kendall et al. 2008). Problems related to large colon sand accumulation have been reported and treatments studied in Central Europe

(Johnson & Keller 2005, Hotwagner & Iben 2009, Cieśla et al. 2011, Graubner et al. 2017, Loschelder & Gehlen 2017). Despite the huge amount of accessible sand in certain areas of Africa and Asia, only a few reports come from these areas. In addition to Egypt (McIntyre 1917), sand enteropathy has been recognised in Israel (Granot et al. 2008). Many authors might publish in languages other than English (Yang et al. 2005, Loschelder & Gehlen 2017), and thus, these reports are not readily accessible for all researchers.

Similar to regional differences reported with sand accumulations in USA, there are also regional differences in much smaller and climate-wise more uniform countries, such as Sweden, where most of the sand colic cases are clustered in the south and are rare in central Sweden (Kendall et al. 2008). Similarly in Britain, cases of sand colic have shown small-scale spatial clustering (Packer et al. 2008). Based on reports all over the world, there are areas where horses seem to be more prone to accumulate sand in their GI tract, whereas sand accumulation might be under-diagnosed in some areas.

2.2.2 RELATIONSHIP OF EXTERNAL FACTORS TO SAND ACCUMULATION

Geophagia and sand accumulations have been linked to managemental factors including not only nutrition, type of soil and pasture (Nieberle & Cohrs 1967, Ragle et al. 1989a, Hanson 2002, Husted et al. 2005), but also to weather and season (Rollins & Clement 1979, Specht & Colahan 1988, Kaikkonen et al. 2000, Graubner et al. 2017). In sandy soil areas of southern USA, up to 30% of colic cases have been related to sand accumulation (Lieb 1997), and it has been proposed that geographical areas with loose sandy soils predispose horses to sand colic (Ragle et al. 1989a, Hanson 2002). Flooded areas and muddy or sandy watering places may predispose horses to accidental geophagia and subsequent colonic sand accumulation (Nieberle & Cohrs 1967). In a study in Florida, most (31/48) of the horses were on pasture at the time colic developed, and all horses with sand colic came from areas where annual rainfall was >120 cm (Specht & Colahan 1988).

Problems related to large colon sand accumulation are suggested to cluster during certain seasons or weather conditions (Rollins & Clement 1979, Specht & Colahan 1988, Kaikkonen et al. 2000, Graubner et al. 2017). The connection seems to vary from one report to another, with reports coming from areas with different climates. Most of the reports suggesting an association between large colon sand accumulation and season or weather have concentrated on acute sand colic or fecal sand excretion without confirmed large colon sand accumulation. In Arizona, most cases of sand colic occurred between May and October (Rollins & Clement 1979). The authors speculated that the reason might be higher ambient temperatures during that time period relative to the other half of the year. However, in Florida, most of the sand colic cases presented in October, and two-thirds of the horses were admitted to hospital between July and December (Specht & Colahan 1988). Lieb & Weise (1999) found no differences in faecal sand excretion between farms or seasons when collecting faeces from five farms in Florida for one year. In northern climates, most sand accumulations have been diagnosed between November and March (Kaikkonen et al. 2000), which is very similar to Central Europe (Switzerland), where cases are clustered to winter and autumn (Graubner et al. 2017). In Britain, a case-control study did not find significant temporal clustering, but in descriptive data cases primary occurred in the period from November to February (Packer et al. 2008). Since sand accumulations are sometimes cleared in the pasture with proper grass (Kaikkonen et al. 2000), it might be a protective factor and, therefore, accumulations cluster outside pasture season. Currently it remains unclear whether the reason for clustering is the actual season, weather changes or the possible managemental changes associated with either of them.

2.2.3 LARGE COLON MOTILITY AND ITS DISTURBANCES

Disturbances in the GI motility may be associated with sand accumulation in some horses (Korolainen & Ruohoniemi 2002, Fintl et al. 2004). The equine caecum and colon serve as a reservoir of digesta, allowing the microbiota to hydrolyse cellulose and synthetize protein for 48-72 hours (see review by Sellers & Lowe 1986). Both the caecum and large colon have not only retrograde and normograde movements, which exchange digesta from haustrum to haustrum, but also less frequent coordinated longitudinal and circular muscle contractions, responsible for mixing and moving the digesta through in the GI tract (Sellers & Lowe 1986). Junctions between the ventral and dorsal large colons at the pelvic flexure and the right dorsal large colon and small colon appear to be major locations where movement of digesta may be delayed (Argenzio et al. 1974). Large particles are retained in the large colon longer than smaller particles and are subject to retrograde movement in order to be retained within the colon for ongoing digestion (Argenzio et al. 1974). Different areas of the colon have different functions; the dorsal colon retains larger particles to a much greater extent than the ventral colon (Argenzio et al. 1974), but also the pelvic flexure has retrograde activity moving large particulate digesta back into the left ventral colon for further digestion (Sellers & Lowe 1986).

In all mammalian species, the interstitial cells of Cajal (ICC) serve as pacemakers, creating the bioelectrical slow wave potential that leads to contraction of the smooth muscle in all regions of the GI tract. Equine ICC have been described and reported by Hudson et al. (1999). Earlier, ICC have been referred to as enteric pacemakers or neuronal plexuses (Burns & Cummings 1991). The highest density of myenteric neuronal plexuses is at the pelvic flexure of the large colon (Roger & Ruckebusch 1987, Burns & Cummings 1991). Feeding increases enteric plexus activity. There is a greater initial increase, most likely due to the gastrocolic reflex and cholinergic stimulation (Roger & Ruckebusch 1987), and later lesser increase, probably resulting from an enteral response to stretching of the stomach (Merrit et al. 1995). Due to aforementioned reflexes, administering laxatives as a bolus might increase intestinal movement and enhance the effect of laxatives.

Although generalized intestinal inflammation and diseases, such as equine grass sickness, have been shown to affect neurons and motility of the GI tract, the mechanism of simple large colon impaction is not fully understood (Sellers & Lowe 1986, Fintl et al. 2004). Associations with other factors like poor quality hay, dental disease and inadequate water intake have been established (Sellers & Lowe 1986). The two most common sites for impaction are at the pelvic flexure and in the right dorsal colon, both of which are sites of narrowing of the GI tract (Sellers & Lowe 1986). Loss of ICC could lead to less enteral movement and potential predisposition to impaction colic (Fintl et al. 2004). Horses that underwent colic surgery due to large colon impaction or displacement had fewer ICC in the pelvic flexure compared to horses with small intestinal problems or healthy control horses (Fintl et al. 2004). Whether the loss of ICC in the large colon was the reason for colic, or result of it, was not shown in the study. The density of ICC in horses with large colon sand accumulation has not been studied specifically; however, 5/6 horses with colon impaction in the study by Fintl et al. (2004) had sand accumulation.

2.2.4 SITE OF ACCUMULATION

The exact site of sand accumulation in the GI tract may be difficult to localize in a live animal, but often can at least partially be determined with radiography or ultrasonography, and most accurately at exploratory laparotomy (Specht & Colahan 1988, Ragle et al. 1989a, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Kendall et al. 2008, Keppie 2008, Hardy 2017).

Sand can accumulate anywhere in the equine large colon (Specht & Colahan 1988, Hardy 2017) and transverse colon (Colahan 1987, Hanson 2002). Particle size is more important than its weight for intestinal transit. Regarding sand accumulations, larger particles have been identified more commonly in the dorsal colon, whereas smaller particles have been located in the ventral colon (Snyder & Spier 1990, Hammock et al. 1998). Being heavy, sand is deposited on the ventral wall of the bowel (Nieberle & Cohrs 1967). The sites of sand accumulation in the large colon that have been confirmed surgically or at post-mortem are listed in Table 2. Many horses have multiple sites of sand accumulation, the proportion varying from 34% to 54% (Specht & Colahan 1988, Granot et al. 2008). Horses younger than two years appear to have multiple accumulations more often than older horses (Kaikkonen et al. 2000). Besides the colon, sand accumulation in the caecum has been reported in some foals (Campbell et al. 1984).

Contrary to surgical reports, radiographically most of the large colon sand accumulations are described as being in the ventral colon (Bertone et al. 1988, Ruohoniemi et al. 2001). However, radiography does not always allow determination of location due to the lack of depth evaluation and possible dislocation of the sand filled part of intestine due to its weight (Ruohoniemi et al. 2001). The aforementioned reports of surgically managed sand accumulation describe horses with acute colic. The ventral colon is the widest part of the colon, and thus, less likely to have an impaction causing complete obstruction and acute colic (Dabareiner & White 1995, Sanchez 2017).

Table 2. Surgically or post-mortem confirmed sites of sand accumulation in the GI tract.

Site of accumulation	Reference
Ventral colon	Specht & Colahan (1988), Ragle et al. (1989a)
Pelvic flexure	McIntyre (1917), Colahan (1987), Specht & Colahan (1988), Ragle et al. (1989a)
Left dorsal colon	Specht & Colahan (1988), Ragle et al. (1989a), Gilroy & Bellamy (1998)
Sternal flexure	Specht & Colahan (1988), Ragle et al. (1989a), Hanson (2002)
Right dorsal colon	Colahan (1987), Specht & Colahan (1988), Ragle et al. (1989a), Ragle et al. (1992), Gilroy & Bellamy (1998), Hanson (2002), Maxwell (2003)
Transverse colon	Colahan (1987), Specht & Colahan (1988), Ragle et al. (1989a), Maxwell (2003)
Small colon	Ragle et al. (1989a)

Although the large colon is the most common place for sand accumulation in horses, there are also a few reports of gastric sand accumulation in horses diagnosed with pyloric stenosis and oesophago-gastroduodenal ulceration (Heidmann et al. 2004, Bezdekova 2009). Heidmann et al. (2004) did not conclude on the causality of the clinical problems, but Bezdekova (2009) speculated that passage of sand through the stomach might have produced inflammation with secondary fibrosis of the pylorus leading to obstruction.

2.2.5 SIGNALMENT OF HORSES WITH SAND ACCUMULATION

Sand accumulation has been reported in multiple horse breeds. However, some breeds, such as Quarter Horse (Ford & Lokai 1979, Udenberg, 1979, Ramey and Reinertson, 1984, Keppie et al. 2008, Hart et al. 2012, Kilcoyne et al. 2017), Shetland pony (Graubner et al. 2017), Miniature horse (Ragle et al. 1989a, Ragle et al. 1992, Granot et al. 2008, Keppie et al. 2008, Hart et al. 2013) and Finnhorse (Kaikkonen et al. 2000, Ruohoniemi et al. 2001), seem to present with large colon sand accumulation more often than others. Thoroughbred horses appear to rarely develop sand accumulations within the GI tract (Ragle et al. 1989a, Yang et al. 2005, Granot et al. 2008, Hart et al. 2013, Kilcoyne et al. 2017). Kaikkonen et al. (2000) showed that Finnhorses had larger sand accumulations than Warmbloods at the time of hospital presentation. The association between horse breed and size of the sand accumulation has been infrequently investigated and possible relationships between breed should consider breed-related factors such as use and associated management.

In general, the sex of the horse does not appear to be associated with sand accumulation (Keppie et al. 2008, Hart et al. 2012, Kilcoyne et al. 2017). Foals and young horses are less commonly diagnosed with sand accumulation, but reports of problems related to sand exist in this demographic (Ramey and Reinertson 1984, Ruohoniemi et al. 2001). In one study, young Icelandic horses tended to pass more sand with their faeces, but their geophagic behaviour was not recorded, nor was sand accumulation diagnosed (Hustedt et al. 2005).

2.2.6 BODY CONDITION

Although weight loss in horses with large colon sand accumulation has been noted (McIntyre 1917, Ramey & Reinertson 1984, Bertone et al. 1988, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Hart et al. 2013, Graubner et al. 2017), the causality has not been reported. Some of the horses presented to hospital due to sand colic also had a history of weight loss (Ruohoniemi et al. 2001, Hart et al. 2013), but the actual body condition score (BCS) of these horses was not reported. Icelandic horses with lower BCSs than the herd average of 5/9 (Henneke et al. 1983), had a tendency to have more sand in the faecal test (Husted et al. 2005). However, this group of lower BCS consisted of only 7 horses, and it was not reported whether any horse had a large colon sand accumulation (Husted et al. 2005).

2.3 CLINICAL SIGNS RELATED TO SAND ACCUMULATION IN THE LARGE COLON

Horses can have small sand accumulations without any clinical signs (McIntyre 1917, Kaikkonen et al. 2000, Kendall et al. 2008). No exact knowledge exists on what size of large colon sand accumulation causes clinical problems in a horse, but it might vary individually and be dependent on the horse's size (Bertone et al. 1988, Keppie et al. 2008, Hart et al. 2013). Clinical signs vary, possibly depending on the size and density of accumulation, from mild, such as loose faeces, to more severe clinical problems such as diarrhoea and acute or recurrent colic (McIntyre 1917, Kaikkonen et al. 2000).

Large colon sand accumulation has been reported to cause irritation (Buergelt 2013), chronic mucosal inflammation (McIntyre 1917, Nieberle & Cohrs 1967, Ramey & Reinertson 1984), diverticulum formation (Nieberle & Cohrs 1967) and sometimes weakening and rupture (Buergelt 2013) of the colon wall. Sand accumulation of up to 27 kg (Udenberg 1979) or even 68 kg (Ford & Lokai 1979) has been found during post-mortem examinations, combined with intestinal wall oedema and haemorrhage (Udenberg 1979), or mucosal necrosis (Ford & Lokai 1979). Acute colon wall changes that may be related to accumulation of sand in the large colon can be difficult to distinguish from those related to impaction of ingesta or displacement or volvulus of the colon (Senior et al. 2011). In one horse, mild clinical signs with changes in faeces had been observed for two months after the sand had been removed, which led the authors to suggest that inflammation persisted and intestinal repair after chronic inflammation caused by sand accumulation would be a slow process (Ramey & Reinertson 1984). Contrary to this, mucosal healing after acute induced ischemia has been shown to occur in 4-7 days (Blikslager & Roberts 1997).

2.3.1 COLIC

Colic is the most acute and severe clinical sign related to equine large colon sand accumulation. Sand accumulation can cause both acute and recurrent colic (Gilroy & Bellamy 1998, Kaikkonen et al. 2000, Korolainen et al. 2003). Horses with sand colic often require veterinary intervention, and sometimes it is not possible to save the animal (Ford & Lokai 1979, Udenberg 1979, Specht & Colahan 1988, Ragle et al. 1989a, Hart et al. 2013). Many clinical reports mention colic as the main clinical sign associated with sand accumulation (McIntyre 1917, Ferraro 1973, Udenberg 1979, Specht & Colahan 1988, Ragle et al. 1989a, Gilroy & Bellamy 1998, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen et al. 2003, Granot et al. 2008, Kendall et al. 2008, Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017). However, often colic is not the first sign of sand accumulation, but can be preceded (or accompanied) by other clinical signs (McIntyre 1917, Ferraro 1973, Kaikkonen et al. 2000, Korolainen & Ruohoniemi 2002, Hart et al. 2013).

The most common finding in horses with sand colic is a large colon impaction caused by complete intraluminal obstruction with sand and feed material, diagnosed sometimes only at laparotomy or necropsy (Nieberle & Cohrs 1967, Udenberg 1979, Specht & Colahan 1988, Ragle et al. 1989a, Ruohoniemi et al. 2001, Hart et al. 2013). Complete intraluminal obstruction leads to large colon and/or caecal distension (Specht & Colahan 1988, Ragle et al. 1989a). Sometimes the impaction is accompanied by large colon displacement (McIntyre 1917, Colahan 1987, Specht & Colahan 1988, Ragle et al. 1989a, Hardy 2017, Kilcoyne et al. 2017). The observation of dilated large colon mesenteric vessels in necropsy by McIntyre (1917) might have been more related to colon displacement than sand accumulation itself. Whether the cause of vascular changes could be the weight of the sand, as suggested by Colahan (1987), has not been confirmed. Small intestinal distension is not commonly reported with sand colics, but can occur (Hart et al. 2013).

Colic signs caused by sand accumulation can be intermittent (Sullins 1990) and they can manifest in various ways. Some older reports state that in the later stage of colic that the horse lies down as a pathognomic sign for large colon sand accumulation (McIntyre 1917, Ferrero 1973, Ford & Lokai 1979). Another suggested sign has been standing in a stretched position for a prolonged time in order to reduce the tension in the intestine (Sullins 1990). However, current reports describe no specific signs which would distinguish sand colic from other types of colic (Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017). The severity of colic is generally evaluated by specific signs of abdominal pain, heart rate and measures of perfusion, together with absence of fecal production, abdominal distension and changes in peritoneal fluid. In uncomplicated sand colic cases, the heart rate is close to being within normal limits (Ferraro 1973, Hart et al. 2013, Kilcoyne et al. 2017) and signs of pain are mild (McIntyre 1917, Hart et al. 2013, Kilcoyne et al. 2017). Data from a recent study showed no difference in packed cell volume, blood lactate concentration or peritoneal fluid lactate concentration between medically and surgically managed horses with sand colic (Kilcoyne et al. 2017). In a previous study, an increase in blood lactate concentration was associated with non-survival in horses with sand colic (Hart et al. 2013).

Horses demonstrating colic signs had larger and more sand accumulations on abdominal radiographs than those with other clinical signs (Kaikkonen et al. 2000). Furthermore, horses hospitalized for colic had larger sand accumulations than control horses hospitalised with orthopaedic problems (Kendall et al. 2008). However, Kilcoyne et al. (2017) did not find any association between the size of sand accumulation and the severity of colic or the need for surgery; findings on abdominal palpation per rectum and colonic distension were more useful indicators of the requirement for surgery.

2.3.2 CHANGES IN FAECAL CONSISTENCY

Large colon sand accumulation is one of the differential diagnoses for chronic diarrhoea and changes in faecal consistency (Hines 2018, Sanchez 2018), together with parasites (including cyathostomiasis), dental problems and various inflammatory conditions (Hines 2018). However, many horses with chronic diarrhoea remain without a specific diagnosis with the current methods of investigation (Hines 2018).

Chronic diarrhoea and loose faeces are commonly associated with large colon sand accumulation either alone or in combination with other clinical signs (McIntyre 1917, Ferraro 1973, Ramey & Reinertson 1984, Bertone et al. 1988, Ragle et al. 1989a, Gilroy & Bellamy 1998, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Korolainen et al. 2003, Granot et al. 2008, Hart et al. 2013, Hines 2018). For example, in a Finnish study, 11 of 52 horses with large colon sand accumulation presented with a history of diarrhoea and another 9 with diarrhoea and colic (Kaikkonen et al. 2000). Diarrhoea has been suggested to be an earlier clinical sign of sand accumulation than colic (McIntyre 1917, Ferraro 1973). Diarrhoea has also been reported as a side effect of colic treatment in horses with sand accumulation, both with medical (nasogastric intubation with laxatives) and surgical treatment (Granot et al. 2008, Kilcoyne et al. 2017). A case report described the persistance of diarrhoea for some weeks after removal of sand accumulation in one weanling, but other possible causes of diarrhoea were not ruled out (Ramey & Reinertson 1984).

2.3.3 POOR PERFORMANCE

Poor performance can be defined as exercise intolerance or the horse's inability to perform at a level previously observed or at a level to be expected based on the horse's training and physical character. In racehorses and endurance horses, it can be assessed by loss of speed or prize money. In endurance horses, poor performance has been associated with a high exercising heart rate or delayed heart rate recovery after performance (Flaminio et al. 1996). In sport horses, poor performance can be expressed as reluctance or inability to perform tasks, e.g. dropping fence poles, or lack of elasticity of movements (Dyson 2000).

Gastric ulcers have been shown to cause poor performance in Thoroughbred racehorses (Franklin et al. 2008). However, GI problems as a cause of poor performance have not been thoroughly evaluated. For example, Martin et al. (2000) found a reason for poor performance in only 73.5% of cases (256/348 horses); GI causes of poor performance were not investigated. Most of the studies of poor performance concentrate on various types of lameness, respiratory problems or cardiac disease (Morris & Seeherman 1991, Martin et al. 2000, Parente et al. 2002, Ducro et al. 2009, Girodroux et al. 2009).

Although GI sand accumulation has not been listed as a specific cause in previous studies of poor performance, reluctance to move and exercise intolerance have been reported in some of the horses diagnosed with sand accumulation (Kaikkonen et al. 2000, Hart et al. 2013).

2.3.4 OTHER CLINICAL SIGNS AND CONDITIONS ASSOCIATED WITH LARGE COLON SAND ACCUMULATION

Other less common clinical signs have also been associated with large colon sand accumulation. Most commonly mentioned are weight loss, weakness, and lethargy (Ramey & Reinertson 1984, Bertone et al. 1988, Kaikkonen et al.

2000, Hart et al. 2013). Fever (Hart et al. 2013) and altered intestinal flora (Kaikkonen et al. 2000) may also be associated with sand enteropathy. Large colon sand accumulation has occasionally been associated with gastric impactions with ingesta (Ruohoniemi et al. 2001, Graubner et al. 2017). Another study found gastric ulcers not to be related to sand accumulation (Mönki et al. 2016). Most horses with girth aversion (i.e. resentment to tightening of the girth while saddling the horse, occasionally termed 'girthiness') had gastric ulcers or an orthopaedic problem in a case series, but one horse with girth aversion had sand accumulation, and removal of sand resolved the clinical signs (Millares-Ramirez & Le Jeune 2019).

2.4 DIAGNOSIS OF SAND ACCUMULATION

Large colon sand accumulations can be diagnosed using various methods, but the accuracy of these tests varies, as does the ability to estimate the amount of sand in the colon. Currently, multiple methods are in use depending on the tools available and the veterinarian's preference. The only exact method is to measure the amount of sand at necropsy (Ford & Lokai 1979, Udenberg 1979). In order to evaluate the amount of sand evacuated via an enterotomy during laparotomy, special arrangements need to be made to retain the intestinal contents rather than loosing sand if contents are normally flushed into drains.

2.4.1 RADIOGRAPHY

Sand and other types of geosediment have a distinct shadow of mineral opacity on an abdominal radiograph, which is equivalent to or more radiopaque than ribs (Figure 2) (Keppie et al. 2008). Sand as a material is heavier than feed particles and, therefore is more likely to be radiographically detected in the ventral part of the colon.The particle size of the sand may affect the radiographic opacity, with coarse sand being more opaque than fine sand (Keppie et al. 2008). The more opaque and homogeneous accumulations have been assumed to represent more compact and pure sand accumulations and have been suggested to be most likely associated with sand colic (Keppie et al. 2008). The more heterogeneous and less opaque accumulations, or accumulations of mixed contents (sand and digesta), with some water content have been speculated to be less severe and less likely to cause obstruction (Keppie et al. 2008).

The shape of accumulations can vary markedly, from flat and elongated to round or even with a pointed ventral border (Kaikkonen et al. 2000, Ruohoniemi et al. 2001). The dorsal border of accumulation can vary from distinctive and horizontal to an uneven surface mixed with intestinal contents (Kaikkonen et al. 2000). Some of the types are shown in Figures 2 and 3.

2.4.1.1 Technique

In general, diagnostic radiographs of the entire adult horse abdomen (abdominal width exceeding 70 cm) are not possible, although sand and similar material with high mineral density can be identified (Bargai 1972, Butler et al. 2017). In foals, young horses and small ponies, diagnostic lateral radiographs can be obtained, and even a ventrodorsal view is possible with neonatal foals (Campbell et al. 1984, Butler et al. 2017). Equine sand accumulation has been diagnosed using radiography since the 1980s, first in foals and then in adults (Ramey & Reinertson 1984, Bertone et al. 1988). The radiographic technique for an adult horse was further developed in order to demonstrate sand in the ventral abdomen, with only the ventral abdomen being targeted because of the high exposures required (Kaikkonen et al. 2000, Ruohoniemi et al. 2001). The technique was validated with 20 horses having radiographs of the entire abdomen with sand being detected in the ventral abdomen only (Kendall et al. 2008).

Equine abdominal radiographs need a powerful machine (Ruohoniemi et al. 2001, Kendall et al. 2008) and preferably a gantry for holding the cassette. High exposures are required and special attention must be paid to radiation safety. Use of a grid is still recommended, although not always necessary with the digital technique (Butler et al. 2017). Radiographic techniques described have an anode-cassette distance of 1.5-2 m with the horse standing with its flank next to the cassette (either left to right or right to left lateral view). Exposure settings depend on the machine used and on the horse's size, e.g. 96-117 kV and up to 150 mAs or 150 kV / 100 mAs for an adult horse (Ruohoniemi et al. 2001, Kendall et al. 2008). A view centred on the cranioventral abdomen has been found to be most useful, but another view more caudally may be needed (Ruohoniemi et al. 2001). Large sand accumulations cannot always be visualized in their entirety on one radiograph, although it may be sufficient for obtaining a clinical diagnosis (Ruohoniemi et al. 2001).

2.4.1.2 Size and other evaluated parameters of sand accumulation

The size of the sand accumulation is the easiest measurable variable from a radiograph and can be measured using the current imaging programmes. Other variables evaluated have been opacity, homogeneity, and shape of the accumulation (Korolainen & Ruohoniemi 2002, Kendall et al. 2008). Size, or size relative to the horse's size, has been used to categorize sand accumulations in order to compare these with clinical signs or the accuracy of other diagnostic methods (Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Kendall et al. 2008, Keppie et al. 2008, Hart et al. 2013, Kilcoyne et al. 2017).

The grading system for sand accumulations by Korolainen & Ruohoniemi (2002) is shown in Table 3. This grading system only measured the maximal length and height of accumulation, regardless of magnification, shape of accumulation or size of horse. Some years later, Kendall et al. (2008) refined the scoring system to determine the association between score and colic signs.

Table 3. Scoring systems for grading abdominal sand accumulations by its maximum length and height.

Grade	Korolainen & Ruohoniemi (2002)	Kendall et al. (2008)
0	No sand	No sand
1	Small amount <5 x 5 cm	<5 × 5 cm
2	Small or moderate amount (largest accumulation <15 x 5 cm, or <5 x 15 cm) of sand, relatively ventrally or only a small part of the sand close to the ventral abdominal wall	≤5 × 15 cm or ≤15 × 5 cm
3	Moderate amount of sand ventrally (largest accumulation <15 x 5 cm, or <5 x 15 cm)	≤5 × 15 cm or ≤15 × 5 cm close to the ventral abdominal wall
4	Large (>10 x >10 cm) sand accumulation ventrally	>5 × 15 cm or >15 × 5 cm. If an accumulation was thin (<5 cm) but longer than 15 cm, it was graded as a 4.

To eliminate the effects of magnification and size of the horse, a scoring system using multiple variables was developed (Table 4, Keppie et al. 2008). A ratio of rib width to sand accumulation length and height was used to account for horse size. The opacity of the accumulation was also compared with the rib opacity (Keppie et al. 2008).

Table 4. Objective radiographic assessment scoring by Keppie et al. (2008). Maximum 12 points. Opacity of the sand accumulation was compared with the rib or vertebral body.

Scoring	0	1	2
Location	Other	Cranioventral	-
Number of accumulations	0	1	2; 3 or more = 3
Opacity	Much less	Mix	More, or as opaque
Homogeneity	Heterogeneous	Mix	Homogeneous
Height/rib width	1-3	4-5	>5
Length/rib width	< 10	10-20	>20

A different approach for determining the size of the sand accumulation relative to the horse's size was developed for a retrospective clinical study (Hart et al. 2013). The grading system was based on comparison of the height of the sand accumulation to the width of colon lumen (as a percentage). In this grading system, <30% was grade 1, ≥30% but <60% was grade 2, and ≥60% was grade 3 (Hart et al. 2013).

The correlation between grading the measured sand accumulation size (Korolainen & Ruohoniemi 2002) and adjusted relative grading systems (Keppie et al. 2008, Hart et al. 2013) was evaluated in a recent clinical retrospective study (Graubner et al. 2017). The three grading methods were significantly correlated when using Spearman-Rank correlations; coefficients were r=0.58 between Keppie et al. (2008) and Hart et al. (2013), 0.7 between Keppie et al. (2008) and Korolainen & Ruohoniemi (2002) and 0.89 between Hart et al. (2013) and Korolainen & Ruohoniemi (2002) (Graubner et al. 2017).

2.4.1.3 Radiography in monitoring the clearance of sand

Radiography has also been shown to be suitable for monitoring the changes in the appearance of the sand accumulation during treatment (Ruohoniemi et al. 2001). The shape of the accumulation did not predict the horse's response to medical treatment (Kaikkonen et al. 2000, Ruohoniemi et al. 2001). However, sand accumulations with an uneven dorsal surface mixed with feed seemed to be easier to clear (Kaikkonen et al. 2000). Movement of sand from the ventral to dorsal colon has been observed when a radiograph was taken during treatment (Ruohoniemi et al. 2001). During treatment, the opacity may diminish before there is an actual reduction of accumulation size (Butler et al. 2017).

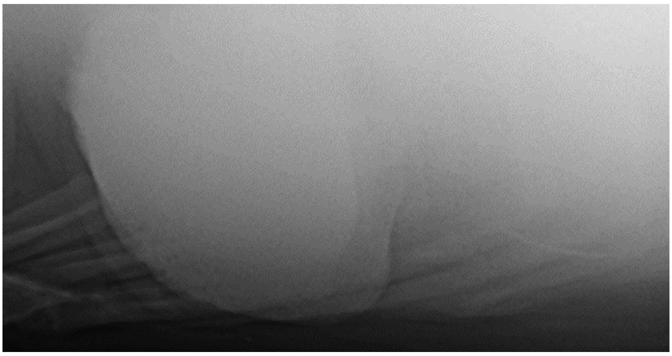


Figure 2. A radiograph of a cranial and deep sand accumulation with rounded ventral border. The accumulation is more radiopaque than the ribs.

Figure 3. A radiograph of a ventral colon sand accumulation with sacculae of the large colon visible, but the dorsal edge is not visible.

2.4.1.4 Radiographic differential diagnoses for sand accumulation

Due to the distinct appearance of large colon sand accumulation observed radiographically, the number of differential diagnoses is limited. Gastric sand accumulation can be very similar to cranial (diaphragmatic flexure) colonic accumulation, especially with concurrent gastric impaction. The diagnosis can be confirmed using gastroscopy (Heidmann et al. 2004). Radiographically, enteroliths can be differentiated from sand accumulation due to their rounded appearance (Kelleher et al. 2014, Butler et al. 2017).

2.4.1.5 Relationship between radiographic findings and clinical signs

Studies attempting to define the clinically relevant size of sand accumulation are scarce. Horses with colic have been reported to have larger sand accumulations than healthy controls. The medians for maximal length and height of the sand accumulation were 26.5 cm (range 7.3–40.0) and 9.0 cm (12–200), respectively, in horses with colic signs and 8.3 cm (7–156) and 0.9 cm (2–86), respectively, in the control horses originally radiographed for an orthopaedic reason (Kendall et al. 2008). Besides the larger median sand accumulation size in horses with colic, the median grade of sand accumulation for colic horses was higher (4/4), than that for control horses (1.5/4, Kendall et al. 2008). Sand accumulations in horses with colic were more homogenous and opaque than those of the control horses, but location of sand was not associated with colic (Kendall et al. 2008). A significant linear relationship was detected between overall radiographic opacity, homogeneity and the clinical diagnosis of sand colic (Keppie et al. 2008). With an objective score higher than 7 out of 12 (Table 4), there was an 83% chance of a true-positive diagnosis of sand colic (Keppie et al. 2008).

2.4.2 ULTRASONOGRAPHY

Use of ultrasonography for the diagnosis of sand accumulation is not as common or as widely studied as radiography. Transabdominal ultrasonography is part of routine colic work-up in some hospitals, and is also used in the diagnosis of horses with chronic or recurrent GI problems (Reef 1988, Busoni et al. 2011, Hines 2018). A convex probe is commonly used for transabdominal ultrasonography, but even a 5 MHz rectal probe can provide an image of the ventral abdomen and the ventral large colon (Korolainen & Ruohoniemi 2002). A fast localized abdominal sonography of horses (FLASH) technique has been developed especially for the assessment of horses with colic, with clinically most useful windows selected into the protocol (Busoni et al. 2011). One of the windows of the FLASH technique is the ventral abdomen, which is the most likely localization for large colon sand accumulation (Korolainen & Ruohoniemi 2002). From the ventral window, one can evaluate the layers of the abdominal wall, the amount of abdominal fluid, the thickness of the colon wall and the movement and sacculation of the colon and its contents (Korolainen & Ruohoniemi 2002, Busoni et al. 2011). Sand-filled large colon is hyperechoic, causing varying acoustic shadowing (Korolainen & Ruohoniemi 2002). The sacculations can be flattened and the colon amotile. In cases of large, heavy sand accumulations, the intestine may lie against the ventral abdominal wall, even compressing the ventral abdominal fat (Korolainen & Ruohoniemi 2002). Transabdominal ultrasonography has also been found to be a practical method to monitor resolution of sand accumulation (Korolainen et al. 2003).

In a comparison of ultrasonographic and radiographic diagnosis of sand accumulation, ultrasonography had a sensitivity of 87.5% and specificity of 87.5% for detecting sand accumulations compared to radiography (Korolainen & Ruohoniemi 2002). However, in 22 colic horses, no cases were diagnosed to have sand accumulation on trans-abdominal ultrasonograpy even though radiography confirmed the diagnosis of sand accumulation in all of them (Graubner et al. 2017). Small sand accumulations and sand accumulations located dorsally can give false-negative results with ultrasonography (Korolainen & Ruohoniemi 2002, Korolainen et al. 2003). Ultrasonography does not measure the height of the accumulation or give as accurate an estimate of the amount of sand as radiography, sometimes overestimating especially flat and elongated accumulations (Korolainen et al. 2003). Ultrasonography is useful in situations where radiographic equipment is unavailable (Korolainen et al. 2003).

2.4.3 AUSCULTATION

Auscultation has been found to be a useful method to diagnose large colon sand accumulation (Rollins & Clement 1979, Ragle et al. 1989b, Hammock et al. 1998). The sounds caused by sand are different from other intestinal sounds, being described as a paper bag partially filled with sand and slowly rotated, or waves hitting the beach (Ragle et al. 1989b). Anecdotally, coarse sand reportedly produced louder sounds than fine sand (Ragle et al. 1989b). However, sounds related to sand accumulation cannot be heard at conventional sites, but at the lowest part of the ventral abdomen, caudal to the xiphoid process (Rollins & Clement 1979, Ragle et al. 1989b).

The amount of sand inducing the aforementioned intestinal sounds has been investigated in research horses. The sounds could be auscultated after horses (500 kg) were administered in total 6.3-10.5 kg of sand over 3-5 days (Ragle et al. 1989b). The amount of sand retrieved from horses in necropsy in other studies has been multiple times this amount, up to 68 kg (Ford & Lokai 1979, Udenberg 1979). The colon must have motility and contact with the ventral abdominal wall to produce sounds, i.e. a large accumulation without motility can be silent (Ragle et al. 1989b).

2.4.4 FAECAL SAND TEST (SEDIMENTATION)

The faecal sand test has been used as a semi-quantitative test to diagnose large colon sand accumulations, but it is poorly related to the presence of sand accumulation in the colon (Edens & Cargile 1997, Korolainen et al. 2003, Hart et al. 2013, Hukkinen 2015). Even clinically normal horses excrete some sand in their faeces, especially in sandy regions (Colahan 1987, Landes et al. 2009, Maceido Filgueiras et al. 2009, Cieśla et al. 2011). Faecal sand excretion has varied from 0.13-0.26 mg of sand/g of feces (Cieśla et al. 2011) to 2.29 mg of sand/g of feces (Landes et al. 2008). When a faecal sedimentation (glove) test was compared with abdominal radiography (gold standard), the glove test had a sensitivity of 83% and a specificity of 71% for diagnosing radiographically evident colonic sand accumulation. The positive predictive value was 91%, while the negative predictive value was only 56% (Hukkinen 2015). Even though the faecal sedimentation test is not reliable for diagnosing sand accumulations, it has proven to be useful in monitoring the efficacy of medical treatment (Ruohoniemi et al. 2001, Korolainen et al. 2003). The onset of passing sand into faeces was often associated with radiographic diminishing of the sand (Ruohoniemi et al. 2001).

Table 5. A scoring system for faecal sand described by Husted et al. (2005): 200 g of faeces dissolved in 1 litre of water in a rectal sleeve, hung up and evaluated after 20 min of sedimentation.

Sand test	Subjective evaluation
0	No sand visually or palpably
1	0.5–5 mm, minimum recognizable layer of sand
2	5–10 mm, minimum of one finger
3	10–20 mm, minimum of one finger
4	>20 mm, minimum of one finger

Multiple variations of the faecal sedimentation (glove) test have been described (Colahan 1987, Husted et al. 2005, Rood 2011). The test involves mixing faeces and water and letting the sand sediment to the fingertips of the disposable glove or the bottom of the bag. More than 6-7 mm of sand in the glove fingers has been suggested to indicate an excessive amount of sand in the faeces (Colahan 1987). This amount corresponded to grade 2 in a later study of faecal sand excretion of horses without radiographically confirmed large colon sand accumulation (Husted et al. 2005, Table 5).

Another study repeated the sedimentation test with 20 g of faeces from Silesian foals (age 9-28 weeks), and used a scale of 0 to 3, where 0 was no sand, 1 visible but impalpable trace of sand, 2 visible and palpable small amount of sand and 3 larger amount of sand visible without palpation (Siwinska et al. 2019). In the sedimentation test, the sand was palpable in the faeces of 57.1% of the foals and clearly visible (grade 2-3) in 42.9% of the foals without any clinical signs (Siwinska et al. 2019). The sedimentation test had a correlation coefficient of 0.47 for detecting sand with transabdominal ultrasonography performed using the FLASH technique (Siwinska et al. 2019). In the quantitative sand assessment by Siwinska et al. (2019) the amount of excreted sand was minimal compared to that previously described by Landes et al. (2008) in adult horses. The author proposed differences to be due to foal and adult colon, and the foals being on a pasture (Siwinska et al. 2019).

2.4.5 OTHER METHODS OF DIAGNOSIS

2.4.5.1 Abdominal palpation per rectum

Abdominal palpation *per rectum* is one of the most important procedures when evaluating a horse with colic. Unfortunately, it is often not useful with sand-related problems, although sand sometimes can be palpated in the faeces or in part of the GI tract such as the pelvic flexure (Ferraro 1973, Ford & Lokai 1979, Colahan 1987, Ragle et al. 1989a, Hart et al. 2013). Sand accumulates most frequently in sites that are not palpable, or sand can displace the affected segment of colon ventrally, thus not being palpable (Colahan 1987). In a retrospective study, 24% of radiographically diagnosed sand accumulations in horses with colic also were confirmed to have sand based on palpation *per rectum* (Hart et al. 2013).

2.4.5.2 Abdominocentesis

There are no reports about changes in the peritoneal fluid specific for sand accumulation, as the samples have been taken from horses with colic, and the findings are most likely reflective of bowel viability (Hart et al. 2013, Kilcoyne et al. 2017). Abdominocentesis with iatrogenic enterocentesis is not an appropriate method to diagnose sand accumulation, but many studies have reported diagnosis of sand accumulation by accidental enterocentesis (Udenberg 1979, Colahan 1987, Specht & Colahan 1988, Ragle et al. 1989a, Kilcoyne et al. 2017). Whether the reason for inadvertent enterocentesis is solely the ventral position of the sand accumulation due to its weight, or changes in the colon wall remains to be elucidated. Even with a large colon sand accumulation, the enterocentesis fluid does not always contain sand (Specht & Colahan 1988). Sometimes sand can be felt with the tip of the instrument during abominocentesis (Ragle et al. 1989a).

2.5 TREATMENT OF ACUTE COLIC CAUSED BY SAND ACCUMULATION

If a horse with abdominal sand accumulation presents with colic signs, the patient must first be treated according to its clinical findings (Colahan 1987, Edens & Gargyle 1997, Hart et al. 2013, Kilcoyne et al. 2017). However, awareness of the presence of sand accumulation is beneficial when planning the best approach to diagnosis and treatment. Abdominocentesis needs to be performed with caution, as it carries the risk of enterocentesis (Specht & Colahan 1988, Ragle et al. 1989a, Kilcoyne et al. 2017), and the colon might be more prone to rupture than non-sand related colonic impactions when lifted from the abdomen during surgery (Ragle et al. 1989a). Current data show that most horses with sand colic can be treated medically (Hart et al. 2013, Kilcoyne et al. 2017). The cornerstone of treatment is to assess of the horse's current systemic status and degree of pain and to change the treatment plan if needed according to clinical changes or unresponsiveness to treatment (Colahan 1987, Hart et al. 2013, Kilcoyne et al. 2017).

2.5.1 MEDICAL TREATMENT OF ACUTE COLIC CAUSED BY SAND ACCUMULATION

The mainstay of medical treatment is to control pain, increase hydration of intestinal contents and promote gastrointestinal motility. This is achieved with fluid therapy, analgesics, cathartics and withholding feed until the impaction is resolved (Rakestraw & Hardy 2012).

Overhydration with a combination of enteral cathartics and fluids is thought to promote rehydration of the digesta in feed impactions (Freeman et al. 1992, Lopes et al. 2002), but has not been investigated in cases of sand impaction. Enteral fluids can be administered by intermittent nasogastric intubation or as a continuous infusion via an indwelling feeding tube. Intermittent nasogastric intubation with enteral fluids also stimulates the gastrocolic reflex, thus increasing colonic motility (Freeman et al. 1992). Enteral fluid therapy should be given as an isotonic electrolyte solution (Lopes et al. 2004, Rakestraw & Hardy 2012). Even though nasogastric intubation is a simple and commonly used procedure, it can cause trauma to nasal and pharyngeal mucous membranes, and in extreme cases even sinusitis (Nieto et al. 2014). Intravenous fluids are needed when dehydration is evident or when a condition such as gastric reflux prevents enteral fluid therapy, and has been suggested to benefit impactions of long (>24 hours) duration (Rakestraw & Hardy 2012).

The early methods of treating acute sand colic with ammonium carbonate, a drench of linseed oil and turpentine (McIntyre 1917), or dioctyl sodium succinate (DSS) (Ferraro 1973, Udenberg 1979) are currently not recommended. Also a more conservative approach has been suggested; if the horse does not have acute signs of abdominal pain, it can be fed laxatives at home instead of being transported to a clinic or hospital for treatment (Ferraro 1973). The current mainstays of treatment, comprising lubrication and breaking down of the obstructing mass, together with relieving pain and maintaining organ function, have been in use for decades and are recommended in textbooks (Ford & Lokai 1979, Udenberg 1979, Colahan 1987, Sullins 1990, Hanson 2002, Rakestraw & Hardy 2012, Hardy 2017, Sanchez 2017). In the most recent retrospective case series (Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017), horses with acute sand colic were treated medically with intravenous and enteral fluids, most of them also receiving laxatives, mainly mineral oil and psyllium (Hart et al. 2013, Kilcoyne et al. 2017). Medical treatment was successful in 52/62 (83.9%) of horses (Hart et al. 2013) and in 109/153 (71.2%) of horses (Kilcoyne et al. 2017); the remainder of horses in these studies either had surgery or were euthanized. The radiographic grade of the sand accumulation was not associated with the horse's survival in a study performed by Hart et al. (2013). When evaluating the efficacy of treatment based on change in radiographic grade, half of the horses (13/27) had improved 1-2 grades with treatment at a median of 5 days (range 1–75 days) after the first radiographs (Hart et al. 2013).

2.5.2 SURGICAL TREATMENT OF ACUTE COLIC CAUSED BY SAND IMPACTION

In current studies, 14-31% of horses with sand colic required surgery (Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017). In an earlier study, 5.2% of horses undergoing colic surgery were reported to have a large colon sand impaction (Johnson & Keller 2005). Impacted sand is generally removed via a pelvic flexure enterotomy similar to impaction of digesta, but the weight of the sand and colonic wall injury caused by sand makes the colon more prone to rupture (Specht & Colahan 1988, Ragle et al. 1989a, Ragle et al. 1992, Maxwell 2003, Granot et al. 2008, Kilcoyne et al. 2017).

The decision to treat colic caused by a sand impaction surgically is usually not an emergency. In one of the earlier studies, the median duration of colic prior to surgery was 2.7 days (Ragle et al. 1989a), 20 years later 1.8 ± 1.67 days (Granot et al. 2008). In most current studies, horses were taken to surgery within 24 hours of arrival at one hospital (Kilcoyne et al. 2017), whereas at another hospital the mean time from hospital admission to surgery was 5 days (Hart et al. 2013). The amount of sand is not a criterion for surgical intervention, concurrent clinical findings being more important (Rakestraw & Hardy 2012, Kilcoyne et al. 2017). However, in a study performed by Kilcoyne et al (2017), colic cases treated surgically had larger sand accumulations and a greater number of accumulations compared to colic cases managed medically in their population.

Removal of the sand accumulation through a pelvic flexure enterotomy is the mainstay of surgical treatment and so a ventral midline approach is recommended (Sullins 1990, Rakestraw & Hardy 2012). For situations where lifting the colon up onto a colon tray is impossible, Specht & Colahan (1988) describe inserting both a nasogastric tube and a hose together into the colonic lumen via the pelvic flexure enterotomy with a minimally exteriorized colon. The hose provides water and the nasogastric tube egress flow of water, sand and digesta. It is often impossible to remove all of the sand from the right dorsal and transverse colon (Ragle et al. 1989a). If all of the impaction cannot be removed, it needs to be broken down with fluid and massage as much as possible to prevent re-impaction. In a miniature horse, multiple enterotomies were needed to enable removal of the sand from the right dorsal colon (Maxwell 2003).

Reported complications during surgery include tears and ruptures of the heavy sand-filled colon, especially when operating on a chronic case (Specht & Colahan 1988, Granot et al. 2008, Rakestraw & Hardy 2012, Hardy 2017, Kilcoyne et al. 2017). Complications after surgery include endotoxaemia (Specht & Colahan 1988), diarrhoea (Specht & Colahan 1988, Granot et al. 2008, Rakestraw & Hardy 2012), fever (Kilcoyne et al. 2017), septic peritonitis (Specht & Colahan 1988, Ragle et al. 1989a, Rakestraw & Hardy 2012), incisional hernia (Specht & Colahan 1988, Granot et al. 2008, Kilcoyne et al. 2017), laminitis (Ragle et al. 1989a, Granot et al. 2008), internal bleeding (Granot et al. 2008) and adhesions (Ragle et al. 1992).

The prognosis for treating horses with sand accumulation and related colic in recent studies has been good; up to 90-95% of horses survive with medical or surgical treatment, compared with 75% short-term survival after surgery some decades ago (Specht & Colahan 1988, Ragle et al. 1989a, Granot et al. 2008, Hart et al. 2013, Kilcoyne et al. 2017). Long-term survival of up to 100% for discharged horses has been reported (Granot et al. 2008). Some horses have been described to have recurrent sand colic during the follow-up period, considered most likely due to ongoing sand ingestion (Ragle et al. 1989a, Kilcoyne et al. 2017).

2.6 COMPOUNDS USED FOR REMOVAL OF LARGE COLON SAND ACCUMULATION

Although the most important action is to prevent access to sandy soil and gravel, treating the sand accumulation using laxatives is common (Colahan 1987, Sullins 1990). In some horses, limiting access to sand is enough, and pasture turnout seems to help to clear the sand accumulation (Ragle et al. 1989a, Hammock et al. 1998, Ruohoniemi et al. 2001). If therapy is needed, it must be continued for a sufficient period of time and efficacy of treatment preferably evaluated using radiography, as often clinical signs can resolve even though sand accumulation is still present in the colon (Colahan 1987).

Clinicians have found laxatives and cathartics useful when treating large colon sand accumulation and related clinical signs (McIntyre 1917, Ferraro 1973, Bertone et al. 1988, Ruohoniemi et al. 2001, Hart et al. 2013, Kilcoyne et al. 2017). However, there are differences between laxatives, not only in the mechanisms, but also in their usefulness regarding sand accumulation removal. Both laxatives and cathartics promote defecation, but laxatives do so by promoting elimination of soft-formed stool, whereas cathartics produce more fluid evacuation into the colon and loss of fluid with faeces (Boothe & Jenkins 1995). Laxatives can be divided into lubricant laxatives (mineral oil), which are not absorbed or metabolized, and bulk-forming laxatives (psyllium), which absorb water to produce a gel. Cathartics can be osmotic (magnesium sulphate, later $MgSO_4$) or irritant (castor oil, DSS), but both have the same mechanism of drawing water into the intestinal lumen and enhancing movement of the intestine (Boothe & Jenkins 1995).

In healthy horses, nasogastric intubation and the gastrocolic reflex linked to the streching of the stomach wall may produce some of the early intestinal responses to laxatives, instead of them arising from any specific compound (Freeman et al. 1992). The effect of gastrocolic reflex has not been examined in conjunction with treatment of sand accumulations; however, when half of a small group of research ponies refused to eat psyllium, no difference was detected in outcome whether psyllium was administered via nasogastic intubation or fed (Hammock et al. 1998).

2.6.1 PSYLLIUM (PLANTAGO OVATA)

Medical psyllium (*Plantago ovata)* is a group of the *Plantago* species. It is produced mainly for its mucilage content, which is obtained from the husk (*Testa ispaghula*). Psyllium seed is categorized as a soluble fibre, compared with corn and wheat, which are insoluble (Pirotta 2009). It contains multiple active substances, e.g. 4-O-methylglucuronic acid, aucubin, b-sitosterol, campesterol, L-asparagine, L-cystine, linoleic acid, mucilage, oleic acid, palmitic acid, polysaccharides, rhamnose, sterol, tannins and arabinoxylans with high gel-forming property (Mehmood et al. 2011).

Currently, psyllium is widely used in humans for both treatment of constipation and diarrhoea. It has also been investigated in human irritable bowel syndrome (Pirotta 2009), intestinal inflammation (Rodriguez-Cabezas et al. 2003) and amoebic dysentery (Zaman et al. 2002). The variety of effects of psyllium in the GI tract seems to increase the more psyllium is studied. Originally, the action of psyllium was proposed to be based on the bulk effect of poorly fermented fibre (Bourquin et al. 1993, Campbell et al. 1997). The unfermented gel component of psyllium seed husk has been shown to act as a lubricant and promote laxation in humans (Marlett et al. 2000). It makes faeces bulkier and increases the water content (Marlett et al. 2000). On the other hand, in a lactulose-induced diarrhoea model in humans, psyllium delayed gastric emptying due to increased meal viscosity and slowed transit in some parts of the large intestine (Washington et al. 1998). Contrary to this, psyllium did not delay colon transit in patients with constipation (Ashraf et al. 1995).

In humans, psyllium has several other uses, such as lowering the plasma cholesterol concentration, and inhibiting colonic inflammation (Anderson et al. 1988, Pastors et al. 1991, Rodriguez-Cabezas et al. 2003). Psyllium has been shown to lower post-prandial insulin concentration, likely due to reduced absorption of glucose (Moreno et al. 2003). Other recent *in-vitro* studies with psyllium involve its effect on cytokines (Yakoob et al. 2016) and its use as a drug carrier to the colon (Singh et al. 2007, Singh et al. 2008, Rosu 2014).

In both live rats and the isolated intestine of the rabbit and guinea pig, the gut stimulatory effect of psyllium is mediated through muscarinic receptors and partially by 5-HT receptors, which could explain more of the laxative effect than sole high fibre and mucilaginous content (Mehmood et al. 2011). However, psyllium also has gut movement inhibitory activity, most likely by blocking Ca^{2+} channels and by activating NO-cyclic guanosine monophosphate pathways. The responses of equine intestinal tissue to psyllium have not been investigated, but horses have a similar type of hind-gut digestion and fermentation to the rabbits and guinea pigs investigated by Mehmood et al. (2011). Horses could respond to psyllium similarly to humans and laboratory rodents.

Adverse effects of psyllium have been reported. An Arabian mare had a gastric bezoar formed from psyllium which obstructed the pylorus and duodenum. The horse was euthanized due to gastric rupture (Bergstrom et al. 2018). She had been fed pelleted psyllium (1.13 g/kg body weight (BW) q24 h) for four days, which was above the manufacturer's recommendation of 0.31 to 0.46 g/kg BW, q24 h. Moreover, the Food and Drug Administration (FDA) of USA has forbidden humans to use granular formulations of psyllium, due to their propensity to cause oesophageal obstruction and bezoars (Noble & Grannis 1984, Shuren et al. 2007, Hefny et al. 2018).

2.6.1.1 Experimental studies in research horses

In horses, experimental studies have shown either no effect (Lieb 1997, Hammock et al. 1998, Lieb & Weise 1999) or some effect (Hotwagner & Iben 2008, Landes et al. 2008, Cieśla et al. 2011) of psyllium on large colon sand accumulation clearance or fecal sand output. The psyllium doses, route of administration and duration of treatment are shown in Table 6.

Lieb (1997) compared the effect of feeding single doses of psyllium (0.5 g/kg BW), wheat bran (1 g/kg BW) or mineral oil (8 g/kg BW of a ½ mineral oil ½ water mixture) in a 4x4 Latin square design study, and measured the amount of administered sand (5 kg via nasogastric tube (NGT)) excreted in faeces after each treatment. Control horses without any treatment had better sand clearance in 5 days (78% sand recovered) than horses with any of the treatments (70-73% sand recovered). However, psyllium increased the faecal sand content during the first 24 hours (Lieb 1997).

When sand was surgically placed into the caecum, and the ponies were either having no treatment (6 ponies), fed psyllium (3 ponies), or psyllium was administered via nasogastric intubation (3 ponies), ponies treated with psyllium had more sand left (39%) than untreated controls (27%, $P<0.05$) (Hammock et al. 1998). The most recent study with artificial sand administration investigated the effect of mineral oil with and without psyllium (dose 500 g q12h) (Hotwagner & Iben 2008). Combined mineral oil and psyllium administration resulted in a significant increase in crude ash excretion (mean 51% of administered sand mass) compared to mineral oil alone (mean 26%, $P<0.001$) (Hotwagner & Iben 2008). However, these studies might not reflect the response of naturally acquired sand accumulation to treatment.

Some research groups have studied response to psyllium by measuring faecal sand excretion in healthy horses (Table 6), without experimentally administered sand or known large colon sand accumulation (Landes et al. 2008, Cieśla et al. 2011). The studied treatments have been psyllium alone (Cieśla et al. 2011), or in combination with pre- and probiotics (Landes et al. 2008). Both studies showed increased faecal sand excretion after psyllium administration. A small amount of psyllium increased sand excretion in faeces threefold compared to an untreated control group (Cieśla et al. 2011). Psyllium administered with pre- and probiotics increased sand excretion 2.5 times from the baseline excretion in the same horses (Landes et al. 2008). The onset of increased faecal sand excretion varied with Cieśla et al. (2011) observing an increase in sand excretion as early as day 2, whereas Landes et al. (2008) did not observe an increase until day 4.

Table 6. Dose and administration of psyllium given to research horses in selected prospective studies.

Dose of psyllium	Route	Duration	Effect on sand excretion	Reference
1 g/kg BW	Feed/ NGT	11 days	No effect	Hammock et al. (1989)
0.5 g/kg BW	Feed	1 day	No effect	Lieb (1997)
225 g/horse	Feed	6 days	No effect	Lieb & Weise (1999)
0.5 g/kg BW	Feed	35 days	Increased	Landes et al. (2008)
2x500 g/horse	NGT	5 days q12h	Increased	Hotwagner & Iben (2009)
3x70 g/horse	Feed	5 days q8h	Increased	Cieśla et al. (2011)

NGT= nasogastric tube

2.6.1.2 Clinical use of psyllium in horses

No randomized clinical trials exist on the efficacy of psyllium for clearing equine large colon sand accumulations, but some case reports have been published (Ramey & Reinertson 1984, Bertone et al. 1988, Ruohoniemi et al.

2001, Korolainen et al. 2003, Hart et al. 2013). The first references regarding psyllium for treatment of equine sand accumulation appear in the 1970s by Ferraro (1973), who recommended flake-form (granular) psyllium 0.5-1 g/kg BW first via nasogastric tubing for acute colic and then a smaller amount given by the owner with feed for one week. A foal with sand-associated diarrhoea was treated with psyllium mucilloid 1 g/kg BW daily, and within 5 days it had improved clinically (Ramey & Reinertson 1984). Ruohoniemi et al. (2001) reported on one case that showed no response in the amount of excreted sand after ingesting psyllium for over six weeks, but the sand accumulation resolved within three days following administration of mineral oil and $MgSO_4$. Hart et al. (2013) and Kilcoyne et al. (2017) reported use of psyllium in their retrospective reports, but the treatment of horses varied too much to compare efficacy of the different laxatives used for sand colic.

Administering psyllium via nasogastric tube is not a straightforward procedure. When mixed with water, it soon becomes gelatinous and no longer passes through the tube. To avoid gelatinization, it needs to be mixed and pumped at the same time (Colahan 1987). Gelatinization can be avoided if psyllium is mixed with mineral oil for administration (Sanchez 2018). Psyllium can also be given to horses with food; 125 g/feeding has been suggested to be palatable when mixed with grain (Ferraro 1973, Colahan 1987). The palatability of psyllium has been questioned with research horses; some take time to start eating it and others refuse to eat it at all (Hammock et al. 1998, Cieśla et al. 2011).

Multiple authors mention the possibility of psyllium losing its efficacy to remove sand accumulations when used long-term (Edens & Gargyle 1997, Hammock et al. 1998, Hanson 2002, Mair 2002, Hardy 2017). Hammock et al. (1998) based their concern about feeding psyllium for too long on laboratory studies *in vitro* with human fecal inoculum (Bourquin et al. 1993, Campbell et al. 1997). However, in horses, Landes et al. (2008) have demonstrated that 0.5 g/kg BW psyllium (when fed with pro- and prebiotics daily) enhanced faecal sand excretion from baseline even on day 35 after starting the psyllium administration. Many studies have used psyllium for months in rats and in human patients, but the laxative effect has not been the main target of these studies, and possible reduction of laxative effect of psyllium has not been investigated.

2.6.2 MAGNESIUM SULPHATE

In horses, magnesium is absorbed predominantly passively from the small intestine. In one study, 46-56% of the total magnesium was absorbed before reaching the caecum, with very little absorbed from the caecum or colon (Hintz & Schryver 1972). The total magnesium digestibility was found to be 52% in the high concentrate diet and 61% in the high forage diet (Hintz & Schryver 1972). Renal function regulates the body magnesium content (Fettman 1995). Absorption normally begins one hour after administration and can last up to eight hours. Mg^{2+} is absorbed from the small intestine by passive concentration-dependent paracellular diffusion and by transcellular active transport (Hintz & Schryver 1972).

Saline cathartics such as $MgSO_4$ have been speculated to enhance fluid accumulation in the colon, enhancing sand removal (Colahan 1987). $MgSO_4$ is a commonly used osmotic laxative for large colon impactions. It promotes fluid secretion into the colonic lumen and stimulates intestinal motility. Based on a study with rats, the laxative effect produced by $MgSO_4$ has been proposed to not simply be the result of changes in osmotic pressure but also to be associated with the increased expression of a water channel, aquaporin-3, in the mucosal epithelial cells of the colon (Ikarashi et al. 2011). In horses, the commonly recommended dose for enteral treatment via nasogastric intubation is 0.5-1 g/kg BW/day mixed with water. A dose of 1 g/kg BW of $MgSO_4$ resulted in a greater total weight of faeces excreted and faecal water excretion than a lower dose (0.5 g/kg BW), DSS or water alone (Freeman et al. 1992). A dose of 1 g/kg BW of $MgSO_4$ also increased horses' water consumption at 12 hours after administration (Freeman et al. 1992).

An overdose of $MgSO_4$, or administering it to a horse with renal failure and abnormal magnesium excretion can lead to high serum magnesium levels and clinical signs of toxicity. In a clinical report of two horses with an overdose (2.0 g/kg BW) of $MgSO_4$, clinical signs comprised sweating, trembling, tachycardia, tachypnoea, circulatory changes and, in the end, recumbency with signs of flaccid paralysis (Henninger & Horst 1997). Both horses might have been predisposed to overdose, as one horse had azotemia and the other had been medicated with DSS, which alters mucosal permeability (Clark & Becht 1987, Edens & Cargile 1997). Magnesium toxicity is treated by increasing renal flow to enhance excretion and using calcium salts to replace magnesium (Fettman 1995, Henninger & Horst 1997).

In clinical studies of large colon sand accumulations, even with repeated administrations of $MgSO_4$, diarrhoea has not been a problem (Ruohoniemi et al. 2001). The effect of $MgSO_4$ alone on clearance of naturally acquired sand accumulation has not been reported, but it has been used in various combinations with other laxatives (Ruohoniemi et al. 2001, Hart et al. 2013, Kilcoyne et al. 2017).

2.6.3 MINERAL OIL

Mineral oil (paraffin oil) is a mixture of aliphatic hydrocarbons. Because of its limited absorption, it is used as an intestinal lubricant and as a marker of intestinal transit (Rakestraw & Hardy 2012). The recommended dose is 5 to 10 mL/kg BW, and normal transit time is 12 to 24 hours. Mineral oil is still commonly used for treatment of sand accumulations (Hart et al. 2013, Sanchez 2017), but many authors have been concerned that it may by-pass firm sand impactions (Ferraro 1973, Ramey & Reinertson 1984, Colahan 1987, Sullins 1990, Rakestraw & Hardy 2012, Hardy 2017). Use of mineral oil (2 L for 0.5-1 g/kg BW of psyllium) may facilitate the administration of psyllium by preventing gelatinization (Sanchez 2017). In a study comparing mineral oil alone (2 L oil/500 kg horse q24h) and combined with psyllium (500 g q12h), the combination removed more (mean of 51%, SD ±20.5% and oil alone 26.1%

±17.7%) of the previously administered sand during the five-day treatment period (Hotwagner & Iben 2008). The effect of mineral oil in naturally occurring sand accumulations remains to be investigated.

2.6.4 OTHER LAXATIVES AND FOOD SUPPLEMENTS

DSS has been used as a laxative in horses (Ferraro 1973, Udenberg 1979), but its use currently is infrequent and is not recommended (Rakestraw & Hardy 2012, Talcott 2017). DSS reduces surface tension and allows water to penetrate impacted material, increases intestinal secretion and alters mucosal permeability (Clark & Becht 1987, Edens & Cargile 1997). Use of DSS via nasogastic intubation has been reported with sand colic (Udenberg, 1979), and also as an intraluminal injection during surgery to relieve small colon faecal impactions in American Miniature Horses (Ragle et al. 1992).

DSS is an irritant and is possibly injurious to intestinal mucosa (Moffat et al. 1975). The therapeutic dose (10-66 mg/kg BW) is markedly lower than the reported toxic level (200 mg/kg BW), but there seems to be individual variation, and horses have been reported to have toxic signs even at low doses (Freeman et al. 1992, Southwood et al. 1999, Talcott 2017). High doses have resulted in severe diarrhoea, dehydration and death (Moffat et al. 1975). The use of $MgSO_4$ and DSS in combination may increase the absorption of magnesium from the intestine and lead to magnesium toxicity (Henninger & Horst 1997).

Linseed (*Linum usitatissimum*) is a traditional laxative that has been recommended for use in horses with colonic sand accumulation (McIntyre 1917). Prophylactic use of boiled lindseed (227 g/horse once a week) was noted to decrease the occurrence of colic cases (McIntyre 1917). Compared with other laxatives, linseed has been studied less frequently in horses, but at least one report has suggested that linseed is a reasonable option for treating sand accumulation (Särkijärvi et al. 2010). The area of sand accumulation measured from radiographs of horses receiving 1 g/kg BW of a linseed-based supplement for 11 weeks decreased on average from 145 to 41cm^2 in the treatment group (N=8). The change in the untreated control group (N=8) was from 158 to 95cm^2. However, no significant difference could be detected (P=0.095) between groups (Särkijärvi et al. 2010).

Chia seeds are very similar to linseed and are sometimes used as an alternative to psyllium for sand removal, although there is no evidence of their efficacy. Feeding bran has been recommended previously since it was suggested to be effective and harmless (Colahan 1987).

Multiple pro- and prebiotic formulations are advertised as a method of removal of intestinal sand accumulations and to have other beneficial effects on the intestine. Combining psyllium with probiotics and prebiotics (*Saccharomyces cerevisiae, Lactobacillus acidophilus* and *Enterococcus faecium*) led to increased faecal sand excretion during supplementation (Landes et al. 2008), but whether one ingredient was more effective than another remains unknown. Researchers suggested that pro- and prebiotics could contribute to sand clearance by reducing the inflammation caused by the sand, thus improving the health and motility of the intestine (Landes et al. 2008). However, in another study psyllium alone resulted in a similar increase of sand excretion compared to the combination of psyllium and pre- or probiotics (Cieśla et al. 2011).

2.7 PREVENTION OF LARGE COLON SAND ACCUMULATION

Research on prevention of large colon sand accumulation is scarce. Authors from previous studies have suggested ensuring adequate food and mineral supplementation (Rollins & Clement 1979, Ramey & Reinertson 1984, Bertone et al. 1988, Ragle et al. 1989b), preventing food from dropping onto the sandy ground (Udenberg 1979, Ragle et al. 1989b) and for horses at pasture ensuring that there is sufficient feed, either by rotation of fields or supplemental feeding

(Snyder & Spier 1990, Sullins 1990). A reasonable amount of hay (1.5 - 2.5 % BW) seemed to help some horses reduce large colon sand accumulation (Lieb 1997, Lieb and Weise 1999). Feeding sand-free hay indoors before letting the horse out to pasture has been proposed to reduce the intensity of grazing activity and subsequent exposure to accidental sand ingestion (Hanson 2002).

Some horses might seek the salty taste in soil, and therefore, sodium supplementation may stop geophagia in these individuals (McIntyre 1917). There are no controlled studies about preventive methods for sand accumulation. However, anecdotal experience exists, and several authors have suggested various methods to prevent horses eating sand (Udenberg 1979, Colahan 1987, Lieb & Weise 1999, Hanson 2002). One author recommended not feeding horses on the ground or even discarding nets or overhead slatted bunkers, where food falls down and horses eat part of it from the ground (Udenberg 1979). Placing rubber mats under feeding containers and keeping them clean of sand should help unless the horse is especially seeking to ingest sand (Lieb & Weise 1999). Pasture with a good grass cover is recommended (Lieb & Weise 1999). In extreme cases, if sand eating cannot be otherwise prevented, the horse must be confined to a stall or muzzled while at pasture (Colahan 1987).

3 OBJECTIVES OF THE STUDY

The overall hypotheses of the study series were that naturally occurring equine large colon sand accumulation manifests itself with various clinical signs, and affected horses respond to treatment with laxatives. In the questionnaire study (I), the hypotheses were that clinical signs would be associated with the amount of colonic sand accumulation in affected horses, and management and behavioural factors would be related to the degree of sand accumulation. The hypothesis for the retrospective study (II) was that nasogastric intubation of laxatives in hospital would be more efficient for sand removal than feeding psyllium at home. The hypothesis for the prospective randomized clinical trials (III, IV) was that the combination treatment with psyllium and $MgSO_4$ would remove more sand than either treatment alone or with no treatment.

The overall objectives were to identify clinical signs associated with large colon sand accumulation and to determine an optimal method of medically treating large colon sand accumulation in horses.

Detailed objectives were as follows:

1. To describe associations between equine large colon sand accumulation and clinical signs (I, II).
2. To investigate associations between equine large colon sand accumulations and behavioural traits and management-related factors (I).
3. To compare the efficacy of feeding psyllium with laxative administration via a NGT for removal of equine large colon sand accumulations (II).
4. To determine which of the studied medications (oral psyllium, nasogastric intubation of psyllium, $MgSO_4$ or their combination) is the most effective for removal of sand accumulations in the equine large colon (II-IV).

4 MATERIALS AND METHODS

Study I was an internet-based owner questionnaire, Study II was a retrospective clinical study and Studies III and IV were prospective randomized controlled clinical trials.

4.1 ANIMAL WELFARE AND ETHICAL EVALUATIONS

Due to the questionnaire-based format of Study I and the retrospective nature of Studies I and II, they did not require an ethical approval. In Study I, owners gave the information voluntarily and anonymously.

Both prospective studies (III, IV) were evaluated by the Ethics Committee of the University of Helsinki (III) or the Eläinkoelautakunta (ELLA, Evaluation of Animal Research Board, IV) and were approved by the Finnish Medicines Agency (Fimea), due to psyllium and $MgSO_4$ being used as a medicine. Informed consent from the owner, or the trainer acting as an agent for the owner, was obtained at the time of enrolment onto the study. The owners were blinded to the treatment group assignment until the end of the follow-up period in Studies III and IV.

4.2 RECRUITMENT OF HORSES AND RECORDED DATA

4.2.1 QUESTIONNAIRE

Study I analysed questionnaire responses from owners / carers of horses that had undergone previous radiographic examination for suspected sand accumulation. The horses were assigned to categories based on the amount of sand that had been observed on the radiograph (no sand / small accumulation

/ moderate accumulation / large accumulation). There was no limit to the amount of sand, but the respondent had to remember the estimation of sand area given by the treating veterinarian. A modified version of the grading by Korolainen & Ruohoniemi (2002) was given as a guideline: no sand, small: less than 5 x 15 cm, moderate: 5 x 15 - 10 x 20 cm, large: more than 10 x 20 cm.There were no restrictions regarding horses' size or age. Horses with an unknown amount of sand or radiographs taken before the year 2009 were excluded from Study I.

The questions used in Study I were obtained as part of a larger questionnaire, but only the relevant behavioural and management issues were included in the present study (Table 7). In some of the questions, e.g. questions about clinical signs and stereotypic behaviour, the respondents had an option of open answers.

Table 7. Questionnaire for owners of horses radiographed for abdominal sand accumulation in 2009-2016. These questions were used in Study I.

Question	**Response options**
Breed	Warmblood / Standardbred / Arabian / Finnhorse / Other coldblood breed / Icelandic horse / Pony / Other
Sex	Mare / Gelding / Stallion
Body score	1-9
Age	Numeric
Year of radiograph	Numeric
Season taken*	Autumn / Winter / Spring / Summer
Sand accumulation size	No sand / Mild / Moderate / Large / Presence of sand, but amount unknown** / Height and length in centimetres
Reason for abdominal radiography	Recommendation of veterinarian / Owner has seen horse eating sand / Owner suspicion/ Not known
Potential clinical signs observed by owners	Diarrhoea, loose faeces or free faecal water / Colic / Hyperaesthesia to touch of the abdominal area / Poor performance / Do not know / Other, what?
Feeding management in the paddock	Feeding directly on the ground / Feeding on mats / Feeding from trough / Use of feeding net / No feeding outdoors
Roughage feeding behaviour	Horse does not eat all of the roughage / Horse does not eat roughage that is mixed with soil / Horse eats all of the roughage

Salt lick/ rock	Yes/ No, but salt is added to food / No / Do not know
Horse grouped in paddock	No / Yes, with one horse / Yes, with many horses / Do not know
Position in herd hierarchy	Dominant / Neutral / Subordinate / Horse is not grouped
Use	Pleasure riding / Competition / Riding school / Breeding / Foals and yearlings / Resting / Other
Housing	Stable / Walk in-walk out / Other
Stereotypic behaviour	Oral / Weaving / Other / Do not know / No

**Seasons: Autumn months 9-11, winter 12-2, spring 3-5 and summer 6-8*
*** Horses with unknown amount of sand were excluded from the study*

The survey was made accessible via a dedicated web page (in Finnish) and some horse shows for respondents for 15 months, between September 2015 and November 2016.

4.2.2 RETROSPECTIVE DATA

In Study II, information on horses was gathered from medical records of the University of Helsinki Veterinary Teaching Hospital and Oulu Equine Clinic between 1 January 2009 and 31 December 2014. A total of 1097 horses and ponies had abdominal radiographs taken during this period. Foals less than 6 months of age were excluded. The minimum area of sand accumulation observed on the first radiograph had to be at least 75 cm^2 for Study II inclusion. The horses treated at home had to be re-radiographed within 40 days, and those treated in hospital within 8 days from initiation of treatment. Clinical signs or owner's request for abdominal radiographs were obtained from patient records. The date of the radiograph was used for classification of the season (Autumn months 9-11, Winter 12-2, Spring 3-5, and Summer 6-8).

The included horses (N= 246) were grouped according to their recorded treatment (feeding psyllium at home / nasogastric intubation with laxatives once, followed by feeding psyllium at home / daily nasogastric intubations in hospital). Owners had been given detailed instructions on their horse's medication at home. The Oulu Equine Clinic did not monitor the horses during nights, but in both University and Oulu clinics horses had a clinical exam 1-3 times a day and were monitored for signs of colic or other side effects of treatment, such as diarrhoea or high serum magnesium concentration.

4.2.3 PROSPECTIVE CLINICAL STUDIES

Prospective studies III and IV involved 34 and 40 horses, respectively. Small ponies and miniature horses (body mass <300 kg) and horses younger than 1 year were excluded. The owners of clinical cases presented to the hospital were invited to take part in the study if the horse had a sufficiently large sand accumulation observed on the abdominal radiographs (see section 4.3) and fulfilled other inclusion criteria. Some of the horses with colonic sand accumulation were diagnosed in another clinic and were referred to the university hospital for treatment and participation in the study. In order to be included in the study, horses must not have undergone any treatment for sand accumulation in the previous 24 hours, had shown no acute signs of colic and had normal blood magnesium (<1.20 mmol/L) and creatinine (<170 µmol/L) concentrations.

Horses were randomly assigned to experimental groups in both studies. Treating veterinarians were not blinded to experimental group assignment, but the radiographic evaluation at the end of Study IV was performed by a veterinary radiologist who was blinded to the treatment group.

Horses in Studies III and IV were monitored regularly and their status was evaluated three times a day by students and once a day by one of the investigators. Consistency of faeces was routinely observed in Studies III andIV, but was not consistently recorded, and thus, not reported. Severe colic, diarrhoea, and high plasma magnesium concentration checked on day 4 in Study III were exclusion criteria.

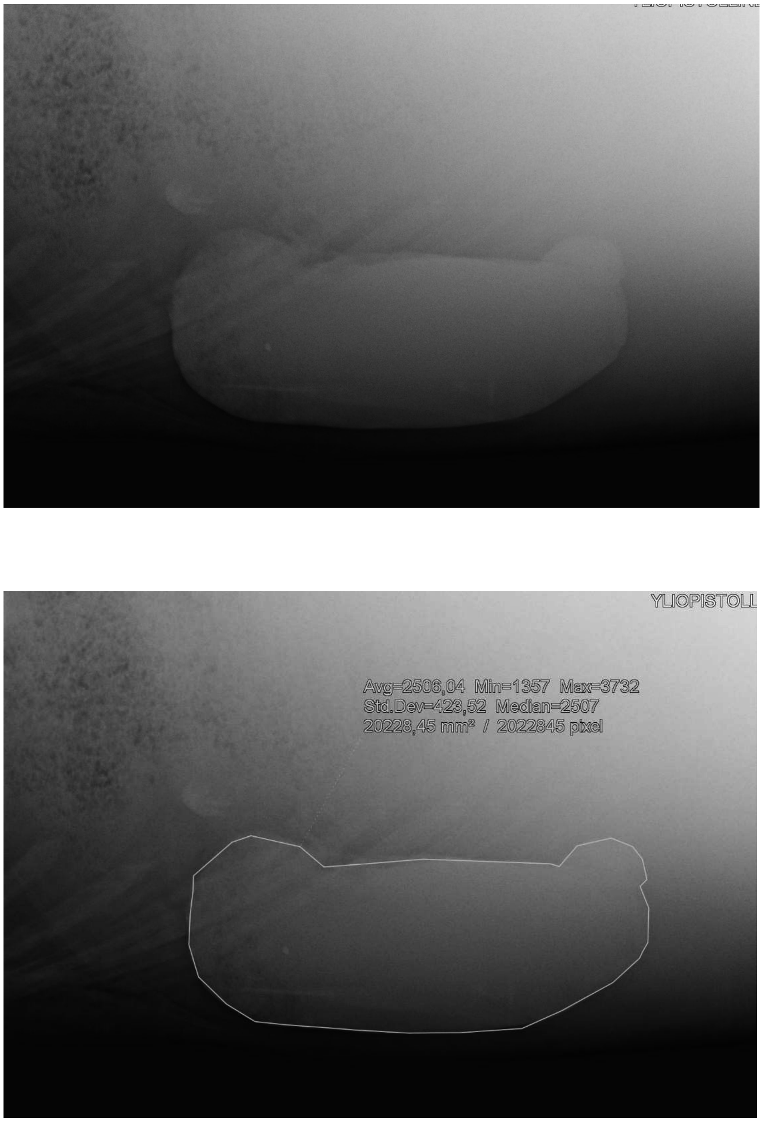
All horses had free access to water at all times. Horses were fed hay ad libitum unless they showed signs of colic, and they were either allowed to go out onto a concrete paddock or were hand-walked daily. In Study IV, access to hay was denied two hours before nasogastric intubation for horses in the treatment group.

4.3 RADIOGRAPHY

The diagnosis was confirmed and the area of sand accumulation was measured from abdominal radiographs. The minimum area of sand on the radiograph in order to be included in Studies II and III was 75 cm^2 (e.g. circa 15 cm x 5 cm) (Kendall et al. 2008) and in Study IV 100 cm^2. In Studies II-IV, the sand accumulation was categorized as resolved if the area of sand was <25 cm^2 on follow-up radiographs.

In Study II, radiographs were taken either at Oulu Equine Clinic or at the University of Helsinki Veterinary Teaching Hospital. In Studies III and IV, all of the radiographs were taken at the University of Helsinki Veterinary Teaching Hospital. The horses were sedated with detomidine (5-10 µg/kg BW), if required. The cranioventral abdomen was radiographed using a right-to-left standing lateral view, with the cassette placed in a ceiling-mounted wall stand with a grid. If the sand accumulation exceeded the cassette size, another radiograph was obtained in most horses.The anode-cassette distance was 1.5 m. A computed radiography system (Shimadzu UD150B-40) was used with maximum tube voltage of 131 kV and current of 80 mAs.

Radiographic evaluation was similar in Studies II and III. The circumference of the sand accumulation was drawn individually for each radiograph, and the area of sand calculated using the system's software tool (Jivex, VISUS Technology Transfer) (Figure 4). In the case of multiple accumulations, their areas were summed. In both studies, the area of sand was measured before and after treatment.



Figures 4a,4b Latero-lateral radiograph of the cranial abdomen in a horse with a sand accumulation in the ventral colon. The area of sand (202 cm^2) was calculated by drawing an outline around the sand accumation (yellow line in Figure 4b).

In Study IV, only the most cranial radiograph of each horse was evaluated (Figure 5), as some of the horses did not have a radiograph of the most caudal part of a large accumulation, or the edges could not be properly visualized. To ensure safe work procedures with radiation, only one image was taken for many horses if the accumulation did not seem to extend much further, or we could calculate the size sufficiently with one image. This image showed the relative size of the accumulation in all horses.

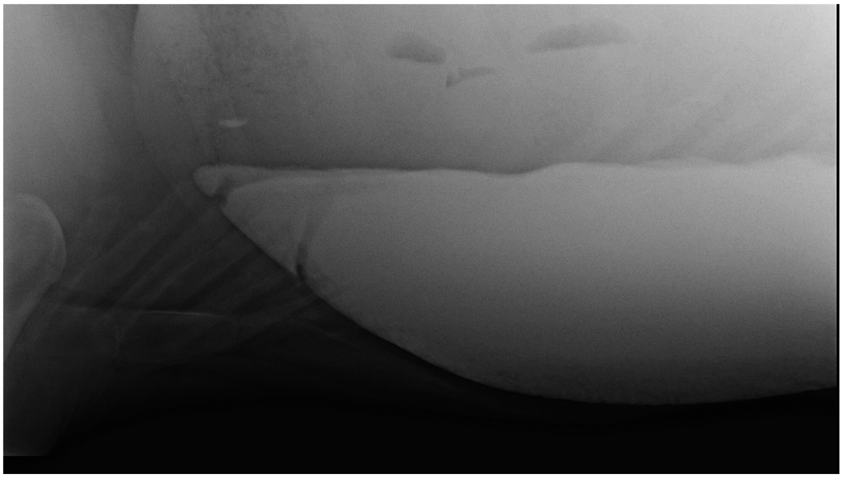


Figure 5. *Latero-lateral view of the cranioventral abdomen in a horse with a large distinct sand accumulation. The caudal aspect of the accumulation extends outside the view, but the relative size of the accumulation can be estimated.*

All the radiographs, taken on days 0, 4, and 7, were evaluated in detail after all horses had been treated in Study IV, despite previous area calculations performed during clinical management. The radiologist was blinded to the horse, date and treatment during the evaluation, which included measuring the height and length of the accumulations and recording their homogeneity, shape and location. After these evaluations, the measured height and length of the accumulations and the previously measured area were compared to confirm the size of the accumulations. In case of discordancy between the measurements, the corresponding radiographs were re-evaluated and a consensus was reached between the treating veterinarian and the veterinary radiologist.

4.4 TREATMENTS

The details of medical treatment in Studies II-IV are presented in Table 8. The treatment regimen used in in the hospital consisted of once daily laxatives administered via nasogastric intubation. Horses receiving laxatives via nasogastric intubation were treated for 3-8 days once a day in Study II and 4-7 days in Studies III and IV, based on the radiographic findings on day 4. Day 4 was chosen for the timing of the follow-up radiograph, based on clinical experience and results of previous studies (Sullins 1990, Ruohoniemi et al. 2001).

Table 8. Medications used for treatment of sand accumulations in Studies II-IV. The nasogastric intubations were administered with 15 mL/kg BW water once a day.

Study	Medication	Route
II	Psyllium 1 g/kg BW with food	Oral
	Combination of psyllium and $MgSO_4$, both 1 g/kg BW	NGT
III	Psyllium 1 g/kg BW	NGT
	$MgSO_4$ 1 g/kg BW	NGT
	Combination of psyllium and $MgSO_4$, both 1 g/kg BW	NGT
IV	Combination of psyllium and MgSO4, both 1 g/kg BW	NGT
	Control; no medication	
II-IV	**Other medication if needed**	
Analgesia	Flunixin meglumine 1.1 mg/kg BW Detomidine 5–20 µg/kg, and butorphanol 10–20 µg/kg BW	i.v. i.v.
Sedation	Detomidine 5-10 µg/kg BW	i.v.

NGT= nasogastric tube

In Study II, the instruction for the oral psyllium dose fed at home was 1 g/kg BW daily, but the actual dose consumed by the horse was not verified. In Study II, the treatment regimen used in the hospital mainly comprised of a combination treatment of psyllium and $MgSO_4$ administered via nasogastric intubation, but if needed the dose was adjusted individually (e.g. horses with high creatinine concentration received a lower dose or no $MgSO_4$). The group with single nasogastric intubation received the combination of $MgSO_4$ and psyllium.

In Study III, horses received a weight-based dose of either $MgSO_4$ or psyllium, or both via nasogastric intubation. In Study IV, horses either received no treatment (controls) or received the combination of $MgSO_4$ and psyllium.

In the hospital, the commercial brand of psyllium was chosen based on the form of psyllium; the granular type was the only available form at the beginning of Study III. Therefore, a similar form was chosen for Study IV to ensure repeatability of dosing, although there were also other forms available, i.e. powder and whole seed.

For nasogastric intubation, the horses were placed in stocks and/or restrained with a nose twitch in their own box stall. The nasogastric tube was placed routinely and the presence of reflux was ruled out before medication was administered. Psyllium (and $MgSO_4$) was mixed with water (15 mL/kg BW) only when ready to administer and was then whisked while pumping to avoid gelatinization. The side of intubation was alternated to prevent mucosal damage.

4.5 STATISTICAL METHODS

In Study I, the descriptive categorical data are presented as percentages. Data about the BCS are presented as median (minimum - maximum).

Differences in the amount of sand between the subcategories of the categorical variables were tested with the Mann-Whitney U test or the Kruskal-Wallis test. Associations between the ordinal scale variables and the size of the sand accumulation were tested using Spearman's rank correlation with Bonferroni correction. The effect of the four pre-selected clinical signs, namely diarrhoea, colic, decreased performance level and hyperesthesia to touch of the abdomen, on the category of the sand accumulation were assessed with cumulative logit-models (SAS System for Windows, version 9.3; SAS Institute Inc., Cary, NC, USA). In addition to assessing the effects of the individual signs, the effects of the two-way combinations of the signs were analysed, as the frequencies of clinical signs were not high enough for multivariate analysis. The cumulative logit-models included the sign or combination of signs as the sole fixed effect. The effects were quantified with odds ratios (ORs) and their 95% confidence intervals (CIs).

In Study II, the the area(s) of sand on the radiographs before and after the treatments were compared with Kruskal-Wallis analysis of variance and the Mann-Whitney U test. Pearson's Chi-squared test was used to compare the proportion of resolved (area less than 25 cm^2 yes/no) sand accumulations between horses in treatment groups 1 to 3, and both Chi-square and Fisher's exact test for pairwise comparisons between the groups.

In Studies III and IV, differences between treatments were considered to be clinically significant if 75% of cases resolved by day 4 in one experimental group and 25% another experimental group. In Study III, using a 95% confidence interval and a power of 80%, the number of cases required per group was calculated to be 13 for two-tailed analysis. In Study IV, 16 horses per group were needed to attain the 5% type I error rate (α) and a power of 90% in two-tailed analysis.

In Study III, the differences in the area of sand in the radiograph were analysed with the Kruskal–Wallis and Mann-Whitney U tests. Pearson's Chi-squared test was used to compare the numbers of resolved horses between treatments on day 4.

In Study IV, the areas of the sand accumulations in the large colon were compared between groups before treatment and on day 4 using Mann-Whitney U test. Two-sided Fisher's exact test was used to compare the number of resolved cases between groups on day 4.

If not mentioned otherwise, all data were analysed using SPSS analytical program for Windows (IBM SPSS statistic version 23 (Studies II–IV) or version 24 (Study I); SPSS GmbH, Munich, Germany). The normality of the data was analysed with the Shapiro-Wilk test in all studies. In all studies, *P*-values of <0.05 were considered to be statistically significant. Due to the non-parametric tests used for the size of the accumulation, ordinal comparisons were used for statistical analysis, thus medians were used instead of means.

5 RESULTS

5.1 SIGNALMENT OF HORSES WITH LARGE COLON SAND ACCUMULATION

Data from 447 horses were included in Study I, and a total of 320 horses (II:246, III:34, IV:40) were included in clinical studies II-IV. Age, weight and sex showed no significant association with the size of the large colon sand accumulation in any of the studies (I-IV).

In Study I the distribution of the size categories of sand accumulation differed significantly between breeds (P=0.002). Table 9 shows the proportion of large accumulations (defined as: >10 x 20 cm) within different breeds. Finnhorses had significantly higher category of sand accumulation than other breeds in Study I (P<0.001). In pairwise comparison between all breeds, the category of accumulation size was significantly higher in Finnhorses than in Warmbloods (Bonferroni corrected P=0.006). The effect of breed was not analysed in Study II. In Studies III and IV, approximately half of the cases were Finnhorses (17/32 and 20/40, respectively), but they were equally divided between the experimental groups.

Table 9. Distribution of breeds and percentage of horses with large sand accumulations (defined as >10 x 20 cm sand in radiographs in Study I. The breed of 2/447 horses was not known.

Breed	Number of horses	% of horses	% of horses with large accumulations within breed
Finnhorse	215	48	43
Warmblood	85	19	20
Pony	59	13	30
Icelandic horse	38	9	8
Standardbred	19	4	30
Other coldblood breed	19	4	47
Other than these	10	2	50
Total	445	99	33

In Study I, the BCS of horses varied from 3 to 9, with a median of 5, and there was no significant association between the size of sand accumulation and BCS. The use of the horses in Study I varied: 295 horses (66%) were used for hobby riding, 78 (17%) for competition, 14 (3%) for riding school, 32 (7%) were foals or yearlings, 10 (2%) were broodmares, 9 (2%) were resting and 9 (2%) were other than these. The categories of sand differed significantly between different use of the horses (*P*=0.018). However, the only statistically significant difference was foals and yearlings having a higher category of sand accumulation than broodmares (*P*=0.03) or competing horses (*P*=0.037).

5.2 CLINICAL MANIFESTATIONS OF LARGE COLON SAND ACCUMULATION

Table 10 shows the reported clinical signs in each sand category.

Table 10. Reported clinical signs in Study I categorized by the size of sand accumulation. One horse may have multiple signs.

Clinical sign	No sand	Mild	Moderate	Large	Total
N of horses	89	79	130	149	447
Colic	26	23	39	76	164
Diarrhoea	41	39	75	86	241
Poor performance	23	25	55	71	174
Hyperaesthesia	33	30	41	62	166
Miscellaneous GI signs	7	13	29	31	80
No problems reported	8	8	5	8	29

In Studies I and II, horses with moderate or large sand accumulation presented with various owner-observed clinical signs, the most common of which are presented in Table 11. Hyperaesthesia was an option only in Study I; in Study II, it was grouped with behavioural changes, and thus, the exact frequency of this sign is not known. In Study II, most horses were brought to hospital because of acute colic. Many horses (52/246 in Study II) had multiple clinical signs.

Table 11. Clinical signs reported in horses with moderate or large sand accumulation (data from Studies I and II). One horse could present with multiple signs.

Clinical sign	Study I (N=273)	Study II (N=246)	Total (N=519)
Colic	115	161	276
Diarrhoea	161	66	227
Poor performance	126	27	153
Hyperaesthesia (I)	103	not reported	103
Behaviour (II)*	not reported	11	11
Miscellaneous GI signs	60	not reported	60
Weight loss	6	10	16
No signs reported	13	15	28

* Hyperaesthesia included.

The frequencies of combinations of clinical signs in Study I are reported in Table 12. In general, the number of horses expressing three or more clinical signs was too low for multivariate analysis.

Table 12. Frequency of incidence of different clinical signs combined (Study I).

Variable	Reported N	%	Not reported N	%
Colic and diarrhoea	85	19.0	362	81.0
Colic and poor performance	54	12.1	393	87.9
Colic and hyperaesthesia	57	12.8	390	87.2
Diarrhoea and poor performance	84	18.8	363	81.2
Diarrhoea and hyperaesthesia	104	23.3	343	76.7
Poor performance and hyperaesthesia	80	17.9	367	82.1
Colic, diarrhoea and hyperaesthesia	41	9.2	406	90.8
Colic, diarrhoea and poor performance	32	7.2	415	92.8
Diarrhoea, hyperaesthesia and poor performance	47	10.5	400	89.5
All four clinical signs	21	4.7	426	95.3
Any three clinical signs	78	17.4	369	82.6

In Study I, the clinical signs of colic and poor performance, but not diarrhoea or hyperaesthesia, were significantly associated with the amount of sand when assessed alone (Table 13). When colic was combined with poor performance, diarrhoea or hyperaesthesia, the odds (probability) of larger sand accumulation were higher than in horses without combinations of these signs. These significant differences were more pronounced for the aforementioned combinations than for individual signs. Also horses with diarrhoea combined with poor performance had significantly greater probability of a larger amount of sand (Table 11). When Finnhorses were evaluated alone, the same combinations of signs were significantly associated with larger sand accumulations.

Table 13. Combination of certain clinical signs compared with size of sand accumulation in all horses, and Finnhorses and other breeds separated (Study I).

Clinical signs	*P*	OR	95% CI
All horses			
Colic	<0.001	2.03	1.42-2.91
Diarrhoea	0.058	1.39	0.99-1.94
Poor performance	<0.001	1.89	1.33-2.68
Hyperaesthesia	0.49	1.31	0.80-1.60
Colic and poor performance	<0.001	4.52	2.54-8.13
Colic and diarrhoea	<0.001	2.64	1.69-4.13
Colic and hyperaesthesia	<0.001	2.69	1.54-4.66
Diarrhoea and poor performance	<0.001	2.12	1.42-3.44
Diarrhoea and hyperaesthesia	0.22	1.29	0.86-1.92
Finnhorses only			
Colic and poor performance	<0.002	3.90	1.67-9.23
Colic and diarrhoea	0.005	2.52	1.32-4.82
Colic and hyperaesthesia	<0.009	2.74	2.73-5.80
Diarrhoea and poor performance	<0.001	4.23	2.04-8.79
Diarrhoea and hyperaesthesia	0.029	2.00	1.07-3.74
Horses of other breeds than Finnhorses			
Colic and poor performance	<0.001	5.48	2.44-12.28
Colic and diarrhoea	0.002	2.68	1.42-5.04
Colic and hyperaesthesia	0.063	2.21	0.96-5.10
Diarrhoea and poor performance	0.189	1.47	0.83-2.64
Diarrhoea and hyperaesthesia	0.846	0.95	0.55-1.62

5.3 INFLUENCE OF SEASON, MANAGEMENT AND HORSE TRAITS

Most horses (63%) in Study II were presented to the clinic between October and December, and a similar tendency towards cases clustering in autumn was observed in Study I (Table 14). However, no significant association was detected between the size category of the sand accumulation and the season (Study I).

None of the feeding or management factors investigated in Study I (method of feeding roughage, salt lick provision, grouping, housing: Table 7) were significantly associated with the size category of the sand accumulation. The distribution of the categories of sand accumulation was not associated with observed sand eating (P=0.228). Owners had seen only 69 horses eating sand. However, the most common reason for abdominal radiography of the horse had been owner's suspicion of sand accumulation in the horse's intestine (262 responses).

Table 14. Time of abdominal radiographs divided into seasons in Study I. Seasons: autumn months 9-11, winter 12-2, spring 3-5 and summer 6-8.

	No sand	Mild	Moderate	Large	Total
Autumn	35	36	64	74	209
Winter	26	25	36	48	135
Spring	18	11	17	19	65
Summer	10	7	13	8	38
Total	89	79	130	149	447

In the questionnaire responses of Study I, roughage was given directly on the ground in 190 (42.5%), fed from a trough in 158 (35.3%), placed on a mat in 16 (3.6%) and fed from a feeding net in 56 cases (12.5%). Furthermore, 27 (6%) of the horses were not fed outdoors. The method of feeding roughage was not associated with categories of sand accumulation. Salt supplementation was given in some form to 439 horses (97.2%), and 311 horses (69.6%) received vitamin supplementation, but neither of these was significantly associated with the size category of sand accumulation.

All horses except one had daily access to a paddock. Altogether 296 horses (66.2%) were grouped during turnout, and 151 horses (33.8%) were never kept in groups (Study I). Two behavioural traits were significantly related to the size of sand accumulation. Dominant behaviour and high position in the hierarchy protected the horse (Spearman rho 0.170, P=0.002), whereas greedy and precise eating of all roughage was associated with larger sand accumulation (Spearman rho 0.184, P<0.001). No difference was detected in the category of sand accumulation between horses with and without reported stereotypic behaviours (P=0.810).

5.4 EFFICACY OF MEDICAL TREATMENTS – RADIOGRAPHIC FINDINGS

In Study II, repeated nasogastric intubation with psyllium and $MgSO_4$ was significantly more effective in removing sand than feeding psyllium at home (Table 15). Sand accumulation was cleared by 4/19 horses treated with psyllium and $MgSO_4$ once and then treated with psyllium at home. Overall, the median area of sand accumulation post treatment in this group was approximately one fourth of that measured on the pre-treatment radiographs (Table 15).

In Study III, nasogastric intubation with a combination of psyllium and $MgSO_4$ for four days was more effective than either treatment alone (Table 15), when comparing the radiographic area(s) of sand at the beginning and at the end of the study. When evaluating the proportion of horses having an area of sand accumulation < 25 cm^2 on day 4, the combination of psyllium and $MgSO_4$ treatment was superior to treatment with psyllium (P=0.012), or $MgSO_4$ (P=0.03) alone (Table 15). Twelve horses with unresolved sand accumulations at day 4 were further treated with laxatives via nasogastric intubation until day

7. Of these 12 horses, the accumulation was cleared in 3/7 in the psyllium group, in 1/4 in the $MgSO_4$ group and in 0/1 in the combination group.

Table 15. Areas of sand at the beginning and end of Studies II-IV. No significant differences were detected between the groups in each study at the beginning. A horse's sand accumulation was classified as 'resolved' if the area of sand was less than 25 cm² in the follow-up radiograph. The other treatments differed significantly from $PsMgSO_4$ ($P \leq 0.05$ always).

Group	Number of horses	Sand at start, median (cm²) (range)	Sand at end, median (cm²) (range)	Number of resolved horses
II				
Ps	57	227 (75–1205)	103 (0–810)*	14*
NGT+Ps	19	456 (76–1241)	106 (0–792)**	4***
$PsMgSO_4$	170	285 (79–1021)	6 (0–828)	91
III				
Ps	12	369 (74–682)	105 (0–708)***	3†
$MgSO_4$	10	424 (87–1028)	212 (0–895)***	2††
$PsMgSO_4$	12	303 (83–712)	2 (0–149)	9
IV				
$PsMgSO_4$	20	250 (109–710)	0 (0–336)	15
Control	20	285 (128–665)	176 (0–558)*	4†††

* $P \leq 0.001$
** $P = 0.09$
*** $P < 0.05$
† $P = 0.012$
†† $P = 0.003$
††† $P = 0.004$

Ps — *Study II Oral psyllium at home, Study III, Psyllium via NGT*
NGT+Ps — *Study II One nasogastric intubation with $MgSO_4$ and psyllium, then oral psyllium*
$MgSO_4$ — *Study III $MgSO_4$ via NGT for 4 days*
$PsMgSO_4$ — *Study II-IV Combination of psyllium and $MgSO_4$ via NGT for 4 days*
Control — *Study IV Control, 4 days without treatment*

In Study IV, the horses treated with a combination of psyllium and $MgSO_4$ via nasogastric intubation cleared the sand accumulation more efficiently than the control horses without treatment (Table 15). However, not all treated horses responded to even extended treatment. The original radiographs of the remaining four treated horses with unresolved accumulations, which were treated further with psyllium and $MgSO_4$, revealed accumulations with a height of approximately 8 cm and the length ranging from 26 to 36 cm.

Extended treatment diminished the amount of sand in all four horses and all accumulations fragmented into smaller pieces. However, none of these horses fulfilled the criteria for removal of sand by day 7. In all four horses, the height of the accumulation remained between 5 and 8 cm and the length remained between 7 and 27 cm. None of them had clinical signs during the extended treatment.

Four horses in the control group cleared the accumulation on their own, without any treatment within the four days. These horses had relatively high initial accumulations (10–17 cm) with length between 16 and >41 cm. Of the 16 untreated control horses that still had sand accumulation on day 4, ten horses had a smaller accumulation, two horses had an accumulation size similar to the original and four horses had a larger accumulation than in the initial radiograph. After completion of the 4-day study period, 15 control horses were treated four times according to the same protocol as the initial treatment group. One horse was discharged from the hospital without follow-up radiography. On day 7, the sand accumulations in 9 of these 15 horses (60%) had resolved.

5.5 OTHER MEDICATIONS, SIDE EFFECTS AND CLINICAL REMARKS

Sedatives and analgesics were administered as needed during Studies II-IV; however, in Study II data about their use were not compared between treatments, since the possible administration of analgesia at home was not known.

In Study III, four horses were sedated for nasogastric intubation; detomidine and butorphanol was administered to two horses once (one horse in each of the groups $MgSO_4$ and Psyllium + $MgSO_4$) and to the other two horses on multiple occasions (one horse in each of the groups $MgSO_4$ and Psyllium + $MgSO_4$). Of these, one horse (from group Psyllium + $MgSO_4$) was classified as resolved, two horses cleared more than half of the original amount and one horse ($MgSO_4$) maintained nearly the same accumulation. In Study IV, most horses in both groups required sedation for radiography, but this did not appear to affect the clearance of sand. Two horses in the treatment group also required sedation for nasogastric tubing, but in both cases the accumulation was resolved during the four-day study period.

In Study III, 1-3 doses of flunixin were administered to seven horses (2 treated with $MgSO_4$, 3 with Psyllium, 3 with Psyllium + $MgSO_4$). Two horses received also a dose of detomidine (0.02 mg/kg i.v.) along with flunixin to resolve signs of abdominal pain (one in MgSO4 group and one in Psyllium + $MgSO_4$ group). In Study IV, four horses in the treatment group required a single dose of flunixin meglumine (1.1 mg/kg bwt i.v.) during the study (one horse because of fever and the other three for alleviation of mild abdominal discomfort). One horse in the control group received one dose of flunixin during the study period.

In both Studies III and IV, all horses tolerated the medications with nasogastric intubations well for four days, and no clinically relevant adverse effects were observed. The most common side effect of treatment was softening of the stools or loose faeces, which resolved within 24 hours of discontinuation of the medication in all cases. Based on clinical impression, MgSO4 alone caused more loose faeces than other treatments, but no significant difference was detected in Study III (P=0.594). Some horses developed nasal mucosal irritation because of repeated nasogastric tubing. This was noticed clinically as bloodstained serous or mucopurulent nasal discharge. Such nasal irritation was not considered a reason for exclusion from the study, and resolved in all cases following cessation of treatment.

Although no adverse effects were detected during the initial four days of Study III, three horses had complications when the treatment with nasogastric intubation was continued. One of the horses (Psyllium group) developed a right dorsal displacement on day 6 of treatment and was euthanized for financial reasons. Another horse ($MgSO_4$ group) developed colic and had an exploratory laparotomy after discontinuating treatment on day 4. This horse had an unusual cement-like sand accumulation that had to be broken into pieces before getting them out. The horse died in recovery and no necropsy was performed. The third horse (originally $MgSO_4$ group) developed acute colic when administered psyllium and $MgSO_4$. The horse had undergone colic surgery previously and when intensive medical treatment did not resolve the clinical signs, the owner elected for euthanasia to be performed. No other medical treatment for sand was attempted due to acute colic.

In clinical studies III and IV, many of the horses came as referrals, and there were no reliable records of the original complaint. In Study IV, 13 horses had been examined in other clinics before they were admitted to hospital. In the hospital, 16 horses had further examinations; six horses had either an abdominal examination per rectum or a routine colic examination (at least examination per rectum and nasogastric intubation, most often also transabdominal ultrasonographic evaluation), five horses had a lameness examination (at least two horses unrelated to GI problems, some for reluctance to move), four horses had a dental examination, three horses had a gastroscopy and two horses had an unrelated respiratory endoscopy.

There were no changes in serum magnesium concentration, haematological values or other biochemical parameters in Study III. In Study IV, 37 horses had a complete blood count and routine serum panel (not only magnesium and creatinine) evaluated on admission. Nine of them had mild leucopenia (leucocytes $< 5 \times 10^9$ /L), one mild leucocytosis and three slightly increased fibrinogen (4 to 5 g/L, normal under <4 g/L). In four horses, plasma creatine kinase activity was slightly above the reference range (<350 U/L).

6 DISCUSSION

6.1 BREED OF THE HORSE

Sand accumulation has been reported in multiple breeds (Udenberg, 1979, Ramey & Reinertson, 1984, Ragle et al. 1989a, Ragle et al. 1992, Granot et al. 2008, Keppie et al. 2008, Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017). In Study I, the breed of the horse was significantly associated with the size category of sand accumulation; a high proportion of Finnhorses had accumulations categorized as large. In Finland, Standardbred trotter is the most common breed (34% of population; Hevostalous lukuina 2017), which was not the case in the population of Study I. On the contrary, Finnhorse (25% of population in Finland) and Icelandic horse (5%) seemed to be over-represented. The Finnhorse and Icelandic horse are considered 'stoic' breeds. A 'stoic' nature is attributed to coldbloods in general or native breeds e.g. Shetland ponies, which are adapted to cope with adverse environments (Taylor et al. 2002). Signs of pain also may not be easily detected in stoic horses. For example, when the severity of lameness was compared with findings on radiographic or ultrasonographic assesment, the scores were not related, but 'stoic' horses showed the lameness less readily than horses described as 'extroverts' (Ijichi et al. 2014).

Additionally, racing Standardbreds were virtually absent from Studies I, III and IV, despite being the most common breed in Finland. The Standardbred trotter has been underrepresented in the University of Helsinki Veterinary Teaching Hospital population in general, and this might affect the breed distributions in Studies III and IV, but does not explain the low numbers in Study I. The Thoroughbred has been reported to be less affected by sand accumulation than other breeds (Ragle et al. 1989a, Graubner et al. 2017, Kilcoyne et al. 2017). Whether the breed itself, or breed specific use and management of the horses e.g. lack of pasture turnout, protects against sand accumulation merits further investigation. However, in Study I the use of adult horses was not associated with the category of sand accumulation.

6.2 CLINICAL SIGNS OF LARGE COLON SAND ACCUMULATION IN HORSES

In Studies I and II, the most common clinical manifestations of large colon sand accumulation were colic and changes in faecal consistency, as also reported in previous studies (McIntyre 1917, Ferraro 1973, Udenberg 1979, Ramey & Reinertson 1984, Specht & Colahan 1988, Ragle et al. 1989a, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen et al. 2003, Granot et al. 2008, Kendall et al. 2008, Hart et al. 2013, Kilcoyne et al. 2017). In Study I, there was no significant association between size category of sand accumulation and diarrhoea alone, but an association emerged when combined with other signs such as colic or poor performance. Some authors have described more subtle clinical signs, e.g. weight loss and diarrhoea, before colic signs (McIntyre 1917, Ferraro 1973), but in Studies I and II the order of appearance of clinical signs was not recorded.

The weak connection between diarrhoea and sand accumulation in Study I was unexpected since a quarter of the horses in Study II presented with the complaint of diarrhoea or loose faeces. However, there are multiple other differential diagnoses for chronic diarrhoea, for example parasites, chronic salmonellosis, and inflammatory bowel disease (Hines 2018), and these were not systematically ruled out in Studies I-IV. On the other hand, changes in faecal consistency or free faecal water commonly occur without a specific clinical diagnosis even after clinical examinations (Hines 2018). No consistent changes were found in basic haematology or serum biochemistry related to sand accumulation (Studies III and IV). As sand has been proposed to cause chronic mucosal inflammation (McIntyre 1917, Nieberle & Cohrs 1967, Ramey & Reinertson 1984), one might expect to find some changes in leucocyte count and fibrinogen concentration. However, Aytekin et al. (2010), for instance, found only a tendency for lower haemoglobin and no specific changes in serum biochemistry in horses with geophagia.

Poor performance was a common complaint in Studies I and II, although it has not been commonly observed in previous literature describing naturally occurring sand accumulations (Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Hart et al. 2013). The manifestation of signs varied and could be subtle such as change in quality of canter. In endemic areas, large colon sand accumulation should also be listed as a differential diagnosis for poor performance, especially if more common reasons have been ruled out.

Hyperaesthesia of the abdomen when being touched has not been reported in horses with sand accumulations previously, although it was often mentioned as a clinical sign in Studies I and II. Most commonly, owners described it as hyperaesthesia of the abdominal skin or the flank areas or problems when tightening the girth. In a study investigating girth aversion, sand accumulation had previously been diagnosed in one horse, but gastric ulcers and orthopaedic problems were more common underlying reasons (Millares-Ramires & Le Jeune 2019). The avoidance of touch or tightening of the girth could suggest secondary hyperalgesia from referred pain, which in humans is visceral pain presenting at a somatic site (Kansal & Hughes 2016). However, there is debate about whether referred pain is a true sign of GI pain in horses (Taylor et al. 2002). In humans, hyperalgesia is usually confined to the muscles, but may extend superficially to subcutaneous tissue and skin. Pain referral from internal organs is likely to result from a process of central sensitization, involving viscero-somatic convergent neurons (Kansal & Hughes 2016). However, in horses, the evidence of certain clinical signs, such as hyperalgesia related to ulcers, is currently scarce, and gastroscopy is always recommended (Sykes et al. 2015). In clinical cases of abdominal hyperalgesia, both gastric ulcers and sand accumulation should be ruled out (Millares-Ramires & Le Jeune 2019).

Witnessing the horse eating sand is not a certain indication for accumulation; some of the horses observed to have ingested sand had no accumulations, and vice versa in Study I. It is not known why sand tends to accumulate in some horses, while others have no problems despite eating it. Sand accumulation may be related to insufficient intestinal motility, similar to susceptibility to large colon impaction in horses that have fewer ICC at the pelvic flexure than healthy horses (Fintl et al. 2004). Individual and breed variability may play a role in how horses show pain (Taylor et al. 2002), and it may be difficult to notice the discomfort that this gradually progressing condition causes in an individual horse. Persons involved in the daily care of the horse tend to underestimate impairments because of the horse's expressions of well-being (Lesimple & Hausberger 2014). Some horses had large sand accumulations without their owner noticing any clinical signs. This might be related to differences in owner observation, but also to an individual way of showing pain or discomfort as well as to a higher pain threshold in some horses.

6.3 INFLUENCE OF SEASON, MANAGEMENT AND HORSE TRAITS

The results reinforced the clinical impression of sand accumulation being a problem in Finland, especially during autumn, but also during the spring months, since most horses were presented between October and December (Study II). The seasonality is most likely connected to the habit of horses being kept in sand/dirt paddocks and possibly fed on sand. The problem seems to be less problematic during the summer when horses are on pasture, having access to grass throughout the day. Ruohoniemi et al. (2001) reported one horse that did not respond to medical treatment, but cleared the sand accumulation when on pasture. The effect of ingesting fresh grass with less bulk might alter motility and intestinal contents beneficially, promoting removal of sand. The northern latitude with frozen ground and snow prevents sand consumption during the winter. The phenomenon of global warming might eventually change the occurrence of sand accumulation, increasing the incidence during winter in the area studied, in case the period of nonfrozen ground becomes longer. In more temperate areas, the effects of climate change might diminish access to proper pastures, and thus, make sandy soils more common, predisposing the horses to intestinal sand accumulations (Ramey & Reinertson 1984, Colahan 1987, Mair 2002). Nevertheless, the time required for the sand to accumulate in the colon is not known and is likely to be highly variable. Poor pastures might cause sand eating and accumulations in persistent feeders (Ferraro 1973), which seemed to be a significant factor also in Study I.

Dominant behaviour was a protective factor for sand accumulations in Study I. Group behaviour and social relationships always play a role in the way individuals are able to eat in a herd (Albro Houpt & Keiper 1982, Ralston 1986, Rutberg & Greenberg 1990, Burla et al. 2016), but this issue has not been connected to sand accumulations in previous studies. An Icelandic study showed that dominant horses ate the better hay during winter, while subservient horse ate more of the poor winter grass (Ingólsdóttir & Sigurjónsdóttir 2008). Furthermore, horses with increased faecal water were observed to defend their food against other horses (resource guarding) less than a control group without faecal water (Kienzle et al. 2016). In group hierarchy, 40% of horses with faecal water were considered to be last or second to last, compared with 4% in the control group without faecal water. These results suggest that social stress may play a role in the development of faecal water (Kienzle et al. 2016). In Study I, when loosening of fecal consistency were combined with colic or poor performance, the horse was more likely to have sand accumulation. Whether social stress and free faecal water are related to sand accumulation remains to be elucidated. However, one option to prevent the sand accumulations could be to observe the dynamics within herds and give special attention to horses with low social status or excessive greediness.

No significant connections between feeding or management and sand accumulation were found in Study I. Therefore, no new recommendations can be offered to avoid the problem beyond the previous suggestions of muzzling horses and not having horses turned out on a sandy surface (Colahan 1987). Greediness was associated with sand accumulation in Study I. Greedy horses are prone to eat every piece of hay; therefore, potentially accidentally ingesting sand (Ferraro 1973). Earlier suggestions of providing supplemental feed, feeding off the ground and not feeding outside are reasonable to prevent sand accumulation (Udenberg 1979, Colahan 1987, Hanson 2002, Hardy 2017); however, greedy horses may need special consideration of management strategies to minimize the potential for sand ingestion.

6.4 RESPONSE TO MEDICAL TREATMENT OF SAND

6.4.1 EFFICACY OF DIFFERENT LAXATIVES

Studies III and IV were the first prospective randomized studies to assess the efficacy of a combination of psyllium and $MgSO_4$ to remove naturally acquired sand accumulations. Lieb (1997) fed sand and then various laxatives to a small number of research horses and reached a similar conclusion to Hammock et al. (1998) that psyllium treatment did not differ from no treatment. However, Hotwagner & Iben (2009) showed a positive effect of psyllium on faecal sand output relative to mineral oil alone in research horses. Prior to Studies II-IV, the simultaneous use of a combination of psyllium and $MgSO_4$ had not been reported. The improved efficacy of combining these treatments could be due to a double dose of laxatives in combination or they may also potentiate each other. The osmotic laxative $MgSO_4$ draws water into the intestine (Boothe & Jenkins 1995), which may help the bulk-forming laxative psyllium to reach its full efficacy.

Moreover, in Study IV with clinical cases, repeated nasogastric intubation for four days with psyllium and $MgSO_4$ was more effective than solely preventing sand intake. Most of the unresolved, untreated control horses cleared the accumulated sand when treated with nasogastric intubation of psyllium and $MgSO_4$ for four days after the initial four-day control period. In addition to excessive soil ingestion, naturally acquired large colon sand accumulations may result from intestinal dysfunction. These horses perhaps cannot clear the sand by themselves, like the presumably healthy research horses used in some studies (Lieb 1997, Hammock et al. 1998, Hotwagner & Iben 2009). Similar to those healthy research horses, many of the control horses in Study IV could either clear the sand accumulation without any specific treatment or at least the sand accumulation changed site or shape based on radiographic assessment. Besides the variation of colonic motility from horse to horse, the consistency of sand most likely has an effect on the way that a horse can clear it. Based on Studies II- IV, the response to treatment cannot necessarily be predicted and thus, radiography is required to be repeated to confirm resolution of sand accumulation in clinical cases.

The response of different types of accumulations to medical treatment may vary. Studies have graded the accumulations mainly by size (Korolainen & Ruohoniemi 2002, Kendall et al. 2008, Hart et al. 2013), but also according to other properties such as opacity (Keppie et al. 2008). None of the studies have evaluated the effect of accumulation type on the response to treatment. In Study IV, all four horses that were unresponsive to treatment had elongated sand accumulations with an initial height of around 8 cm. It is possible that such accumulations were not high enough for laxatives to truly mix with the sand, but were still sufficiently heavy to prevent proper intestinal movement. Furthermore, the consistency of the accumulation cannot be reliably assessed using the current diagnostic methods. For example, one horse in Study III did not respond to medical treatment, and in surgery the accumulation turned out to have a cement-like consistency. It would therefore seem plausible that medical therapy would have little effect on these types of accumulations and that owners of horses with persistent sand accumulations should be aware that these may become problematic and potentially require surgical management.

In Study III, some horses could clear accumulated sand when treated with psyllium alone for longer than the initial four days. Mehmood et al. (2011) showed both prokinetic and inhibitory properties of psyllium in the GI tract, which might explain the delay in effect. Landes et al. (2008) fed psyllium with pro- and prebiotics to healthy research horses, and the supplement increased the faecal sand excretion only on day 4 of treatment. A delay in faecal sand excretion was also reported by Lieb & Weise (1999). Hotwagner & Iben (2009), by contrast, detected an increase in faecal sand excretion as early as day 1 when giving psyllium and mineral oil. However, they used administered sand, which might have been moving through the GI tract before treatment (Hotwagner & Iben 2009). The dosage of psyllium has varied in previous reports, but the effect of optimal dose on faecal sand excretion and the efficacy of sand removal remain to be investigated. The different formulations of psyllium (whole seeds, pellets, granules) should also be compared in future studies.

In Study III, horses seemed to have loose faeces more often when administered $MgSO_4$ alone. The stool-softening effect of $MgSO_4$ has been shown to be dose-related, and 1 g/kg increased faecal water content effectively (Freeman et al. 1992). If the sole effect of $MgSO_4$ is to draw water into the intestinal contents, one option could have been to treat the control horses of Study IV with plain water. That would have offered an opportunity to evaluate the role of hydrating the intestinal contents and the effect of the gastrocolic reflex induced by filling the stomach via nasogastric intubation (Freeman et al. 1992). However, Hammock et al. (1998) did not detect any improvement in sand clearance when psyllium was given via nasogastric intubation rather than fed with food. In Study III, nasogastric intubation of client-owned horses without actual medication was considered unethical.

6.4.2 COMPARISON OF NASOGASTRIC INTUBATION OF LAXATIVES WITH PSYLLIUM FEEDING

Based on Studies III and IV, once a day nasogastric intubation of a combination of both psyllium and $MgSO_4$ for four days is the most efficient treatment of the studied regimens for removing sand accumulations from the large colon. If the horse tolerates repeated nasogastric intubation and a veterinarian is available daily or the owner is willing to let the horse be treated in a hospital, this is the treatment of choice, as it removed the sand accumulation more frequently and efficiently than feeding psyllium (Study II). Moreover, not all horses eat psyllium willingly (Hammock et al. 1998) or they only eat small amounts daily (Colahan 1987). Whilst nasogastric intubation itself is somewhat unpleasant for the horse, it is a routine veterinary procedure and the benefits would outweigh the associated transient discomfort and potential risks associated with it.

Repeated treatment with laxatives via nasogastric tube in hospital was more effective than feeding psyllium at home, both considering number of resolved horses and the actual size of the sand accumulation after treatment (Study II). Psyllium feeding nevertheless had a beneficial effect on the size of the accumulation; the median size halved as an outcome of psyllium treatment. In the home-treated group, it was not controlled whether owners restricted access to sand or could feed their horses the psyllium properly. This might have affected the size of radiographic sand accumulation. Psyllium feeding is commonly recommended (Colahan 1987, Hanson 2002, Hardy and Rakestraw 2012), but the evidence of its efficacy varies. Case reports with psyllium feeding have shown resolution of large colon sand accumulations, but reports have had a single or only a few horses (Ramey & Reinertson 1984, Bertone et al. 1988, Ruohoniemi et al. 2000). Prospective studies have been conducted with research horses without any known sand accumulation, and the efficacy was evaluated using faecal excretion of sand only (Landes et al. 2008, Cieśla et al. 2011) and not radiographic evaluation.

In Study II, a small group of horses was treated once with nasogastric intubation of psyllium and $MgSO_4$, and then went home to eat psyllium for various time courses. Although not significantly different, the median of the initial accumulations was the largest in this group of horses. This had probably tempted the veterinarians to try at least one nasogastric intubation in addition to feeding psyllium. While the median size of accumulation was much smaller after treatment in this retrospective study, very few horses were graded as resolved, and thus, no benefit could be shown compared with solely feeding psyllium at home.

In Study II, psyllium was fed for 10 to 40 days, which is similar to or slightly longer than textbook recommendations (Edens and Gargyle 1997, Hanson 2002). Limiting the duration of psyllium feeding has been suggested in order to avoid loss of its laxative effect (Hammock et al. 1998, Hanson 2002, Hardy 2017). The concern was based on in vitro studies of human colonic bacteria, which indicated that colonic flora could start to degrade psyllium; 4-26% of psyllium fibre was fermented, but longer (up to 24 hours) fermentation increased the production of short chain fatty acids (Bourquin et al. 1993, Campbell et al. 1997). However, there is no clinical evidence of psyllium losing its effect in equids?, only laboratory studies showing increased bacterial fermentation of psyllium after one day of administration (Bourquin et al. 1993, Campbell et al. 1997). On the contrary, Landes et al. (2009) showed increased excretion of sand in faeces even at the end of their study on day 35. It cannot therefore be assumed that psyllium would lose its effect on sand removal within the length of the treatment period used in Study II. Thus, longer periods of treatment may be effective.

6.4.3 SIDE EFFECTS OF MEDICAL TREATMENT

In general, horses in Studies II-IV tolerated the medications well. Softening of the faeces was the only side effect, and was associated with the use of $MgSO_4$. High blood magnesium concentration and its related clinical signs have been reported due to magnesium overdose (Henninger & Horst, 1997).

Since sand accumulations may cause changes to the intestinal wall (Nieberle & Cohrs 1957, Buergelt 2013), absorption of magnesium could have been enhanced, thus causing magnesium toxicity. No such signs or even hypermagnesemia were detected in Studies II-IV. Minor nasal bleeding and mucosal irritation was observed in some horses after repeated nasogastric intubation (Studies III and IV). This side effect can be minimized with experienced veterinarians and handlers, adequate lubrication and proper equipment.

When treatment was continued after the initial 4-day study period, three horses in Study III (two in $MgSO_4$ group, one in Psyllium group) demonstrated severe signs of colic, resulting in death of the horses. The outcome of Studies III and IV is in line with recent case studies which report a good prognosis for recovery from sand colic with medical treatment, although some horses had died or had been euthanized either for medical reasons or financial constraints (Hart et al. 2013, Graubner et al. 2017, Kilcoyne et al. 2017). A retrospective study reported a high OR (28.5, 95% CI 2.37 - 342.61) for non-survival in horses needing surgery for sand colic, but also not treating the horse with laxatives increased the risk of death (OR 6.13, 95% CI 1.05–35.83) (Hart et al. 2013). By contrast, a later report had a more favourable outcome for horses with sand colic requiring surgery, probably because the mean duration of colic before surgery was much shorter (Kilcoyne et al. 2017). The authors of both studies recommend timely surgery to improve the prognosis. The decision to undertake surgery was made based on presence of persistent pain or failure to respond to medical management (Hart et al. 2013, Kilcoyne et al. 2017). Colic surgery is not always an option for owners; two of the horses that died / were euthanased in Study III were candidates for surgery, but the owners declined surgical management.

The medical treatment of sand accumulation carries risks of other complications, although they are rare and were not observed in the present studies. Although no written cases of psyllium-related equine oesophageal obstructions exist, there is anecdotal experience of oesophageal obstruction in horses following ingestion of psyllium. Not included in Studies II-IV, one horse had to be euthanized in the University of Helsinki Veterinary Teaching Hospital due to gastric rupture after nasogastric intubation with psyllium (powder type, which swells rapidly). Since this incident, a short fasting period (2 hours) before nasogastric medication has been added to the treatment protocol and was in use during Study IV. Intestinal obstruction also has been reported in both humans and horses (Bergstrom et al. 2018, Hefny et al. 2018). The administration of psyllium with nasogastric intubation in Studies II-IV required generous amounts of water to prevent tube obstruction, which probably also reduced the risk of intestinal obstruction.

6.5 LIMITATIONS OF THE STUDIES

The population in Study I was a selected one and did not represent the general horse population because all horses must have had some indication for abdominal radiography. Study I was retrospective, and therefore, the respondents may have recalled details incorrectly. Due to the high number of responses in Study I, minor errors most likely would not have caused a substantial bias. A case-control study including matched control horses from the same stable or paddock. A case-control study including radiographs and questionnaire information from matched control horses without clinical signs from the same stable would have been ideal. Unfortunately, this would not only have been expensive, but also caused much unnecessary exposure to radiation. Nevertheless, Study I was useful for gathering information on a large number of confirmed cases of large colon sand accumulation with details of management, which normally are not included in veterinary patient records.

In Study II, differences between clinics and treating veterinarians' preference might have influenced the way horses were treated. Owner's preferences, including financial issues, are also likely to have had an effect to the choice of the treatment in some horses. Since the study was retrospective, the sizes of treatment groups were not controlled, nor were the distributions of horses between treatments randomized. When horses were treated at home, implementation of treatment was continued for a longer period and the administration of psyllium could not be controlled. There was also a risk for horses to continue eating the soil or not to eat the psyllium properly. However, the situation in Study II reflected “real life”, where treatments sometimes have to be adjusted or owners might not follow instructions.

A small sample size is a common problem in equine research, and the same applied to prospective studies III and IV, although these studies had larger groups than many previous studies with research horses (Hammock et al. 1998, Hotwagner & Iben 2009). In Studies III and IV the numbers of horses required per group were estimated by power calculations. Despite rather small groups, significant differences between treatments were demonstrated. Since the sand accumulation had been naturally acquired, the duration of accumulation prior to diagnosis was unknown. The gradually increasing amount of sand may have affected GI motility and consequently, the rate of sand clearance. However, the study protocol was designed to be applied to clinical cases and the studies yielded relevant information on how to treat naturally occurring sand accumulations.

Using radiographic area of sand accumulation (Studies II-IV) or assigninig it to a broad category (Study I) is not a precise method to evaluate the quantity of large colon sand accumulation. Even though an abdominal radiograph is a two-dimensional image for estimation of a three-dimensional phenomenon, it is still more accurate than other methods, such as ultrasound (Korolainen & Ruohoniemi 2002) or ventral abdominal auscultation (Ragle et al. 1989b), and is also more likely to detect large accumulations than measuring the amount of feacal sand excretion (Reichelt & Lischer 2011, Hukkinen 2015). Furthermore, the areas of sand on each radiograph in Studies II-IV were only evaluated by one clinician, which potentially reduces accuracy. However, for an experienced clinician, the actual calculation of the radiopaque area using current softwares is unlikely to reduce accuracy.

Occasionally, the entire large sand accumulation was not visible in the radiographs. However, the large size of the accumulation could be reliably detected in all cases, thus no further radiographs were taken to avoid unnecessary radiation. Consequently, nonparametric tests making ordinal comparisons were used for statistical analysis. Therefore, not being able to measure the actual area of some very large accumulations exactly probably did not cause a marked bias. It is possible that some caudally located accumulations may have been missed, as the cranioventral abdomen was the main focus in all Studies. The width of the adult horse usually prevents radiographic visualization of the entire abdomen (Butler et al. 2017).

Study III lacked a negative control group, which was then included in the following study (IV). None of the studies (I-IV) had a thorough exclusion to rule out GI problems other than sand accumulation. Therefore, some horses might have had another underlying problem, which could have affected their clinical signs (I, II) or the rate of sand removal (II-IV). However, in Study IV other diagnostic procedures for horses' clinical signs were recorded.

All horses in hospital were likely exercised less than usual. A rapid reduction in exercise predisposes horses to impactions (Hillyer et al. 2002). Many horses were sedated for radiography and some for nasogastric intubation. Alpha$_2$-adrenoceptor agonists have a tendency to decrease intestinal movement (Merrit et al. 1998, Elfenbein et al. 2009) and therefore could have inhibited sand removal. However, the frequency of sedation was similar between groups in Studies III and IV, thus probably affecting the groups similarly. Whether sedation and lack of exercise predisposed some of the horses to colic or delayed the removal of sand in Studies II-IV is unknown.

6.6 CLINICAL IMPLEMENTATIONS AND FURTHER STUDIES

Use of psyllium has been recommended for removal of sand accumulation in textbooks for decades, although the efficacy of psyllium has been questioned. This work confirms that psyllium has a role to play in successful management of natural sand accumulations causing clinical signs of disease. Study II demonstrated that tubing with restricted access to sand was more effective than feeding psyllium at home. Studies II-IV have established that treatment with laxatives administered via nasogastric intubation (i.e. combination of psyllium and $MgSO_4$) once daily at least for four days is merited in the management of clinical cases of large colon sand accumulation. The results of Studies II and III are already being implemented into textbooks (Hardy 2017, Southwood 2018). Studies I and II have revealed a wider range of clinical signs related to sand other than colic or diarrhoea. This will help veterinarians to remember sand accumulation as one differential diagnosis for subtle signs such as changes in performance.

Although the clinical signs of large colon sand accumulation (Studies I and II) and its medical treatment (Studies II-IV) have been studied in the present thesis, it is still not known why horses acquire sand in their large colon. Most likely there are various individual reasons for geophagia as well as differences in the ability to clear the sand ingested either intentionally or accidentally. Some future avenues for investigation could be to analyse the soil that horses prefer when eating sand or to look for any trace mineral deficiencies in horses. Investigating the motility of the large colon and the number of pacemakers in horses with large colon sand accumulation could identify the individuals predisposed to problems related to GI motility and thus in the ability to clear sand. The investigation would require intestinal biopsies to count the pacemakers in horses with sand accumulation as well as in controls during surgery or when collecting samples after euthanasia.

7 CONCLUSIONS

1. The owner-recognized clinical signs of colic and poor performance were related to the size of large colon sand accumulation. Horses with combinations of clinical signs, such as diarrhoea and poor performance or colic and diarrhoea, had a higher probability of a larger amount of sand (I).
2. Some behavioural traits of the horses affected the probability of having sand accumulation. Dominant position in the herd was protective for large colon sand accumulations, while greediness predisposed the horse to sand accumulation. No association was detected between the studied management-related factors and large colon sand accumulation (I).
3. Nasogastric intubation with psyllium and $MgSO_4$ once daily for 3-7 days was a more effective treatment for large colon sand accumulation than feeding psyllium at home (II).
4. Nasogastric intubation once daily for four days with a combination of 1 g/kg of psyllium and 1 g/kg of $MgSO_4$ was a more effective medical treatment for large colon sand accumulation than either treatment alone or restricting access to sand (III-IV).

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