

**THE DIAGNOSTIC ACCURACY OF THE PLASTIC
GLOVE TEST FOR DIAGNOSIS OF SAND
ENTEROPATHY IN THE HORSE**

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<p>Sand enteropathy is a common form of equine colic in Finland. Detection of sand in the faeces of a horse is a widely known diagnostic method for intestinal sand accumulations. The plastic glove test is a faecal sand sedimentation test recognized by many equine practitioners. However, the diagnostic accuracy of the test is unknown, which restricts the use of the test.</p> <p>The main objective of this licentiate thesis was to determine the diagnostic accuracy of the plastic glove test. The most reliable diagnostic method for intestinal sand accumulations is abdominal radiography, and therefore it was used as a gold standard method against which the plastic glove test was compared. The hypothesis was that there is a positive correlation between the results of the plastic glove test and abdominal radiography.</p> <p>The study was conducted by collecting a faecal sample and an abdominal radiograph from each participating horse. A total of 63 faecal samples were collected from 61 different horses. All the horses were patients at the Veterinary Teaching Hospital of the Helsinki University. The cranioventral abdomen of each horse was radiographed, and the length and height of the sand accumulations were recorded. The plastic glove test was performed by mixing faecal material (~200 grams) and water (1 litre) inside a rectal sleeve. The suspension was left to hang, and the amount of sand sediment in the fingertips was recorded after 30 minutes and 24 hours. Each fingertip of the glove was assessed separately on a 0-3 numerical scale and then added up to make a total score out of 15 for each horse. The results were compared in a 2 X 2 table and ROC analysis.</p> <p>According to this study, the sensitivity of the plastic glove test is 82,61% (95% confidence interval 68,58% to 92,18%) and the specificity is 71,43% (95% confidence interval 41,90% to 91,61%). The positive predictive value is 90,48%, while the negative predictive value is only 55,56%. In the ROC analysis the area under the curve (AUC) is 0,771 with a 95% confidence interval of 0,644 to 0,870.</p> <p>The plastic glove test is a moderately accurate diagnostic test. A positive test result is usually correct, but it does not inform the severity of the condition. It can be used as an indication for further examinations or treatment. A single negative test result should not be used to exclude the disease. The plastic glove test can be a helpful tool in the diagnosis of sand enteropathy in the horse, but should not be relied on as the sole method of diagnosis due to the poor specificity of the test.</p> <p>Since the reference population in this study was the patient material of the Equine Hospital, more reliable results could be obtained by using a more comprehensive and randomized representation of the entire Finnish horse population. It should also be noted that 29% of the horses had been treated with psyllium and/or magnesium sulphate prior to the testing.</p>			
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<p>Suoliston hiekkakeräymät ovat yleinen syy hevosten ähkyihin Suomessa. Hiekan osoittamista ulosteessa on jo pitkään käytetty apuna hiekkäähkyjen diagnostiikassa. Muovihanskatesti perustuu hiekan sedimentoitumiseen, kun hevosen ulostetta ja vettä sekoitetaan hanskan sisällä. Testin luotettavuutta ei kuitenkaan ole raportoitu, mikä rajoittaa testin käyttöä.</p> <p>Tämän lisensiaatin tutkielman tavoitteena oli määrittää muovihanskatestin diagnostinen arvo sekä tarjota hevospraktiikkaa tekeville eläinlääkäreille apukeinoja testitulosten tulkintaan. Kultaisen standardin menetelmänä käytettiin vatsaontelon röntgenkuvausta. Hypoteesina oli, että muovihanskatestin ja vatsaontelon röntgenkuvauksen tulosten välillä esiintyy jonkin verran yhdenmukaisuutta.</p> <p>Tutkimus suoritettiin siten, että jokaiselta tutkimushevoselta kerättiin ulostenäyte sekä otettiin etuvatsaontelon röntgenkuva. Yhteensä 63 ulostenäytettä kerättiin 61 Yliopistollisen hevossairaalan potilashevoselta. Röntgenkuvan perusteella mahdollisten hiekkakeräymien leveys ja korkeus kirjattiin ylös ja hevonen luokiteltiin terveeksi tai sairaaksi taudin suhteen. Muovihanskatestiä varten noin 200 grammaa ulostetta ja 1 litra lämmintä vettä sekoitettiin kertakäyttöisen rektaalihanskan sisällä. Tämän jälkeen hanska jätettiin roikkumaan ja testin tulokset tarkistettiin 30 minuutin ja 24 tunnin kuluttua. Hanskan jokainen sormenpää arvioitiin subjektiivisesti ja pisteytettiin hiekkamäärän perusteella asteikolla 0-3. Sormenpäiden pisteet laskettiin yhteen, jolloin saatiin jokaiselle näytteelle arvo väliltä 0-15. Röntgenkuvauksen ja muovihanskatestin tuloksia verrattiin tilastollisesti 2 X 2 taulukossa ja ROC-analysillä.</p> <p>Tutkimuksen perusteella muovihanskatestin sensitiivisyys on 82,61% (95% luottamusväli on 68,58% - 92,18%) ja spesifisyys on 71,43% (95% luottamusväli on 41,90% - 91,61%). Testin positiivinen ennustearvo on 90,48%, kun taas negatiivinen ennustearvo on vain 55,56%. ROC-analysissä käyrän alle jäävän alueen pinta-ala (AUC) on 0,771 (95% luottamusväli on 0,644 - 0,870).</p> <p>Tulosten perusteella muovihanskatesti on kohtalainen diagnostinen testi. Testin herkkyys on hyvä, mutta positiivinen tulos ei varsinaisesti kerro, onko löydös kliinisesti merkittävä vai ei. Tulosta voidaan kuitenkin pitää indikaationa jatkotutkimuksille (röntgenkuvaus), jolloin taudin laajuudesta saadaan tietoa. Koska testin spesifisyys on huono, negatiiviseen testitulokseen ei pidä luottaa. Muovihanskatesti voi olla hyödyllinen apu diagnostiikassa, mutta se ei riitä ainoaksi diagnostiikkamenetelmäksi.</p> <p>Tulosten kannalta on merkittävää, että tutkimuksen viitepopulaationa käytettiin potilasmateriaalia. Näin ollen kliinisesti täysin terveet hevoset puuttuvat tutkimusaineistosta. Lisäksi tulee huomioida, että osaa (29%) tutkimukseen osallistuneista hevosista oli hoidettu psylliumilla ja/tai magnesiumsulfaatilla p.o. ennen näytteenottoa. Psylliumin on aiemmissa tutkimuksissa osoitettu lisäävän suolistonsisäisen hiekan erittymistä ulosteeseen.</p>			
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1 INTRODUCTION

Intestinal sand accumulations are a common cause for equine colic in various parts of the world, and especially in areas with loose sandy soil (Ragle & Meagher 1987, Ragle et al. 1989a, Ragle et al. 1989b, Kaikkonen et al. 2000, Husted et al. 2005, Granot et al. 2008, Kendall et al. 2008, Keppie et al. 2008). There is little information available on the exact prevalence of the condition, but at the Veterinary Teaching Hospital of the Helsinki University, sand colic seems to be one of the most common forms of colic during the autumn. The severity of the condition varies from asymptomatic to life-threatening (Specht & Colahan 1988, Ragle et al. 1989a, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002).

Many diagnostic methods have been described in the literature, and perhaps the cheapest method is to detect sand in the faeces of a horse. The faecal sand tests are generally performed by mixing faecal material with water so that the heavy sand particles (if there are any) sediment to the bottom (Ferraro 1973, Ramey & Reinertson 1984, in the book section by Colahan 1987, in the book section by Edwards 1999, Ruohoniemi et al. 2001, Husted et al. 2005, Granot et al. 2008, Landes et al. 2008, in the book section by Lopes 2009). The faeces and water are often mixed inside a disposable rectal sleeve, and therefore the test is called the plastic glove test. The plastic glove test is based on the assumption that ingested sand is excreted in the faeces within a certain period of time (Husted et al. 2005).

Abdominal radiography is currently considered the most reliable diagnostic method for intestinal sand accumulations (Kendall et al. 2008, Landes et al. 2008). However, there is still need for a simple 'stall side' test, which could be used at the stables in routine practice. Early observation of sand can reduce the examination and treatment costs, as well as the need for invasive treatments such as surgery (Korolainen & Ruohoniemi 2002). When abdominal radiography is not available, the plastic glove test is often performed as an alternative, even though the test has yet not been validated, and therefore, its diagnostic accuracy is unknown.

The aim of this prospective study was to determine the diagnostic accuracy of the plastic glove test (faecal sand sedimentation test) and to give equine practitioners the means to interpret the test results. Abdominal radiography was used as the gold standard

method when comparing the results of the plastic glove test. Our hypothesis was that there is some degree of positive correlation between the results of the faecal sand sedimentation test and abdominal radiography when used to detect intestinal sand accumulations in horses.

2 LITERATURE REVIEW

2.1 The equine gastrointestinal tract

The equine gastrointestinal track occupies the major part of the abdominal cavity. The stomach is relatively small with the capacity of 5 to 15 litres. The small intestine (over 20 meters long) is quite minor and ordinary compared to the large intestine, which is greatly enlarged and modified in horse (in the book by Dyce et al. 2002). The most voluminous part is the ventral colon that can hold up to 150 litres (in the book by Sjaastad et al. 2003). The large intestine is characterized by taenias; bands that are formed by longitudinal muscles and elastic fibres in the intestinal wall. The taenias divide the colon into sacculations/haustra. Only the left dorsal colon is not sacculated. The caecum, located on the right side of the abdomen, is enlarged and may have a capacity of over 30 litres (in the book by Dyce et al. 2002). The complex arrangement and narrow segments of the large intestine, such as the cecocolic junction, the pelvic flexure, and the transverse colon, predispose the tract to obstructions and displacements (in the book by Dyce et al. 2002, in the book by Sjaastad et al. 2003).

Horses are monogastric herbivores, and their gastrointestinal tract is specialized for consuming large amounts of grass and fibre. Their diet contains only small amounts of fat and protein, but plenty of insoluble carbohydrates (cellulose, pectin) that cannot be broken down by the digestive enzymes. The enlarged caecum and colon function as fermentation chambers, where anaerobic micro-organisms degrade these carbohydrates to volatile fatty acids (VFA), which are a direct energy source for horses. The large volume, prolonged transit time and retrograde flow of the caecum and large colon are prerequisites for microbial fermentation (in the book by Sjaastad et al. 2003).

Unfortunately, the same characteristics are likely to cause the accumulation of sand in the most ventral portions of the bowel (Specht & Colahan 1988).

2.2 Sand enteropathy

The pathological condition induced by gastrointestinal sand accumulation(s) is usually referred to as sand enteropathy, sand colic or sand related gastrointestinal disease (SGID). Sand impaction/obstruction of the colon may be involved in the disease.

2.2.1 Pathogenesis

Some horses tend to ingest sand either deliberately or by mistake while grazing or feeding in the paddock. The sand passes through the proximal gastrointestinal tract and may accumulate both in ventral and dorsal colon of the horse (Bertone et al. 1988). “Sand is thought to accumulate in the colon, rather than the small intestine, because the larger diameter and reduced flow apparently allows sand to settle out of ingesta” (Keppie et al. 2008). In addition to the large colon, the caecum, ileocecal junction and stomach have also been reported as sites of sand accumulation (in the book section by Colahan 1987). It is reported that fine sand is likely to accumulate in the ventral colon, whereas coarse sand tends to accumulate in the dorsal colon (Specht & Colahan 1988, Ragle et al. 1989a). It has also been stated that the sand accumulation is most commonly located in the cranioventral part of the abdomen, which comprises diaphragmatic and sternal flexures and some other parts of ventral and dorsal colon (Ruohoniemi et al. 2001). Furthermore, when sand was surgically placed in the intestines of ponies, coarse sand was more likely to be found in the more proximal parts of the intestines at post-mortem (Hammock et al. 1998). Multiple sand accumulations are sometimes seen in horses (Specht & Colahan 1988, Ragle et al. 1989a, Granot et al. 2008). Ragle et al. (1989a) observed in their study that 50% of the horses treated surgically for sand colic had multiple sites of sand accumulation present.

Like other intestinal contents, sand can also cause an impaction/obstruction when its elimination is disturbed. High density and friction between sand particles makes sand especially hard to move through the gastrointestinal tract (in the book section by Lopes 2009). Sand accumulations that are located in the ventral colon are usually not

obstructive (Ramey & Reinertson 1984, Ragle et al. 1989a, Korolainen & Ruohoniemi 2002). While large amounts of sand frequently accumulate in the ventral colon, sand impactions/obstructions typically occur in the dorsal colon, transverse colon and pelvic flexure (Specht & Colahan 1988, Ragle et al. 1989a, in the book section by Lopes 2009). Ragle et al. (1989a) reported that 65% (26/40) of the horses treated surgically for sand colic had sand in the right dorsal colon, which was the most common site of sand impaction. Again, in the study by Specht & Colahan (1988), the pelvic flexure was the most common location for a single impaction of the gastrointestinal tract.

It has been suggested that when not exposed to sand continuously, a healthy horse should be able to eliminate most of the accumulated sand by normal intestinal motility (Hammock et al. 1998). However, “a large volume of sand in the gastrointestinal tract may overwhelm the ability of the tract to clear it” (Bertone et al. 1988). It is also possible that a small amount of sand in the intestines may affect gastrointestinal peristalsis and thereby contribute to the accumulation of larger amounts of sand (Ruohoniemi et al. 2001). Or it is possible that other gastrointestinal disturbances that affect intestinal motility could impair the excretion of normal amounts of sand ingested with the feed (Bertone et al. 1988).

Sand causes chronic inflammation of the intestinal mucosa (Ramey & Reinertson 1984). Extensive mucosal damage and abrasion may then result in severe colitis (in the book section by Lopes 2009). Bertone et al. (1988) suggested that the weight and abrasiveness of the sand mass may decrease the integrity and change the function of the gastrointestinal tract, possibly leading to peritonitis.

Intestinal sand may also become wedged in the narrowed segments of the bowel and cause an impaction. The obstructed bowel quickly becomes distended with gas, fluid and ingesta (in the book section by Colahan 1987). Consequently, the pressure and distention may result in necrosis of the colon (in the book section by Colahan 1987, Specht & Colahan 1988). This chain of events creates a risk for rupture of the damaged and necrotic intestinal wall of the colon (in the book section by Colahan 1987, in the book section by Lopes 2009).

The heavy weight of the intestinal sand may also lead to ventral displacement of the large colon and development of a volvulus/torsion (Specht & Colahan 1988, Ragle et al. 1989a, in the book section by Lopes 2009). A displacement can also occur due to the

massive gaseous distension of the obstructed bowel (in the book section by Colahan 1987). Concurrent large colon displacement and/or torsion/volvulus have been reported to be present in 54% (Specht & Colahan 1988), 25% (Ragle et al. 1989a), and 32% (Granot et al. 2008) of the horses treated surgically for sand colic. Severe displacement or perforation of the bowel will eventually result in shock (in the book section by Colahan 1987). Signs of endotoxemia are sometimes seen in horses with sand impaction (in the book section by Edens & Cargile 1997, Ruohoniemi et al. 2001).

The quantity of sand required to cause a clinical disease is unclear and possibly varies among individuals (Ramey & Reinertson 1984, Bertone et al. 1988, Ragle et al. 1989a, Kendall et al. 2008). According to older literature colic symptoms appeared in small horses after accumulation of 20 kg of sand in the intestines (Ragle et al. 1989b). Under experimental conditions horses have been given up to 10 kg of sand via nasogastric tube without seeing any clinical signs of colic or diarrhoea (Ragle et al. 1989b). On the other hand, 8 kg of sand has been enough to cause an impaction that requires surgery (Ragle et al. 1989a). There also seem to be physiopathological differences between individual horses (Bertone et al. 1988, Husted et al. 2005). The fact that within a herd under identical conditions only single horses have been affected and shown clinical signs suggests that some horses cannot tolerate as much sand as others in the same population (Bertone et al. 1988).

2.2.2 Risk factors

Environmental and managerial risk factors

Geographic variation in the incidence of sand enteropathy has been frequently mentioned (Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002). Sand related colic is a common problem in the Southern United States (in the book section by Ott 1997). Geographically, areas with loose sandy soil, such as California, Florida, Michigan, Arizona and, the desert southwest of the United States, have been reported to have a higher incidence for sand enteropathy (Ragle et al. 1989a, Ragle et al. 1989b, Keppie et al. 2008). In Europe, researchers from Scandinavia have primarily been interested in sand colic, suggesting that the condition is more common there than elsewhere (Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Husted et al. 2005, Kendall et al. 2008).

Seasonal variation in the occurrence of sand enteropathy has also been reported (Ragle & Meagher 1987, Specht & Colahan 1988, Korolainen & Ruohoniemi 2002). Ragle & Meagher (1987) reported that there are seasonal peaks in sand colic cases in northern California in midsummer and midwinter. In Florida however, 66,6% of the horses admitted for surgical sand colic were admitted between July and December, with the greatest number in October (Specht & Colahan 1988). It was also noticed that all of the admitted horses were from an area with large annual rainfall (≥ 120 cm/year) (Specht & Colahan 1988). It has been stated that flooding may increase the ingestion of sand, as the grass becomes covered by silt (in the book section by Colahan 1987). On the contrary, some authors have suggested that drought conditions could have contributed to the development of sand colic (in the book section by Ott 1997, Korolainen & Ruohoniemi 2002). Either way, season and weather conditions may predispose horses to sand colic. In Finland the disease is generally recognized in autumn. The number of cases decline in winter when the ground becomes covered with snow, presumably because snow and seasonally frozen ground prevent horses from ingesting sand (Kaikkonen et al. 2000).

Soil type (in the book section by Colahan 1987, Ragle & Meagher 1987, Ragle et al. 1989a, Ragle et al. 1989b, in the book section by Ott 1997, in the book section by Edwards 1999, Husted et al. 2005), pasture quality (in the book section by Colahan 1987, Specht & Colahan 1988, in the book section by Ott 1997, Husted et al. 2005), and feeding aspects (Udenberg 1979, Ramey & Reinertson 1984, in the book section by Colahan 1987, in the book section by Ott 1997, Husted et al. 2005) are all considered potential risk factors. In particular, loose or light sandy soils are thought to predispose horses to sand colic (in the book section by Colahan 1987, Ragle & Meagher 1987, in the book section by Ott 1997). Overly grazed pastures, and plants or roots with soil attached to them expose horses to ingesting sand (in the book section by Colahan 1987). Feeding from the ground (Udenberg 1979, Ramey & Reinertson 1984) and an insufficient supply of roughage (Husted et al. 2005) have also been associated with increased sand intake. Even lack of fresh water may lead horses to drink from puddles and muddy pools, and thus contribute to the accumulation of sand in the intestines (in the book section by Colahan 1987).

Husted et al. (2005) analysed risk factors for faecal sand excretion as an indicator of sand ingestion, and found out that neither soil type nor feeding practice alone were

statistically significant risk factors, but when combined with pasture quality, they became relevant. They stated that the risk for faecal sand excretion was increased when horses were on pasture with sparse grass and coarse sandy soil, or pasture with long grass and fine sandy soil. The interaction between feeding practice and pasture quality was the following: “Feeding directly on the ground increased the risk in paddocks with no or sparse grass. No feeding in paddocks with sparse grass also increased the risk significantly” (Husted et al. 2005).

Individual risk factors

Age, sex, breed, and body condition may be predisposing factors for different conditions. According to previous studies, there is no significant age-, breed- or sex-related distribution of sand colic. The condition has occurred in both males and females of different ages and different breeds (Specht & Colahan 1988, Ragle et al. 1989a, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Granot et al. 2008, Hart et al. 2013). It has been stated that underfeeding (low body condition score) could increase the risk for sand enteropathy (Ramey & Reinertson 1984, Ragle et al. 1989a, in the book section by Ott 1997). However, when studying risk factors for faecal sand excretion in clinically healthy Icelandic horses, age, sex, or body condition were not found to be significant (Husted et al. 2005).

Geophagic behaviour is a considerable risk factor for sand colic (in the book section by Colahan 1987, Specht & Colahan 1988, Husted et al. 2005). The reason for such behaviour is not always clear, but boredom and salt deficiency are possible causes in some horses (in the book section by Colahan 1987). High concentrations of iron and copper in the soil have also been associated with geophagic behaviour (McGreevy et al. 2001). In particular, foals tend to ingest sand deliberately (in the book section by Colahan 1987, Specht & Colahan 1988). Husted et al. (2005) studied faecal sand excretion and noticed a tendency for greater quantities of sand to be found in young groups of horses (0-3 years). This may imply that young horses or foals have been eating sand on purpose.

Other individual dependent risk factors for developing sand enteropathy include physiopathological differences, such as the ability to tolerate and excrete sand from the gastrointestinal tract (Bertone et al. 1988, Husted et al. 2005). Since ingestion of sand

seems to be common even in clinically healthy horses (Husted et al. 2005), the physiopathological differences should be an interesting research topic.

2.2.3 Clinical signs and symptoms

The clinical signs of sand enteropathy vary from asymptomatic cases to fatal obstructions (Specht & Colahan 1988, Ragle et al. 1989a, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002). The clinical signs are often nonspecific, and may vary between individuals (Kaikkonen et al. 2000, Korolainen & Ruohoniemi 2002).

The most commonly reported symptoms are abdominal pain (colic) and diarrhoea (Udenberg 1979, Ramey & Reinertson 1984, in the book section by Colahan 1987, Bertone et al. 1988, Ragle et al. 1989a, in the book section by Edwards 1999, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Maxwell 2003, Granot et al. 2008, Hart et al. 2013). The episodes of colic may be acute, chronic, intermittent or recurrent (in the book section by Colahan 1987, Korolainen & Ruohoniemi 2002). Weight loss or inability to gain weight has also been related to sand in the gastrointestinal tract (Ramey & Reinertson 1984, Bertone et al. 1988, Kaikkonen et al. 2000). Anorexia (Ragle et al. 1989a), depression and poor performance are sometimes seen in affected horses (Udenberg 1979, Ramey & Reinertson 1984, Ragle et al. 1989a, Kaikkonen et al. 2000). Additionally, increased wear of the teeth (Specht & Colahan 1988) and poor coat condition (Kaikkonen et al. 2000) have been reported in horses that ingest sand.

In cases where the intestinal sand has caused an impaction and possibly also a displacement/torsion, the clinical signs may be severe abdominal pain that does not respond to analgesics, deteriorating cardiovascular signs, and gaseous distention of large colon and/or caecum (Specht & Colahan 1988, Granot et al. 2008). Signs of endotoxemia and shock are often present in horses with complicated sand impactions (in the book section by Colahan 1987, in the book section by Edens & Cargile 1997).

In most cases sand has been accumulating in the intestines over a prolonged period of time before clinical signs appear (in the book section by Colahan 1987). Furthermore, Ruohoniemi et al. (2001) reported that the signs of colic often settle before all the sand has disappeared in the radiographs. They also reported that when the sand accumulations started to resolve as a result of treatment, a few horses showed new

episodes of pain, presumably due to the sand moving through the intestinal tract (Ruohoniemi et al. 2001).

2.2.4 Diagnosis

From the perspective of this study, the most essential diagnostic methods for intestinal sand accumulations are the faecal sand test and abdominal radiography. Examination of faeces for the presence of sand is mentioned as a diagnostic method in several publications (Ramey & Reinertson 1984, in the book section by Colahan 1987, Ragle & Meagher 1987, Bertone et al. 1988, Ragle et al. 1989a, Ragle et al. 1989b, in the book section by Edens & Cargile 1997, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Kendall et al. 2008, in the book section by Lopes 2009). Abdominal radiography, in turn, is currently considered the most useful diagnostic method for sand colic (Kendall et al. 2008, Landes et al. 2008). Both of these methods are described in detail in a later paragraph, while the remaining diagnostic methods are presented here.

As with other diseases, the history, clinical signs, and a thorough physical examination are essential for diagnosis (in the book section by Colahan 1987). Rectal palpation as a part of the physical examination may sometimes reveal intestinal sand masses (Specht & Colahan 1988). However, sand accumulations are often located in segments of bowel that are beyond reach in rectal palpation (in the book section by Colahan 1987). Such segments include the right dorsal colon and transverse colon. It may also be difficult to differentiate whether the palpated firm mass is sand or dry ingesta (Keppie et al. 2008). Consequently, the usefulness of rectal palpation is debatable.

Auscultation of the ventral abdomen has been used to detect sand in the intestine (Rollins & Clement 1979, Ragle et al. 1989b). Abdominal auscultation is based on the sounds produced by the sand particle friction in the bowel during intestinal muscle contractions (Ragle et al. 1989b). The friction between sand particles has been described to sound like a paper bag that was partially filled with sand and rotated (Ragle & Meagher 1987, Ragle et al. 1989b). It should be noted that auscultation is only effective when sand-filled intestines are in contact with the ventral abdominal wall (Ragle et al. 1989b).

Ultrasonography through the ventral abdominal wall has been reported to be a practical and reliable diagnostic method for sand colic when radiography is not possible (Korolainen & Ruohoniemi 2002). Immobility and ventral localization of the intestines, as well as hyperechoic surface are considered as criteria for positive diagnosis (Korolainen & Ruohoniemi 2002). In the previous study by Korolainen & Ruohoniemi (2002), both the specificity and sensitivity of ultrasonography were 87,5%. Transrectal ultrasonography has also been mentioned as a diagnostic method (in the book section by Lopes 2009).

Additionally, accidental enterocentesis (perforation of the bowel) during abdominocentesis may sometimes lead to the correct diagnosis (Ragle et al. 1989b, Granot et al. 2008). However, enterocentesis is not a recommended procedure, because it involves a risk of laceration of the colon and development of peritonitis (Specht & Colahan 1988). Occasionally, none of the above-mentioned methods have revealed the true situation, and explorative laparotomy has been necessary for the diagnosis (Specht & Colahan 1988).

2.2.5 Treatment

Medical treatment

Medical treatment of sand enteropathy includes the use of laxatives (usually administered via nasogastric tube), fluid therapy and analgesics (in the book section by Colahan 1987). Systemic antibiotics may be necessary for horses with fever or elevated plasma fibrinogen concentrations (Ruohoniemi et al. 2001). Laxatives such as magnesium sulphate ($MgSO_4$), mineral oil, psyllium hydrophilic mucilloid, and dioctyl sodium sulfosuccinate (DSS) are commonly used for removal of intestinal sand accumulations in horses (Udenberg 1979, Ramey & Reinertson 1984, in the book section by Colahan 1987, Bertone et al. 1988, in the book section by Edens & Cargile 1997, in the book section by Edwards 1999, Ruohoniemi et al. 2001, Hotwagner & Iben 2008, in the book section by Lopes 2009, Niinistö et al. 2014). Mineral oil acts as a lubricant for the intestinal contents (in the book section by Edens & Cargile 1997), but does not penetrate the water-soaked sand masses very well (in the book section by Colahan 1987). Magnesium sulphate is an osmotic laxative, which functions by drawing and retaining fluids into the bowel (in the book section by Colahan 1987, in the book

section by Edens & Cargile 1997). It may also be a better stimulus for the gastrocolic reflex than other laxatives (in the book section by Edens & Cargile 1997). Dioctyl sodium sulfosuccinate is thought to reduce surface tension and thereby allow water to penetrate the impacted mass, as well as increase secretion and change permeability of the intestinal mucosa. At high doses, both DSS and magnesium sulphate may be toxic and may induce diarrhoea, and consequently dehydration and hypovolemia (in the book section by Edens & Cargile 1997). Magnesium sulphate in particular, can cause sudden death due to cardiac arrhythmias if overdosed (in the book section by Bonagura et al. 2010), and therefore caution must be exercised when administering this drug.

Psyllium hydrophilic mucilloid is considered the most effective laxative for removal of intestinal sand. Psyllium is a lubricating bulk laxative that forms a gel in the gastrointestinal tract. The gel binds and carries sand along through the bowel (in the book section by Colahan 1987). “Psyllium is believed to have better ability to penetrate, hydrate, and break up sand impactions than the other laxatives” (in the book section by Edens & Cargile 1997). However, the effect of psyllium has been questioned, since an experimental study showed no benefit of psyllium treatment in ponies that had been surgically administered sand during an exploratory celiotomy (Hammock et al. 1998). Recently, Niinistö et al. (2014) demonstrated that the combination of psyllium (1g/kg) and magnesium sulphate (1g/kg) via nasogastric tube once daily for 4 days is an effective way to evacuate intestinal sand.

Surgical treatment

In cases where medical therapy is not successful, surgery is required to remove the sand from the colon (Ragle et al. 1989a). Severe pain, deterioration of cardiovascular signs and significant abdominal distention are considered indications for surgical treatment (Specht & Colahan 1988, Ragle et al. 1989a). A ventral midline laparotomy, pelvic flexure celiotomy/enterotomy and lavage of the bowel lumen are most commonly performed during surgery (in the book section by Colahan 1987, Specht & Colahan 1988, Ragle et al. 1989a, Maxwell 2003, Granot et al. 2008). The large colon can be carefully exteriorized from the abdomen and flushed through the celiotomy site with a garden hose and tap water (Specht & Colahan 1988). Sometimes multiple enterotomies are needed to remove all of the sand (in the book section by Ross & Hanson 1992,

Maxwell 2003). Post-operative complications include recurrent colic, diarrhoea, peritonitis, incisional hernia, incisional wound complications and laminitis (Specht & Colahan 1988, Granot et al. 2008).

2.2.6 Prognosis and prevention

Prognosis

Hart et al. (2013) reported that the short-term survival for horses treated medically for sand enteropathy was 90%. In a retrospective study of 48 horses treated surgically for sand impaction, the mortality was as low as 8,33% (Specht & Colahan 1988). It is comparable with the results of the study done by Ragle et al. (1989), in which the short- and long-term survival rates for horses treated surgically were 86% and 71% respectively. More recently, Granot et al. (2008) reported that the short-term survival rate after surgery was 95%, and the long-term survival rate was 100% in a similar retrospective study. The mortality rate for all horses with sand impaction was 15% (Granot et al. 2008). Early surgical intervention of sand impaction cases is likely to enhance the prognosis after surgery (Granot et al. 2008). In both surgical and non-surgical cases the prognosis is good, but the condition may recur (Ragle et al. 1989a, Granot et al. 2008, Hart et al. 2013).

Prevention

Management practices are important for the prevention of sand enteropathy (in the book section by Colahan 1987, Ragle et al. 1989a). In sandy paddocks the hay should be offered from feed racks or nets instead of the ground (in the book section by Colahan 1987). It is recommended that horses are kept on good quality pastures and fed extra hay when the pastures become overgrazed (in the book section by Edwards 1999). Other preventive practices include providing branches for horses to chew or using muzzles on horses that are turned out. In some cases the prevention of sand ingestion might be a sufficient treatment for sand enteropathy (Hammock et al. 1998).

2.3 Faecal sand tests

Veterinary practitioners in Finland have been using the plastic glove test for years and the subjective evaluation of the quantity of sand in the faeces of horses has been considered a diagnostic method for sand enteropathy over the last few decades (in the book section by Colahan 1987, Bertone et al. 1988). The diagnostic accuracy of the method has not yet been demonstrated however, and so far the test has been considered merely indicative rather than diagnostic.

The faecal sand test is based on the assumption that whenever there is sand present in the intestines of a horse, some of it is excreted in the faeces within a certain period of time. In a previous study, clinically healthy horses that were experimentally administered sand excreted approximately 70% of the given amount in the faeces within 11 days after the administration (Hammock et al. 1998).

The following method for detecting sand in the faeces has been described in the book section by Colahan (1987). Six faecal balls are broken up into a container of water (0,95 litres), and the sand is allowed to gravitate to the bottom of the container. More than 1 teaspoon of sand per 6 faecal balls is indicative of sand accumulation. Alternatively, less quantitative results are obtained by collecting two to four faecal balls with a disposable glove. The glove is then inverted over the faeces and some water is added to make a suspension inside the glove. Again, the sand is allowed to settle for some time before inspection. More than 0,6 cm of sand in the fingers of the glove is considered abnormal (in the book section by Colahan 1987). There are also several other methods described for examining the presence of sand in faecal material mixed with water (Ferraro 1973, Ramey & Reinertson 1984, in the book section by Edwards 1999, Ruohoniemi et al. 2001, Maxwell 2003, Husted et al. 2005, Granot et al. 2008, Landes et al. 2008, in the book section by Lopes 2009). They all have the same basic idea of separating sand from faecal material by sedimentation.

The reliability and functionality of the faecal sand tests have often been questioned. The test is not able to estimate the quantity of sand within the gastrointestinal tract (Kendall et al. 2008). Keppie et al. (2008) stated that detection of sand in the faeces only indicates exposure to sand, and the association between the amount of faecal sand and the amount of intestinal sand is poor in horses with sand enteropathy. Similar observations have been made by other authors; the correlation between the faecal sand

tests and the presence of sand in the colon is poor (in the book section by Edens & Cargile 1997, Ruohoniemi et al. 2001). It has also been reported that the sensitivity of the faecal sand sedimentation test is low (Kendall et al. 2008), and it is supported by previous studies, in which several false negative results were obtained in horses that had radiographic evidence of sand accumulations (Bertone et al. 1988, Ruohoniemi et al. 2001).

Small amounts of sand are a common finding in the faeces of healthy horses, and it has been demonstrated that up to 56,4% of clinically healthy horses pass sand in their faeces (Husted et al. 2005, Keppie et al. 2008). The data consisted of 211 individual faecal samples collected from 19 Icelandic horse stud farms in Denmark. All the horses included were Icelandic horses because they are usually kept outside on pasture or paddock throughout the year. Abdominal radiographs were not included in the study (Husted et al. 2005). The results raise the question, whether some of those horses with sand in their faeces also had an asymptomatic sand accumulation in the colon.

In addition to the diagnostic use of the faecal sedimentation test to identify horses with sand enteropathy, the appearance of sand in the faeces may also indicate the clearance of intestinal sand and therefore provide an indirect measurement of the success of the evacuation treatments (Ruohoniemi et al. 2001).

2.4 Abdominal radiography for detecting intestinal sand accumulations

Abdominal radiography has been used to detect sand in the intestines of horses (Rose et al. 1980, Bertone et al. 1988, Yarbrough et al. 1994, in the book section by Edens & Cargile 1997, Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002, Kendall et al. 2008, Keppie et al. 2008, in the book section by Lopes 2009). It has been demonstrated to be a useful method for diagnosis of sand enteropathy, as well as monitoring the resolution of sand accumulations (Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002). It is also thought to be “the best way to assess the volume and location of sand in the colon” (in the book section by Lopes 2009).

Four overlapping images are needed to cover the entire gastrointestinal tract of an adult horse (Rose et al. 1980, Ruohoniemi et al. 2001). However, the cranioventral abdomen

is considered a predominant location for sand accumulations (Ruohoniemi et al. 2001, Kendall et al. 2008, Keppie et al. 2008), and therefore radiographs are usually focused on that area using a right-to-left standing lateral projection (Ruohoniemi et al. 2001, Korolainen & Ruohoniemi 2002). Sand accumulation typically appears as a radiopaque mass with a horizontal dorsal margin and a convex ventral margin that follows the shape of the sacculations of the colon (Keppie et al. 2008). It is illustrated in Figure 1. An indistinct dorsal margin is thought to be due to sand mixing with other ingesta and therefore becoming less radiopaque (Ruohoniemi et al. 2001, Keppie et al. 2008). Coarse sand may be more opaque in the radiographs than fine sand (Keppie et al. 2008). The number of sand accumulations observed in the radiographs has been reported to have a linear relationship with a positive diagnosis for sand colic (Keppie et al. 2008). Additionally, the size of the radiographic sand accumulation has been demonstrated to correlate with the severity of the clinical signs (Kaikkonen et al. 2000).

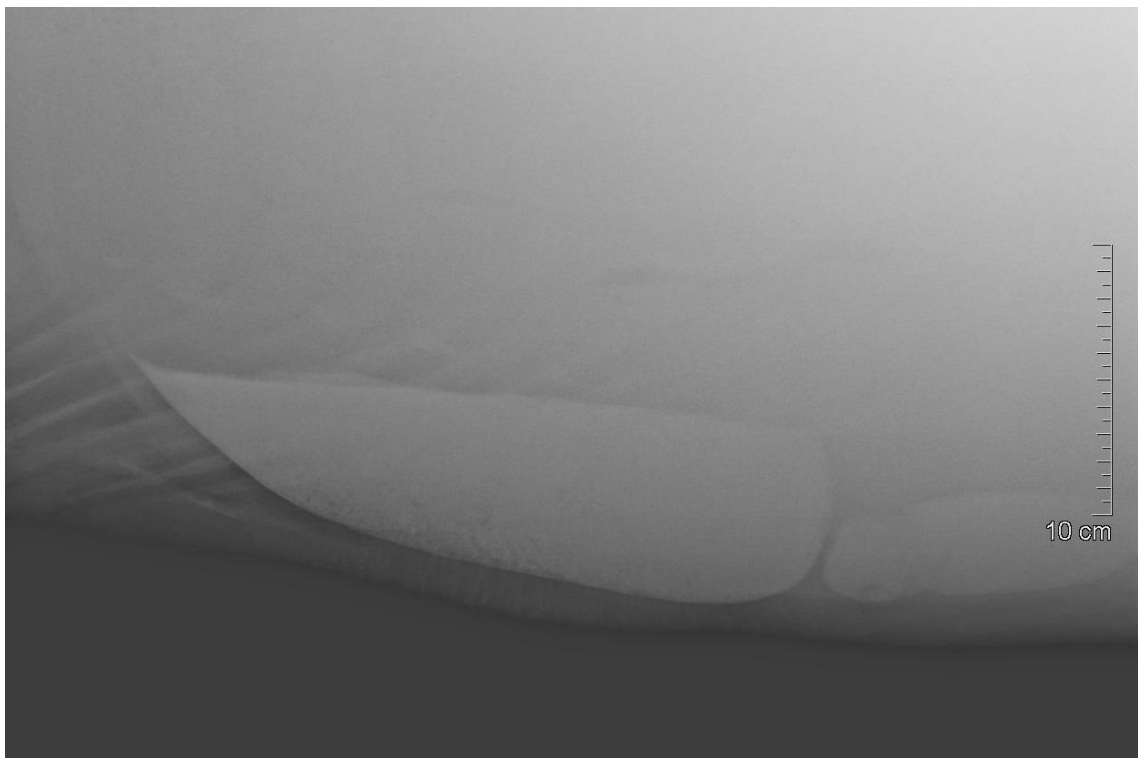


Figure 1. A radiograph showing two sand accumulations in the cranioventral abdomen of a horse.

Radiographic evidence of sand accumulations has been found in the intestines of horses without any gastrointestinal symptoms (Campbell 1984, Kaikkonen et al. 2000, Ruohoniemi et al. 2001, Kendall et al. 2008). To identify the clinically significant cases and to improve the comparability of the radiographic outcome, researchers have suggested tools for evaluation of the radiographs. Korolainen & Ruohoniemi (2002) created a grading system, where the grades (0-4) are given according to the maximum height and length (cm), and location of the suspected sand accumulations. More recently, Keppie et al. (2008) developed an objective radiographic scoring system (0-12) to help assess the severity and quantity of gastrointestinal sand accumulations. The parameters included in the scoring system are location and number of sand accumulations, opacity and homogeneity of the accumulations, the rib width to length of accumulation ratio, and the rib width to height of accumulation ratio. In this system, a score of 7 or higher indicates that the likelihood of true positive diagnosis of sand colic is 83%. A score of less than 7 means that there may be some sand present in the intestine, but it is unlikely to cause clinical symptoms (Keppie et al. 2008).

The main limitation of abdominal radiography is the expense of the equipment, and the fact that it is not routinely available in most private practices (Korolainen & Ruohoniemi 2002). It should be also noted that the evaluation of the radiographs is mostly subjective, even by means of an objective scoring system. Because the abdominal radiographs are only two dimensional, estimation of the exact amount of sand is impossible. The use of a ventrodorsal view is usually not an option except for small foals. Furthermore, the exact location of the sand accumulation is not always possible to determine based on the radiograph (Ruohoniemi et al. 2001). Summation and magnification may also cause false interpretations of the extent and location of sand accumulations (Rose et al. 1980, Kaikkonen et al. 2000, Kendall et al. 2008, Keppie et al. 2008).

2.5 Validation of a diagnostic test

Diagnostic tests are designed to determine the presence or absence of a certain disease. Validation of a diagnostic test is generally considered as the process by which the accuracy and applicability of the test is determined in practice. In other words,

validation demonstrates how well the test fulfils its intended purpose (The World Organisation for Animal Health (OIE) 2013).

A single standard for the validation of diagnostic test has not yet been established in veterinary medicine. However, the World Organization for Animal Health (OIE) has published the principle of validation and certification of diagnostic test methods for infectious diseases in animals, and the latest version is from 2013. Many of those principles are applicable to the validation of other diagnostic methods.

The validation process is generally performed by comparing the diagnostic test to the gold standard method (Enøe et al. 2000). Gold standard can be defined as the most accurate and definitive diagnostic test or method available at a given time. It is used to classify the positive and negative true state of disease (Greiner & Gardner 2000, The World Organisation for Animal Health (OIE) 2013).

2.5.1 Accuracy and precision

The concepts of accuracy and precision are usually taken into account during the validation process. Precision and accuracy measure the performance of a diagnostic test (Greiner & Gardner 2000). Precision can be defined as the degree of dispersion or the consistency of test results within repeated measurements of the same sample under same conditions (Greiner & Gardner 2000, in the book by Riffenburgh 2006, The World Organisation for Animal Health (OIE) 2013). The term accuracy is described as “the ability of a diagnostic test to produce correct test results” and it measures the closeness of the test result to the actual true value of the measured (Greiner & Gardner 2000). It is also considered a synonym for validity (Greiner & Gardner 2000).

The accuracy of a qualitative diagnostic test can be measured by the sensitivity, specificity, predictive values and ROC analysis. The precision of a test can be estimated using confidence intervals of sensitivity and specificity (Cleophas et al. 2008).

2.5.2 Sensitivity and specificity

Sensitivity and specificity can be determined in 2 X 2 table by comparing the test results to the gold standard test results. Sensitivity means the proportion of positive test results

of all the tested animals that truly have the disease (Drobatz 2009). It informs the probability that a diseased individual gets a positive test result (Greiner & Gardner 2000). Specificity is the proportion of true negative test results of all the healthy individuals tested for the disease (in the book by Nienstedt et al. 1997, Drobatz 2009). In other words, it is the probability that a non-diseased individual tests negative for the disease (Greiner & Gardner 2000). “From a strictly diagnostic perspective, a good diagnostic test would never miss an animal with the disease and would always correctly identify animals with the disease being tested for” (Drobatz 2009). In that hypothetical case both the sensitivity and specificity of the test would be 100%.

Sensitivity can be calculated from the following formula:

$$\frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

And specificity respectively:

$$\frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

A test with high sensitivity can be used to rule out a disease, while high specificity is used to rule in a disease (Drobatz 2009). It can be demonstrated with an example; a test with sensitivity of 100% gives always a positive outcome for diseased animals, and therefore a negative test result indicates, that the animal does not have the disease, and it can be ruled out (Drobatz 2009). However, the sensitivity does not depend on the number of false positives, which can be relatively high even with a highly sensitive test. Therefore a positive test result of a highly sensitive test is not useful for ruling in disease. Specificity functions vice versa.

2.5.3 Other parameters and statistical measures

Prevalence of the disease being tested is usually taken into account when calculating the statistical measures for accuracy. Prevalence of a certain disease is the proportion of

diseased individuals in the population at a given time (in the book by Nienstedt et al. 1997, in the book by Riffenburgh 2006, Drobatz 2009).

Like sensitivity and specificity, predictive values are also measurements of accuracy. Positive predictive value describes the likelihood that a patient with positive test result is truly carrying the disease, whereas negative predictive value describes the likelihood that a patient with negative test result is actually negative for the disease (Greiner & Gardner 2000, Drobatz 2009). In summary, predictive values measure the probability that the prediction of the test is correct (in the book by Riffenburgh 2006). Unlike sensitivity and specificity, predictive values are dependent on the prevalence of the disease/condition (Greiner & Gardner 2000, Drobatz 2009).

Likelihood ratios are a way to measure the diagnostic ability of a test. The utility of performing a certain diagnostic test can be assessed with likelihood ratios of the test (Wikipedia 2015). Likelihood ratios are independent of the prevalence, and take into account both the sensitivity and specificity of the test (Greiner & Gardner 2000). Positive likelihood ratio (LR+) means the probability that a sick animal is tested positive in ratio to the probability that a healthy animal is tested positive. It is calculated as $LR+ = \text{SENSITIVITY} / (1 - \text{SPECIFICITY})$. In contrast, a negative likelihood ratio LR- is the ratio of $(1 - \text{SENSITIVITY}) / \text{SPECIFICITY}$. A negative likelihood ratio (LR-) can be defined as the ratio of the probability that a sick animal is tested negative to the probability that a healthy animal is tested negative. Likelihood ratios can be used to calculate the post-test probabilities of the disease (Drobatz 2009, Wikipedia 2015). A likelihood ratio of 1 indicates that the test is not useful for the diagnosis, and the post-test probability of the condition is the same as the pre-test probability (the prevalence of the disease). A likelihood ratio of greater than 1 increases the post-test probability in relation to the pre-test probability and indicates that the test result is associated with the presence of the condition, whereas likelihood ratio less than 1 decreases the post-test probability and indicates that the test result is associated with the absence of the condition (Wikipedia 2015).

2.5.4 ROC analysis

ROC analysis can be used to evaluate the performance of a single diagnostic test, as well as to compare two separate tests with each other. A receiver operating

characteristic (ROC) curve is a graph, which represents the sensitivity as a function of 1 - specificity at various cut off/threshold points (Forsström 1995, Greiner et al. 2000). In many diagnostic tests the outcome varies from qualitative to quantitative on an ordinal, interval, ratio or continuous scale. When the outcome is not dichotomous (binary), a cut-off/threshold value needs to be selected to define the positive vs. negative diagnoses (Greiner et al. 2000). The sensitivity and specificity of the test depend on the selected cut-off value, and the ROC curve can be used to determine the optimal cut-off point to achieve desired sensitivity and specificity. The curve can be created when the false positive rates (x-axis) and true positive rates (y-axis) are calculated for each possible threshold value/data point and recorded in the coordinate system. The area under the ROC curve (AUC) describes the discriminatory power of the test. Hypothetically, in a perfectly discriminatory test, which always correctly distinguishes between positive and negative result, the area under the curve is 1,0. In that case the plot would rise from (0,0) to (1,0), and then continue horizontally to (1,1). In contrast, when the plot passes diagonally from (0,0) to (1,1), the area under the curve is 0,5 and the test has no discriminatory ability (Zweig & Campbell 1993, Forsström 1995, Greiner et al. 2000, Drobatz 2009). The Youden index J (defined as [Sensitivity + Specificity – 1]) is often included in the ROC analysis, and it can also be used to summarize the diagnostic ability of a test (Greiner & Gardner 2000).

2.5.5 Requirements of the validation process

The estimates for accuracy of a certain diagnostic test usually varies among published validation studies. Sampling, reference population, conditions and methods used in the validation process, as well as random and systematic errors, induce variation in the results of rival studies. Also biological factors may cause variation in the sensitivity and specificity within different populations (Greiner & Gardner 2000). For example, the sensitivity of a test may be affected by the stage of the disease, or age and sex of the animals in the target population (Greiner & Gardner 2000, Drobatz 2009). To minimize the errors and biases, the validation process should be standardized.

Greiner & Gardner (2000) have suggested a useful guideline for test validation studies from an epidemiological perspective. A precise validation study determines the purpose and protocol of the particular diagnostic test. The reference test needs to be justified, if

it is other than the gold standard, and the protocol of the reference test/gold standard should be described in detail. Also selection and sampling of the reference population and exclusion/inclusion criteria should be sufficiently described. The reference population should correspond to the target population of the test. Sampling is preferably random or systematic and sample sizes should be determined at the required statistical certainty. The test should be evaluated with patients at different stages of the disease as well as healthy individuals. A reliable validation process should be performed blinded, meaning that the results of test and reference test are evaluated independently. Negative and positive test results need to be properly defined in the test protocol. The results should be presented using the adequate statistical analyses. The uninterpretable and intermediate results or elimination of data must also be reported. Finally, the possible biases and significance of the results, as well as the use and applicability of the test in practice should be discussed (Greiner & Gardner 2000).

3 MATERIAL AND METHODS

The present study was conducted by collecting a faecal sample and an abdominal radiograph from each participating horse. All the horses were clinical cases admitted to the Veterinary Teaching Hospital of the Helsinki University during the study in 2012-2014. The participants were randomly selected from the horses which met the inclusion criteria. In order to participate in the study, the horses needed to be admitted to the hospital for radiography. This could be for a variety of reasons, including colic and musculoskeletal disease. The only proviso was that the owner agreed that an extra radiograph of the abdomen would be obtained for the purposes of our study and that a faecal sample could be collected for analysis. The additional radiograph was free of charge for all horses that met the inclusion criteria.

Because the study was conducted alongside the diagnostic and treatment process of each horse, it should not have caused any unnecessary or significant harm to an individual horse. Generally, all the owners of the patient horses at the Veterinary Teaching Hospital agreed that the horse's medical record could be used as data for

veterinary research. However, if the abdominal radiograph or rectal examination was not included in the diagnostic procedures of the horse, the owners signed an agreement on taking an extra radiograph and a faecal sample. All the data were treated confidentially, and individual animals cannot be identified from this study.

3.1 Material

Sixty three samples were collected from 61 different horses (19 Finn horses, 17 Warmbloods, 3 Standardbreds, 4 Icelandic horses, 5 Shetland ponies, 10 other ponies and 3 horses with unidentified breed). The median age of the horses was 11,5 years (1-27) at the time of the study. Thirty six of the horses were geldings, 19 were mares and 6 were stallions. Most of the horses had signs of colic when they were presented at the hospital. Nine of the horses had been given psyllium at home prior to their presentation at the hospital, and 7 of the horses had already been treated with psyllium (+/-MgSO₄) at the hospital prior to sampling and abdominal radiography.

3.2 Methods

3.2.1 Abdominal radiography

The horses at the hospital were routinely sedated for radiography using either detomidine (Domosedan, Orion Pharma) or a combination of detomidine and butorphanol IV. The cranioventral abdomen of the horses was radiographed (Shimadzu UD150B-40, OneMed) using mostly a right to left standing lateral view. Occasionally the view was from left to right, if it was more convenient to the situation (e.g. the horse was in this position after radiography of the limbs and the horse was too unsteady to move after sedation). The cassette was placed in a ceiling mounted holder. The exposure settings were generally 125 kV/100 mAs, where the current (mAs) was adjusted automatically. The radiographs were viewed with a commercial computer programme (Jivex) and interpreted by the attending veterinarian, who recorded the length and height of the sand accumulation (if present) in the register of the patient.

3.2.2 The plastic glove test

The faecal samples were mainly collected rectally during a clinical examination. In ten cases the faecal sample was collected from the ground for the safety of the collector and the horse. In these cases the sample was taken from a fresh pile of faeces. In addition, there was sawdust base in the stall and no visual signs of sand. Approximately 200 grams of faeces were collected from each horse and placed in a plastic rectal sleeve.

The faecal samples were collected on the same day as abdominal radiography was performed (within 24 hours before or after abdominal radiography). The plastic glove test (faecal sand sedimentation test) was run on the same day or later. If for some reason it was not possible to run the test on the same day as the faeces were collected, the sample was either kept in refrigerated or frozen. A total of 17 samples were frozen.

The plastic glove test was run by adding 1 litre of warm water to the rectal sleeve containing the faeces and gently mixing and rubbing the contents so that the faecal balls were broken up and dispersed within the water. Then the rectal sleeve containing the faecal suspension was left to hang for 30 minutes. The findings were then recorded. The contents of the glove were then again mixed and left to hang for the next day. The findings were again recorded. The plastic glove test results were always read by the same person, who did not know the outcome of the abdominal radiographs.

Each fingertip of the glove was assessed separately and a 0-3 numerical scale was used to record the results of the plastic glove test. The scale was scored as follows:

0 = no sand

1 = possibly some sand visible

2 = small/moderate amount of sand clearly visible and palpable

3 = lots of sand

The score for each finger was then added up to make a total score out of 15 for each horse.

3.2.3 Statistical analysis

For the statistical analysis the plastic glove test results were compared to the radiographic results (gold standard) in a 2 X 2 table (Microsoft Excel). Sensitivity, specificity, predictive values and likelihood ratios (with 95% confidence intervals) were determined. Additionally, a ROC analysis was conducted using MedCalc statistical software, and the method of DeLong et al. (1988) for the calculation of the standard error of the area under the curve.

4 RESULTS

From the 63 original samples 3 had to be deleted from the data, since the plastic glove ruptured before the results were read. This left a total of 60 samples from 58 different horses. The prevalence of radiographic evident sand accumulation in the present data is 76,67% (with a 95% confidence interval of 63,96% to 86,62%). Initially, the threshold value for a positive diagnosis was set to >0 , meaning that the plastic glove test result was categorized as positive if sand was present, and negative if no sand was detected. The sensitivity, specificity and predictive values of the test are presented in Table 1. The 95% confidence intervals of sensitivity and specificity were 68,58% to 92,18%, and 41,90% to 91,61%, respectively. The 95% confidence intervals of predictive values were 77,38% to 97,34% (positive) and 30,76% to 78,47% (negative). The positive likelihood ratio of the test is 2,89 (95% confidence interval 1,25 to 6.69) and negative likelihood ratio is 0,24 (95% confidence interval 0,12 to 0,50).

Table 1. The results of the plastic glove test compared to the gold standard in 2 x 2 table.

		Intestinal sand accumulation confirmed with abdominal radiography (gold standard)		
		condition positive	condition negative	
Plastic glove test outcome	test outcome positive	true positive 38	false positive 4	positive predictive value 90,48 %
	test outcome negative	false negative 8	true negative 10	negative predictive value 55,56 %
	sensitivity 82,61 %		specificity 71,43 %	

The ROC curve of the plastic glove test is shown in Figure 2. According to the ROC analysis, the optimal cut-off/threshold point is >0 . The area under the curve (AUC) is 0,771 with a 95% confidence interval of 0,644 to 0,870 (p-value = 0,0003 meaning that the AUC is significantly different from 0,5).

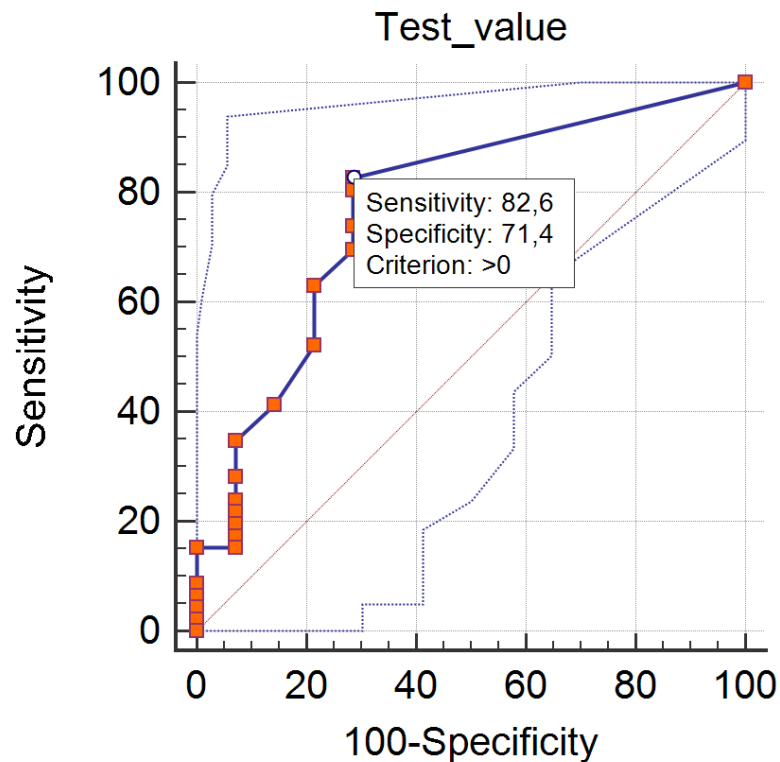


Figure 2. The ROC curve of the plastic glove test. The lighter plots illustrate the 95% confidence interval. The Youden index J for the optimal data point (criterion >0) is 0,5404.

Generally, the amounts of sand in the faeces were small and not always palpable. Even horses with huge sand masses in the radiographs had occasionally just barely positive faecal sand test results. The figures 3 and 4 illustrate the appearance of a typical positive and negative sample in the test. The positive test result was usually more definite when read on the first day. Yet, the outcome of the test on the second day was always consistent with the outcome on the first day. Sometimes dark colouring of the rectal sleeve made detection of sand more difficult.



Figure 3. Sand in the fingertip of a rectal sleeve. Score = 3 (lots of sand)



**Figure 4. The appearance of negative outcome in the plastic glove test. Score = 0
(no sand)**

5 DISCUSSION

5.1 How to interpret the results

Even though several false negative results have been related to the faecal sand sedimentation tests (Bertone et al. 1988, Ruohoniemi et al. 2001), the sensitivity of the plastic glove test is quite good (82,61%) according to the results of our study. The specificity of the test is lower (71,43%), and the wider confidence interval makes the estimate of specificity somewhat more uncertain. The higher sensitivity and lower specificity of the plastic glove test mean that the test is quite reliable to identify a horse with sand accumulation as positive, but not as reliable to identify a healthy horse as negative for the disease. In other words, the test is more likely to provide a false positive than a false negative result.

The high positive predictive value (90,48%) of the plastic glove test indicates that, if the test outcome is positive, the horse most likely has an intestinal sand accumulation evident in the radiographs. Negative predictive value for the plastic glove test is considerably lower (55,56%), and reveals that only 55,56% of the horses tested negative for sand, did not actually have an intestinal sand accumulation. Thus, if the test outcome is negative, there is almost 45% chance that the test result is incorrect. The main conclusion is that a negative outcome of the plastic glove test should not be blindly trusted, since the negative predictive value of the test is low. Then again, it should be noted that the predictive values are affected by the prevalence of the disease (Drobatz 2009). When the prevalence of the condition alters, the predictive values will differ.

The positive likelihood ratio of 2,89 means that a positive plastic glove test result slightly increases the probability of an intestinal sand accumulation to be present. The post-test probability of the disease is therefore greater than the pre-test probability (prevalence of the disease). The positive likelihood ratio of the plastic glove test is not especially high, but it still shows that the test has some diagnostic value and may be useful in diagnostics. The negative likelihood ratio (0,24) of the test is slightly better. It

indicates that a negative plastic glove test result moderately decreases the probability of an intestinal sand accumulation to be present.

Even though the results of the plastic glove test are easy to manage dichotomously (sand vs. no sand), the ROC analysis was performed to see if a higher threshold value (cut-off point) for positive result would have improved the accuracy of the test. However, the plot confirms that our initial decision threshold (>0) is the most optimal (Fig. 2). It also proves that the test has some discriminatory power; the area under the curve (AUC) is 0,72. A diagnostic test that has AUC greater than 0,7 ($0.7 < \text{AUC} \leq 0.9$) is generally considered moderately accurate (Greiner et al. 2000).

According to the data used in this study, the prevalence of intestinal sand accumulation was as high as 76,67%. It may have been elevated by the great number of colic patients in the research material. However, 3 from the 5 non-colic patients included in the study also had radiographic evident sand accumulations. There is no published data on the prevalence of sand colic in Finnish horse population. But in Sweden, Kendall et al. (2008) found out that 66% of the control horses (a complaint unrelated to the gastrointestinal tract) in their study had one or more intestinal sand accumulations confirmed with abdominal radiography. Reportedly, the horses were from an area with a low incidence of sand impactions (Kendall et al. 2008).

5.2 Sample size and sampling bias

Most of the horses in this study were hospital patients with gastrointestinal problems, however a small proportion of patients did not have gastrointestinal symptoms and were hospitalised for other reasons. Consequently, the reference population of the study was actually the patient material of the veterinary hospital. Ideally, the samples would have been collected randomly from the entire Finnish horse population (target population of the test), and without categorizing the horses. However, it was not possible within the resources of this study.

When the sample size is large enough, and the sampling is randomized, the healthy and diseased horses should be represented in the data according to the prevalence of the disease. In our data, the total number of diseased horses (horses with positive sand diagnosis) was considerably larger than the number of non-diseased horses (negative

sand diagnosis confirmed with the gold standard). It most likely results from the above-mentioned fact that the research data was collected only from the patient material of the veterinary hospital. Therefore it is possible that the clinically healthy horses were under-represented, and the reference population used in this study did not correspond to the target population well enough. This may have caused sampling or spectrum bias in the results. The 95% confidence interval for the specificity is very wide, indicating that in regard to non-diseased horses the sample size was too small.

It can be speculated how the results of this study would differ, if the data would have consisted of more equal amounts of clinically healthy (or at least horses with condition unrelated to gastrointestinal tract) and diseased animals. The mere increase in the number of healthy horses (negative diagnosis confirmed with gold standard), would not have changed the sensitivity of the test. But it could have affected the specificity, predictive values and likelihood ratios. If there were significant differences in ability to excrete intestinal sand between healthy horses and horses with any gastrointestinal problem, the lack of clinically healthy horses in the data may have distorted the results. For instance, if the clinically healthy horses with asymptomatic sand accumulations were more effective to excrete sand in the faeces compared to the colic patients, the results could be different.

5.3 Effect of psyllium (and other factors) on the excretion of sand

It has been demonstrated that psyllium increases the clearance and excretion of intestinal sand (Hotwagner & Iben 2008, Landes et al. 2008), and thus makes the faecal sand tests (plastic glove test) more likely to be positive. However, in the study made by Bertone et al. (1988) it was noted that faecal sand excretion was inconsistent during psyllium administration, even though radiographs showed a decrease of intraluminal sand. Psyllium may cause bias in the results of this study, but since we wanted the test to be applicable to horses in different stages of the disease (with previous treatments or not), we decided to also include the horses that had been treated with psyllium. Consequently, it is possible that the sensitivity of the test is lower among horses that have not been treated with psyllium.

In addition to psyllium, some of the horses in this study had been given magnesium sulphate or mineral oil orally prior to performing the plastic glove test. Magnesium

sulphate combined with psyllium (Niinistö et al. 2014) or mineral oil (Ruohoniemi et al. 2001) has been found to be effective to resolve sand accumulations in some cases. Thus it is also possible that these treatments increase the amount of sand excreted to the faeces, and modify the results of the present study.

Other factors that affect the sand excretion of an individual horse might be for instance exercise, feedstuff, other medications, physiological differences, or pathological conditions. Medications or diseases that reduce (or enhance) the intestinal motility may be relevant. Further research is needed to work out this matter.

It is also likely that regardless of the treatments and other affecting factors, excretion of sand into the faeces is a partly random process. Therefore we cannot fully rely on the negative result of the plastic glove test. A positive test result, however, is a quite strong evidence of ingestion of sand, but it actually does not inform, whether the sand is just passing by, or has accumulated, meaning the condition is pathological. A positive result in the plastic glove test may also be a sign of recovery, when the existing sand accumulation has started to resolve (Ruohoniemi et al. 2001).

5.4 Defects and biases of the gold standard method

Abdominal radiography is usually limited to the cranioventral abdomen, and was also the case in this study. Even though the cranioventral abdomen is considered the predominant location for sand accumulations, it has been reported that more sand impactions were found in the pelvic flexure, right dorsal colon and transverse colon (Ragle et al. 1989a, Keppie et al. 2008). If the intestinal sand is located outside the radiographic exposure area, the diagnosis may be false. Consequently, the results of this present study may have been affected by possible false negative results of the abdominal radiography. It is relevant because abdominal radiography was considered as the gold standard diagnostic method against which the plastic glove test was compared.

Additionally, there may be some degree of variation in the interpretation of the gold standard outcome, since the radiographic diagnosis of sand colic was generally made by the attending veterinarian of each horse. Depending on the veterinarian, a very small and narrow sand accumulation may have been either ignored, or reported and classified as a positive condition in the data. The sensitivity and specificity of a test may be

distorted, if the gold standard method is imprecise and inconsistent. To get more consistent and unbiased classification of the radiographic outcome, one and the same person should interpret all the radiographs.

The definition of positive and negative radiographic diagnosis was based on a simple binary scale (sand vs. no sand). However, in practice it seems to be much more complicated to determine if a small sand accumulation is the real cause of illness or not. In many cases clinically healthy or asymptomatic horses have had visible sand accumulations on abdominal radiography (Ruohoniemi et al. 2001, Kendall et al. 2008). Furthermore, the use of previously suggested scoring/grading systems for classifying clinical significance of radiographic evident sand accumulations have demonstrated that it is difficult to define the exact limit between positive and negative diagnosis of sand enteropathy. In one study, a grade 1-2 sand accumulation on a scale from 0 to 4 (grading system by Korolainen & Ruohoniemi 2002) was a common finding in horses that had not recently had any problems related to gastrointestinal tract (Kendall et al. 2008). Since the diagnostics are not so straightforward even with the gold standard method, the plastic glove test cannot be considered as a very qualified method for differentiating the symptomatic cases from the asymptomatic and clinically insignificant cases.

5.5 Other possible biases and limitations of the study

Like the outcome of the abdominal radiography, the plastic glove test results are also open to interpretation. The test is based entirely on the subjective observation of sand, and therefore it is also quick and inexpensive. To avoid subjective bias in this study, the plastic glove test results were always read by the same person, who was unaware of the gold standard outcome. Nevertheless, the estimates for accuracy apply to the conditions and observers used in this study. In practice the subjective differences among veterinarians (and other test performers) may modify the accuracy of the test.

In addition to the subjective observation of sand, we also tried to measure the sand objectively by separating it from the faeces and weighing it. However, in most cases the amounts of sand were so small that the visual inspection appeared to be more accurate. The eye was able to distinguish the individual grains of sand in the fingertips of the glove, while separation of few grains of sand from 200 grams of faeces and 1 litre of

water was unsuccessful even with a centrifuge designed for this purpose. It was also noticed that the colour and particle size of sand influenced on how easily sand was detected. Fine sand was detected more easily by visual inspection than by palpation. In practice the composition of the sand/soil may affect the outcome of the plastic glove test.

In the course of the study, 17 faecal samples were frozen before the plastic glove test was performed. It should not have affected the results, since sand does not change its physical state in the freezer temperatures. Ten of the faecal samples were collected from the ground (instead of rectum), which may theoretically have contaminated the samples, even though the faeces were carefully picked from the centre of a fresh pile on a sawdust bedding. However, 5 of these samples were negative on the plastic glove test.

The average weight of the faecal samples was 204 grams, and it seemed to be an adequate amount. Catching at least some of the intestinal sand within the sample is more likely with greater quantities of faeces. On the other hand, the plastic glove can bear only a limited amount of weight before rupturing. If the quantity of faeces in the sample affects the sensitivity of the test, the smaller (<150g) samples may have caused false negative results and bias in the final results. At least in two cases (faecal sample<150g) the outcome of the plastic glove test was negative, while sand was evident in the radiographs. Six samples contained faeces weighing more than 250 grams, and from these cases one was positive for the plastic glove test, even though no sand was found in the radiographs.

The results of the abdominal radiography and faecal sand sedimentation tests are not always completely comparable, because sand colic may be a rapidly changing condition in horse. Radiographic visible sand accumulation, may be so immobile (petrified), that no sand or just a minimal amount of sand is excreted in the faeces. In turn, a small resolving sand accumulation may remain unrecognized in the radiograph, if the sand is spread and mixed with the ingesta. Yet, the resolving sand makes the plastic glove test positive. Some horses were included in the study at the time of the control radiograph of the abdomen after hospital treatments (psyllium, MgSO₄). In some of these cases the faeces were tested positive for sand, while the control radiographs showed no more sand accumulations. However, the medical record of the patient revealed definite sand accumulations in the initial radiographs that were taken just 3-5 days earlier. This occurrence has caused unnecessary and unjustified false positive results, and raises the

question, whether a longer time frame between the faecal sample collection and abdominal radiography should have been approved in the study protocol (24 hours was the longest interval between the measures/procedures).

5.6 The significance of the plastic glove test in equine practice

According to this study, it is clear that the plastic glove test cannot compete with the more advanced diagnostic methods such as ultrasonography or radiography. However, in absence of these methods, the plastic glove test may sometimes be helpful when combined with other simple diagnostic methods such as rectal examination and abdominal auscultation. In the stable practice the plastic glove test can be used as a routine check for horses with signs of colic, chronic diarrhoea, weight loss or poor performance. A positive result can be used as an indication for abdominal radiography to confirm the diagnosis, or in the absence of abdominal radiography, as an indication for treatment. In mild cases, a positive result for the plastic glove test may be an indication for a change in the management and feeding practice. The advantages of the plastic glove test are its low costs and non-invasive nature. Therefore the test is also easy to repeat to ensure the result.

Additionally, the test can be used as an aid in the follow-up of the recovery from sand colic. The resolution of sand accumulations should increase the faecal sand output. Therefore the plastic glove test may help to evaluate the efficacy of the treatments and medications in cases where radiography is not available. The plastic glove test enables the horse owner to monitor the recovery at home.

5.7 Conclusion

After all, the randomness of the faecal sand excretion process may limit the accuracy of the plastic glove test. Most likely the impact of randomness can be reduced by repeating the test within a certain period of time and using the mean of the results as an aid to the diagnosis. Unfortunately, it would make the simple test time-consuming and impractical. By performing just one faecal sand sedimentation test with negative outcome, one should not exclude the possibility of intestinal sand accumulation. Again, sand in the faeces does not prove that there is a sand enteropathy or intestinal sand

accumulation/impaction, but it does indicate ingestion of sand, which itself is a risk for sand enteropathy.

The methods and results of this study give valuable information about the accuracy and use of the plastic glove test in the diagnosis of sand related gastrointestinal diseases. To get more reliable results, the research material should be a more comprehensive and randomized representation of the horse population. Additionally, the true prevalence of intestinal sand accumulations in Finnish horse population could be a matter of further research. At least during autumn it seems to be one of the most common causes for equine colic in Finland. The plastic glove test can be a helpful tool in the diagnosis of sand enteropathy in the horse, but should not be relied on as the sole method of diagnosis due to the poor specificity of the test.

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