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Tekijä - Författare – Author
Milja Koskinen

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Tiivistelmä - Referat – Abstract

Tämä lisensiaatin tutkielma koostuu kolmesta osasta; kirjallisuuskatsauksesta, kokeellisesta osasta ja liitteistä. Iohexol on ionisoitumaton, trijodattu ja vesiliukoinen röntgenvarjoaine. Iohexolia on hyödynnetty lääketieteessä useita vuosia. Iohexolia on käytetty muun muassa angio- ja myelografiassa, lisäksi iohexolia on hyödynnetty arvioitaessa munuaiskerästen suodattumisnopeutta sekä suoliston läpäisevyyden muutoksia.

Hevosen tulehduksellisessa suolistosairaudessa (Inflammatory bowel disease, IBD) suoliston rakenne ja sen läpäisevyys muuttuu; tyypillistä on tulehdussolujen kertyminen suoliston seinämään ja myös sidekudosmuodostusta saattaa esiintyä. Suolisto muutoksia saatetaan havaita sekä ohut- että paksusuolessa. IBD aiheuttaa hevoselle laihtumista, johtuen ravintoaineiden puutteellisesta imeytymisestä ja proteiinien menetyksestä suoleen suoliston häiriötilan yhteydessä.

Tällä hetkellä IBD:n diagnostiikka perustuu tyypillisiin oireisiin, kliiniseen tutkimukseen, verinäytteisiin, glukoosin imeytymistestiin ja peräsuolesta otettuun koepalaan. IBD:n diagnostiikka on kuitenkin erittäin haastavaa ja tutkimusmenetelmiin liittyy lukuisia ongelmia, jotka vähentävät niiden luotettavuutta IBD:n diagnostiikassa.

Tutkimuksemme tarkoituksena on kehittää hevosen IBD:n diagnostiikkaa entistä helpompaan, luotettavampaan ja turvallisempaan suuntaan. Tämän alustavan tutkimuksen tavoitteet olivat: (1) tutkia voidaanko iohexol havaita hevosen seerumissa oraalisen annostelun jälkeen ja (2) muodostaa iohexolin pitoisuuskuvaaja ajan funktiona terveillä hevosilla.

Materiaalimme koostui kymmenestä terveestä hevosesta, joilla ei ollut havaittu laihtumista tai ripulia. Ennen iohexolin annostelua hevosille suoritettiin kliininen tutkimus ja verinäytteet otettiin maha-suolikanavan sairauden poissulkemiseksi. Hevosille suoritettiin myös mahalaukun tähystys. 16 tunnin paaston jälkeen 1 ml/kg Iohexolia annosteltiin 10 % -liuoksena nenämahaletkulla suoraan mahaan ja verinäytteet otettiin 0, 30, 60, 120, 180, 240, 300 ja 360 minuuttia annostelun jälkeen. Iohexolin pitoisuus määritettiin käyttämällä korkean erotuskyvyn nestekromatografiaa. Iohexolin pitoisuuksista tietyillä ajanhetkillä muodostettiin kuvaaja.

Hevosilla ei havaittu maha-suolikanavan sairauksia. Kaikki hevoset olivat hyvässä kuntoluokassa ja mahalaukun tähystyksessä ei havaittu merkittäviä muutoksia. Verinäytteiden tulokset olivat viiterajoissa. Kaikki hevoset sietivät iohexolia hyvin ja haittavaikutuksia ei havaittu. Iohexol oli havaittavissa seerumissa 60 minuutin kuluttua annostelusta. Kuvaajassa voitiin havaita kaksi huippua. Statistiset menetelmät tukivat löydöksiä.

Iohexol testi oli yksinkertainen suorittaa ja siihen ei liittynyt haittavaikutuksia. Annos 1ml/kg oli havaittavissa seerumissa. Iohexolin pitoisuuskuvaaja muodosti kaksi huippua, ja tämänkaltainen ilmiö on kuvattu kirjallisuudessa aikaisemmin useiden lääkkeiden tapauksessa. Hevosella ilmiö liittyy todennäköisesti maha-suolikanavan rakenteellisiin ja fysiologisiin eroavaisuuksiin ja lisätutkimuksia ilmiön varmistamiseksi tarvitaan. Iohexol näyttää olevan potentiaalinen merkkiaine suoliston läpäisevyyden arviointiin ja lisätutkimuksia IBD:tä sairastavien hevosten seerumin iohexolin pitoisuuksista verrattuna terveiden hevosten seerumin iohexolin pitoisuuksiin on suunnitteilla.

Avainsanat – Nyckelord – Keywords
Horse, inflammatory bowel disease, intestinal permeability, iohexol, malabsorption, oral glucose tolerance test, rectal biopsy, weight loss, xylose absorption test

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Riitta-Mari Tulamo, Yliopistollisen Hevossairaalan johtaja (DVM, MSc, PhD, DIPL ECVS)
Michael Hewetson, Kliininen opettaja (BSc BVSc Cert EM (Int.Med) Dipl.ECEIM MRCVS)

# DETERMINATION OF INTESTINAL PERMEABILITY IN HEALTHY HORSES USING THE CONTRAST MEDIUM IOHEXOL

Milja Koskinen

Lisensiaatin tutkielma

Kliininen hevos- ja
pieneläinlääketieteen laitos

Eläinlääketieteellinen tiedekunta

Helsingin Yliopisto

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CONTENTS

# PREFACE

This presentation consists of three sections: a literature review, a research project and appendixes. In appendix I there is a health assessment sheet according to which a clinical examination was performed. In appendix II there are clinical examination results. Appendix III consists of the results of serum biochemistry and complete blood count. In appendix IV there is an advertisement that was published in Hippos magazine in order to recruit horses with IBD.

# 1 INTRODUCTION

Inflammatory bowel disease (IBD) is an important cause of weight loss in the horse. IBD is characterized by infiltration of inflammatory cells into the intestinal wall and intestinal fibrosis and has many similarities with Crohn's disease in humans. IBD can involve the small or large intestine. Alterations in intestinal barrier function and integrity cause protein-losing enteropathy and malabsorption of nutrients which ultimately lead to progressive weight loss. Other clinical signs that may be associated with IBD include acute or chronic colic, lethargy, poor appetite and chronic diarrhea (Schumacher et al. 2000). Besides information obtained from the history, the diagnosis of IBD is based on results of complete blood cell count and serum biochemical analysis (hypoproteinemia, hypoalbuminemia), results of the oral glucose tolerance test and D-xylose absorption tests and rectal mucosal biopsy (Kemper et al. 2000). However, the antemortem diagnosis of IBD is challenging and there are some fundamental problems with current diagnostic tests which make them less reliable for diagnostic purposes. Even though horses with IBD often have partial or total malabsorption of glucose or xylose (Schumacher et al. 2000), there are many factors which influence the results of oral glucose tolerance test and oral xylose absorption test, making interpretation of the tests difficult and unreliable. For example, an abnormal oral glucose tolerance test will only identify malabsorption in the small intestine and does not provide

any information about malabsorption in the large intestine (Kalck 2009). Furthermore, the analysis of D-xylose can be performed only in limited number of places. Rectal mucosal biopsy is not perfect for diagnosis of IBD either. It has to take into consideration that histopathologic findings at this level of the gastrointestinal system may not represent the entire GI tract and specimens are sometimes difficult to interpret (Lindberg et al. 1996).

In order to overcome these diagnostic limitations related to currently available diagnostic tests used to identify horses with IBD, we propose to develop a simple blood test to help identify horses with IBD using the contrast medium iohexol as a marker of intestinal permeability. Iohexol has been used as a marker of intestinal permeability in several species including humans (Halme et al. 1997) and dogs (Klenner et al. 2009) and has the advantage that it can be used to indentify abnormal intestinal permeability in the small and large intestine (Andersen et al. 2001). Additionally, iohexol is an inert substance and not metabolized (Andersen et al. 2001). Since IBD causes an increase in intestinal permeability, it may be possible to identify horses with IBD by comparing the iohexol serum concentrations to reference ranges for healthy horses following oral administration of a fixed quantity of iohexol. If the concentration of iohexol is above the reference range for healthy horses, then it is likely that the horse has increased intestinal permeability associated with IBD.

The objectives of this preliminary study were: (1) to investigate if the contrast medium iohexol can be detected in equine serum following oral administration and (2) to use this test to estimate intestinal permeability in healthy horses.

# 2 LITERATURE REVIEW

## 2.1 The intestinal barrier and intestinal permeability

The intestine forms the largest interface between an animal and its environment. The integrity of the intestinal barrier is of utmost importantance, since it maintains health; and

prevents tissue injuries and disease (Farhadi et al. 2003). As a filter with selective permeability, the intestinal barrier prevents harmful substances from entering into the organism (Farhadi et al. 2007), while still allowing nutrients to be absorbed into the body at the same time. The concept of absorption relates to carrier- mediated mode of transport, while the concept of permeation describes carrier- unmediated mode of transport (Bjarnason et al. 1995). Hence, the concept of permeability refers to that property of a membrane that enables passage of a solute by unmediated diffusion.

The intestinal barrier is composed of several different structural components including the unstirred water layer, mucosal surface hydrophobicity, the surface mucous coat, epithelial factors (particularly tight junctions) and endothelial factors, from intestinal lumen to serous surface respectively (Farhadi et al. 2003). There are both immunogenic mechanisms e.g. immunoglobulins and mucosal lymphocytes, and non-immunogenic mechanisms e.g. selective intestinal permeability in barrier defenses (Farhadi et al. 2003).

The outermost layer of the intestinal barrier is the unstirred water layer. There is limited information in the literature about this layer and so its role in the permeability of macromolecules remains uncertain (Farhadi et al. 2003). In earlier studies however, it has been observed that this component limits the absorption rate of many different substances, especially lipid-soluble compounds (Thomson et al. 1993).

The barrier component below the unstirred water layer is the mucosal surface hydrophobicity. It may be induced by a surface active phospholipid layer that lines the top of the mucus covering the epithelium and it is essential in maintaining gastric defense as well (Hills et al. 1983). The intestinal mucous hydrophobicity is a very important factor when assessing the overall barrier function of the mucous layer (Qin et al. 2008). Qin et al. (2008) also noticed that decreased mucosal hydrophobicity is associated with increased gut permeability, and thus mucosal hydrophobicity can be a useful method of assessing the intestinal barrier function of the mucous layer.

Below the mucosal surface hydrophobicity is the adhesive mucous gel layer. Mucus protects most surfaces of the body, especially the epithelium. It impedes most bacteria and

many pathogens entering the body, and at the same time it enables the absorption of many nutrients and water (Cone 2009). Regardless, there is no consensus among researchers about what the actual role of the mucous gel coat in intestinal permeability is.

The epithelium is the most intensively studied intestinal barrier component and defects in the epithelium have been associated with various diseases. Substances can permeate the epithelial barrier via the paracellular or the transcellular pathway. The paracellular pathway is the typical route of passive permeation across the intestinal epithelium (Madara 1990). This pathway consists of two different components, the apical junctional complex (consisting of the tight junction, the intermediate junction and the belt desmosome), and the subjunctional space (Madara 1990).

The deepest layers of the intestinal barrier are connective tissue and the endothelial barrier, respectively. The role of connective tissue in intestinal permeability remains unclear; however some believe connective tissue might affect intestinal permeability by modulating epithelial factors. For example, lipopolysaccharides (LPS) were able to alter the transepithelial resistance (TER) and thus also intestinal permeability in the presence of mediators released from lamina propria fibroblasts (Chakravortty et al. 1999). With respect to the endothelial barrier, there is evidence to suggest that the integrity of the capillary endothelial barrier might play a significant role in maintaining intestinal barrier function, and consequently endothelial injury can contribute to the development of intestinal barrier failure (Sun et al. 1997).

### 2.1.1 Factors affecting intestinal permeability

As noted earlier, the intestinal barrier is composed of several different layers, and any alterations in these structures may have influence on intestinal permeability. Disruption of the epithelial architecture can lead to abnormal intestinal permeability, which can bring about serious implications for an animal. An increased permeability of tight junctions may lead to decreased ability of epithelium to maintain electrolyte and non-electrolyte concentration gradients. Consequently, both osmotic balance and ionic homeostasis are disturbed which may even be fatal to animals (Lewis et al. 1995). The physical structure

(composition, thickness, charge, etc.) of the membrane is important in determining intestinal permeability, but it is the physicochemical properties of a solute (shape, charge, thickness, molecular size and solubility) and the solute's interaction with the solvent that ultimately determines the extent of diffusion of a solute across a membrane (Bjarnason et al. 1995).

Mast cells may play an important role in the modulation of intestinal permeability. Research conducted by Stein et al. (1998) suggests that alterations in epithelial barrier may be produced, directly or indirectly, by mediators from activated mast cells. Upon activation, mast cells release a complex mixture of inflammatory mediators (including histamine, cytokines, and platelet activating factor) which can directly increase intestinal permeability (Stein et al. 1998).

Madara et al. (1989) discovered that cytokine interferon (IFN-γ) exposure diminishes the epithelial barrier function if the duration of exposure is adequate. IFN-γ is released from activated lymphoid cells, so there is also an indirect component which alters epithelial properties. Willemsen et al. (2005) evaluated the effect of IFN-γ on intestinal permeability and they were in agreement with Madara et al. (1989) that IFN-γ is a central mediator in the initiation and exacerbation of mucosal inflammation. IFN-γ increases epithelial permeability via disruption of intercellular tight junctions (Willemsen et al. 2005). There is strong evidence that the tight junctions contribute to the altered intestinal permeability in Crohn's disease, one form of inflammatory bowel disease in humans (Vetrano et al. 2009). There is also much speculation on whether the increased epithelial permeability in patients with active inflammatory bowel disease is due to polymorphonuclear leucocytes transmigration along the bowel wall (Nash et al. 1987).

It has been shown that intracellular mediators affect intestinal permeability. For instance, nitric oxide (NO) has an important role in regulating the normal physiology of the alimentary track by affecting both epithelial cells as well as the microcirculation (Alican et al. 1996). Evidence suggests that nitric oxide is a very harmful molecule in inflammatory bowel disease (Alican et al. 1996). Salzman et al. (1995) evaluated the effect of NO on the permeability of tight junctions in an intestinal epithelium model (Caco-2BBe intestinal

epithelial monolayers). They concluded that NO reduces ATP levels and consequently increases the permeability of these junctions in vitro.

Both psychological and oxidative stress increase intestinal permeability. These stresses affect the gastrointestinal track in several ways. Evidence suggests that environmentally induced stress affects the intestinal epithelial barrier by increasing the degranulation of mucosal mast cells and activation of goblet cells, causing epithelial cell separation and detachment, and changing the capillary endothelial ultrastructure (Wilson et al. 1999). As noted earlier, mucosal mast cells are pivotal elements in intestinal barrier function. Santos et al. (2001) demonstrated that chronic stress increased mucosal mast cell numbers and macromolecular permeability in normal rats, whereas no alteration in permeability was discovered in mast cell-deficient rats. Oxidative stress may also significantly alter intestinal function. It has been demonstrated that excessive amounts of reactive oxygen metabolites (ROMs) are produced by inflamed colonic mucosa, which may further lead to tissue injury and loss of integrity of biological tissues (Keshavarzian et al. 1992).

There are several studies about bacterial flora and its effect on the intestinal barrier function. Garcia-Lafuente et al. (2001) investigated whether the colonizing bacteria affect colonic barrier function in the rat. They noticed that certain commensal bacteria, e.g. *Escherichia coli, Klebsiella pneumoniae, and Streptococcus viridans* significantly increased intestinal permeability while *Lactobacillus brevis* reduced permeability. It is important to note however, that permeation of the large molecular size probe dextran was not changed by any of the bacterial strains used in the study. Goldstein et al. (2009) showed that *Clostridium perfringens* epsilon toxin has an influence on intestinal permeability, predominantly by opening the mucosa tight junctions and also inducing further degenerative alterations in the lamina propria of the bowel. It is still not clear whether the changes in intestinal permeability are due to direct bacterial actions or immune system reactions, however (Madara et al. 1989).

Many drugs and diet affect the integrity of the intestinal barrier. Sigthorsson et al. (1998) investigated the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on intestinal permeability. There were 68 patients receiving six different NSAIDs for over six months

and after the period patients underwent combined absorption-permeability tests. Sigthorsson et al. (1998) discovered that the long term ingestion of all conventional NSAIDs except aspirin and nabumetone were associated with small intestinal inflammatory changes. There is continuing debate on the role of diet in the changes of intestinal barrier function. Deitch et al. (1995) observed that the loss of mucosal barrier integrity to both phenol red and *Escherichia coli* seemed greater in the intravenous total parenteral nutrition (IV-TPN) than the elemental diet-fed rats. It is also shown that ingestion of specific foods may have an influence on intestinal permeability in patients with irritable bowel syndrome (Barau et al. 1990).

The effect of genetics and environment on integrity of intestinal barrier has not yet been fully established. Soderholm et al. (1999) investigated environmental and genetic influences on intestinal barrier in both relatives and spouses of patients with Crohn's disease. They concluded that baseline permeability is determined by environmental factors, whereas provoked hyperpermeability is more genetically determined. Not only genetics and environment, but also aging and apoptosis have been shown to influence intestinal permeability. Evidence suggests that intestinal permeability of medium size probes, such as PEG 400, increases with aging in the rat (Ma et al. 1992). Abreu et al. (2000) observed that immune-mediated apoptosis of intestinal epithelial cells may be associated with increased intestinal permeability as well.

Physicochemical properties of solute significantly affect its diffusion across intestinal membranes. Among others the molecular size of a probe determines how it permeates the intestinal membrane. Although the permeability pathways of various molecules are still unclear, evidence suggests that the markers used for assessment of intestinal permeability (E.g. L-rhamnose, PEG 400, lactulose and $^{51}$Cr-EDTA) all permeate the intestinal wall through paracellular junctions, but there are various sizes of tight junctions along the crypt-villus axis (Bjarnason et al. 1995).

Small molecules, e.g. monosaccharides, permeate through the intestinal epithelium via passive transport through the small pores (radius, <6 Å) in the absorbing apical part of the villus, whereas non solvent drag occurs through medium-sized pores (10-15 Å) in the basal

part of the villus. There are also large pores (50-60 Å) in the crypts, but these pores are inaccessible to the luminal content (Fihn et al. 2000). As a consequence, it is suggested that the different permeability rates of the probes are the result of interplay between the accessibility of intestinal contents to the basal part of the villus and the tightness of the junctions in the intercellular space (Hollander 1992).

The osmolarity of a solution significantly affects the permeability of most substances. The permeation of oligosaccharides and lactulose is substantially increased when the osmolarity of the test dose is increased above 1500 mOsm/L (Laker et al. 1977, Wheeler et al. 1978). Hyperosmolar glycerol does not increase the permeation of L-rhamnose, however (Bjarnason et al. 1994). Most researchers promote the use of physiological iso-osmolar solutions, so the test results can be directly compared with each other and there are not so many changes in permeability caused by different osmolarity (Bjarnason et al. 1995). Poorly permeable solutes can retain fluid within the intestinal lumen, which leads to rapid intestinal transit. The contact time of probes with the intestinal wall is decreased and then the permeability of the probes is also reduced without any alterations in the intestinal wall itself (Bjarnason et al. 1994).

### 2.1.2 Factors altering intestinal permeability in horses

Although there are many different conditions which can affect intestinal permeability in horses, IBD is probably the most important due to its significant effect on intestinal barrier function. In the horse IBD is a rare maldigestive and malabsorptive disorder that primarily affects the small intestine, however in advanced disease, the large intestine may be involved as well (Kalck 2009). There are four different types of IBD; idiopathic eosinophilic enterocolitis (EC) and the more severe form of EC is known as multisystemic eosinophilic epitheliotrophic disease (MEED), granulomatous enteritis (GE), lymphocytic-plasmacytic enteritis/enterocolitis (LPE) and lymphosarcoma (Kalck 2009, Schumacher et al. 2000).

Although the classification of IBD is slightly different according to different references, most authors concur that in each type there is an infiltration of the mucosa and submucosa

with abnormal cells. The typical cells depend on the type of IBD. For example, in lymphocytic-plasmacytic enterocolitis, there is moderate to severe infiltration of lymphocytes and plasma cells (Roberts 2004a). The etiology of IBD is unknown, and there is much speculation among researchers about the cause of IBD. In certain IBD cases, there is an abnormal immune response to viral or dietary antigens (Schumacher et al. 2000). IBD may be caused by parasitic infections (Scott et al. 1999) or toxins (Anderson et al. 1983). Scott et al. (1999) noticed that lesions in horses with IBD were located in the terminal branches of arteries or the central of vascular areas, so the pathogenesis of IBD may be also associated with abnormal intestinal blood flow. The prognosis of IBD is poor and most horses with IBD do not survive, although horses with EC have a better prognosis for survival than those with other forms of IBD (Scott et al. 1999).

Besides IBD, there are plenty of other conditions which are associated with decreased body weight in the horse. The pathophysiological mechanisms for weight loss can be categorized as decreased dietary intake, maldigestion and malabsorption, inappropriate hepatic utilization and increased rate of protein and energy loss. Diagnosis of malabsorption is usually made by exclusion of other possible causes of weight loss and wasting, and the diagnosis of IBD is made by ruling out other causes of malabsorption, respectively. In addition to IBD, inadequate absorptive surface area, small intestinal bacterial overgrowth, parasitic, infectious, and immunologic causes, biochemical defects, lymphatic obstruction and miscellaneous causes, such as partial intestinal obstruction and mural thickening, have been related to malabsorption in horses (Roberts 2004b).

There is a wealth of information in the literature about factors which can affect intestinal permeability in the horse. Weiss et al. (2000) discovered that cecal contents incubated with corn starch induced increased in vitro paracellular permeability in the right ventral colon of healthy ponies. The data suggests that fermentation of an excessive amount of carbohydrates may directly induce increased intestinal permeability. On the other hand, it is also possible that the low pH and high lactic acid content may lead to increased intestinal permeability (Weiss et al. 2000). Plummer (2006) noticed that malabsorptive and maldigestive disorders can also be related to salmonellosis in horses as these horses may have an abnormal D-xylose absorption curve. It is probable that the underlying

malabsorptive and maldigestive diseases induce a change in the presentation of nutrients to the large colon and this changes the colonic bacterial flora, leading to colonization and proliferation of salmonella (Plummer 2006).

In addition to the above-mentioned studies done in horses, it is presumable that the same factors affecting intestinal permeability in other species may also affect intestinal permeability in horses.

## 2.2 Intestinal permeability probes in general

The non-invasive assessment of intestinal permeability has fascinated research workers for over 20-years (Bjarnason et al. 1995). Even though the period of time is relatively short, there are many different methods to estimate permeability in a non-invasive manner. Non-invasive absorption tests provide information on the mechanisms of malabsorption and some of them have been used in screening for gastrointestinal diseases. With these tests it may even be possible to predict the prognosis and evaluate the response to treatment (Bjarnason et al. 1995).

There are several criteria that have to be fulfilled for a substance to be considered an ideal intestinal probe. An ideal intestinal probe permeates the intestine by passive diffusion, is not recognized by immune system and is not naturally present in normal body fluids (Ukabam et al. 1983). Measurement of the probe should be sensitive, precise, and effortless (Chadwick et al. 1977). It is also important that the probe is nontoxic, nonnoxious, hydrophilic, lipophobic, rapidly excreted in urine, resistant to intestinal conditions and not metabolized (Chadwick et al. 1977). The degradation site of the probe can be utilized when the affected gut segment has to be localized; i.e. a probe that rapidly degrades in the proximal intestine can be used as a marker of damage only in the very proximal gut; whereas an inert probe can be utilized to estimate the permeability of whole gut (Meddings et al. 1998).

Ethylene glycol polymers (PEGs) have been widely used in different fields, e.g. as solvents, as food additives, as water-soluble ointment base, but PEGs have also been used for

estimation of intestinal permeability in human studies (Bjarnason et al. 1995). PEGs are available as a mixture of various molecular weight polymers. Even though PEG 400 fulfills the previously mentioned criteria of an ideal test substance, more studies are needed to assess its use as an intestinal permeability marker (Chadwick et al. 1977).

Di- and oligosaccharides are another option for use as probes in intestinal permeability studies. Lactulose is the typically used disaccharide probe for estimation of intestinal permeability changes especially in the human intestinal tract. Other suitable oligosaccharides are for instance, melibiose, raffinose, stachyose and dextrans (Bjarnason et al. 1995). Though the above-mentioned oligosaccharides meet most of the criteria of ideal test probe, there is still an analytical sensitivity problem. Some sugars can be metabolized and the test results are not reliable anymore (Hall et al. 1996). In addition, the disaccharide sucrose is degraded rapidly after leaving the stomach and consequently, it can only be used to recognize damage in the very proximal gut (Meddings et al. 1998). In some cases this can be used advantageously, however. For example, permeation of sucrose through the gastric mucosa can be used to detect gastric ulcers in horses (Hewetson et al. 2006). Unfortunately, complicated assays have to be performed to quantify these sugars in serum and because of this limitation; sugar permeability tests are usually done using urine instead of blood (Allenspach et al. 2006). For achieving accurate results urine needs to be collected for several hours and there are technical difficulties related to collecting urine in animals compared to humans which make sugar permeability tests labored and time-consuming.

Monosaccharides L-rhamnose and mannitol are both widely used probes in intestinal permeability studies in humans (Bjarnason et al. 1995). Lactulose and mannitol are good examples of the probes which can bypass the majority of the small bowel, before being degraded by bacterial microflora. This character definitely limits the use of these probes for recognizing small intestinal pathologic alterations (Meddings et al. 1998). Furthermore, Laker et al. (1982) noticed that mannitol is present naturally in urine and this may falsify the test results in humans at least. The ratio of lactulose: L-rhamnose (L: R) has become the standard intestinal permeability test in dogs (Frias et al. 2004). Although the plasma concentrations of lactulose and rhamnose may be achieved with satisfying accuracy, the L:

R blood test requires extensive sample preparation and this may limit the usefulness of this test (Sorensen et al. 1997).

$^{99m}$Tc- DTPA (Technetium-99m labelled diethylenetriamine penta-acetate) and $^{51}$Cr-EDTA (51Cr-Ethylenediaminetetraacetic acid) are nondegraded radiolabeled chelates, commonly used for estimation of intestinal permeability. The probes share many similar properties with oligosaccharides. The disadvantage is that the probes are radioactive, but that makes the measurement easier (Bjarnason et al. 1995). $^{51}$Cr-EDTA remains intact throughout the gut and thus it is possible to recognize colonic damage as well (Meddings et al. 1998). Much more specific assessment of intestinal permeability can be achieved by using $^{51}$Cr-EDTA together with mono- and disaccharides (Bjarnason et al. 1989). Usually $^{51}$Cr-EDTA absorption tests are based on the collection of urine samples and measurement of γ-radiation; however Frias et al. (2004) discovered that a $^{51}$Cr-EDTA absorption blood test is a valuable method for screening intestinal diseases in dogs. This is easier and far simpler to perform than the $^{51}$Cr-EDTA urine test which makes the method more practical for clinical purposes.

Polyvinylpyrrolidine (PVP) is not typically used as an intestinal permeability probe. The backbone of the polymer can be labeled with $^{14}$C, but the use of PVP as an intestinal permeability marker is in its infancy and more studies and experience is needed (Bjarnason et al. 1995). Also sucralose, an artificial sweetener, has been used as an intestinal permeability probe in some studies. Haas et al. (2009) evaluated sucralose excretion as an indicator for altered colonic permeability. They concluded that sucralose excretion is highly variable and no association between gastrointestinal permeability and sucralose excretion was discovered.

### 2.2.1 Diagnosis of IBD in the horse

IBD is difficult to diagnose ante mortem using currently available methods (Kemper et al. 2000). At the moment the diagnosis of IBD is based on clinical findings (weight loss, diarrhea, lethargy), results of complete blood cell count and serum biochemical analysis (hypoproteinemia, hypoalbunemia), results of the oral glucose tolerance test and D-xylose

absorption tests and rectal mucosal biopsy (Kemper et al. 2000). There are several problems with these methods however.

Clinical signs and results of blood samples are not specific, but they provide some suggestive data at least. These clinical signs reflect the gastrointestinal tract condition, possibly caused by intestinal inflammation (Kemper et al. 2000). In addition to weight loss, diarrhea and lethargy, a history of mild, recurrent colic is typical in horses with IBD (Kalck 2009). If the horse has diarrhea, additional diagnostic methods are recommended to rule out *Salmonella* spp and *Clostridium* spp. Besides, *Clostridium* toxin assays are worthwhile to perform (Kalck 2009). Other causes of diarrhea, e.g. sand enteropathy and the use of non-steroidal anti-inflammatory drugs should be excluded as well. Temperature, respiratory and heart rates are normal in most horses affected by IBD (Kemper et al. 2000).

Hypoproteinemia and hypoalbunemia are typical findings in IBD, and as noted; these clinical findings are not specific and can be associated with any cause of protein-losing enteropathy (Kemper et al. 2000). There are several mechanisms responsible for plasma protein leakage across gastrointestinal mucosa. Protein loss can take place due to disordered mucosal cell metabolism or rupture of dilated lymph vessels in mucosa. There can be changes in passive diffusion between cells (increases in pore size or involvement of inflammatory mediators). Plasma protein leakage can also occur because of active secretion by mucosal cells (Roberts 1983). In suspected IBD cases, non-intestinal causes of protein loss have to be excluded by evaluating the results of abdominocentesis and urinanalysis. The results help rule out peritonitis and proteinuria, respectively. Usually the peritoneal fluid is normal in the horse with IBD, although neoplastic cells may be seen in intestinal lymphosarcoma cases (Mair et al. 1992). Additionally in cases of protein loss, thoracic evaluation for pleural fluid and liver function tests have to be taken into consideration (Kemper et al. 2000).

Results of the complete blood count may be normal or may reveal an anemia, neutrophilia or hyperfibrinogenemia. The anemia is typically normochromic or normocytic in horses with IBD (Barr 2006). Kemper et al. (2000) also evaluated serum IgA concentration and they found elevated IgA concentrations in three of four horses. It is possible that serum IgA

concentrations are increased in horses with lymphocytic-plasmacytic enteritis, but this is not a specific method to achieve a diagnosis either.

The oral glucose tolerance test (OGTT) and D-xylose absorption test are not perfect for diagnostics of IBD either. There are several advantages in OGTT; it is simple to perform, inexpensive, and additionally can be performed stall-side (Kalck 2009). After a 12- to 18-hour period of food deprivation horses are assigned glucose at 1g/kg body weight as a 20% solution via nasogastric tube. Following administration of glucose, blood samples are collected every 30 minutes for 2 hours and then hourly for 4 hours (Roberts et al. 1973). Because a continuous blood sample collection over a six-hour period is impractical, there is also a modified OGTT which is based on a single sample taken at 120 minutes after glucose administration (Murphy et al. 1997). If the plasma glucose concentrations at 120 minutes fail to increase by more than 15 per cent above basal glucose concentration, the condition can be considered as total malabsorption (Murphy et al. 1997). An increase below 85% of basal glucose concentration is considered to be evidence of partial malabsorbtion.

There are several factors that may influence the OGTT test results in the normal horse, including gastric emptying rate (gastric impactions), colonic cyathostominosis (Murphy et al. 1997), intestinal motility and transit time (Roberts et al. 1973), and changes in bacterial populations in the stomach and small intestine (Foreman 2004). The dietary history can also affect the test results. Horses grazing pasture had significantly greater maximum glucose concentrations in comparison to horses fed a stable diet (Jacobs et al. 1982a). Because metabolic and endocrinological factors can affect glucose peak and curve shape, it is really important that horses are tested in a relatively quiet environment, without gratuitous noise and movement. There can be a false-negative result in the horse with abnormal absorption if the horse is overly stressed due to an artificially elevated plasma glucose, making the recognition of a flat line curve impossible (Jacobs et al. 1982a). A false-positive result (a flat curve) may result from the catabolic state of the horse with normal intestinal absorption because of the rapid use of the administered glucose (Kalck 2009). Furthermore, Church et al. (1997) noticed that transient flat OGTT curves may occur in horses without obvious intestinal lesions. Additionally, the OGTT test results can

also be influenced by certain diseases, such as polysaccharide storage myopathy and equine motor neuron disease (De La Corte et al. 1999, Nout 2010).

The procedure and interpreting of results of the D-xylose absorption test are quite similar to the OGTT. Even though D-xylose absorption test may be slightly more reliable compared with the OGTT and may provide a more accurate assessment of absorption, there are still several different factors that can falsify the test results and in some cases even result in a false-positive flat curve (Taylor 2002). Freeman (1993) discovered that metabolism affects the results of xylose absorption test. There is also evidence that insulin affects the volume of distribution of D-xylose in humans (Segal et al. 1957). Hindmarsh (1976) noticed that reduced xylose metabolism in cases of liver disease definitely affected the test results. In addition, intestinal motility, intraluminal bacterial overgrowth and gastric emptying (Roberts et al. 1979), the state of renal function and age (Hindmarsh 1976) may all affect the results of xylose absorption test as well.

Evidence suggests that the duration of the food deprivation prior to administration of xylose may also significantly affect the results, so this should be taken into consideration when the results are interpreted (Freeman et al. 1989). The diet prior to oral administration of xylose may also affect the test results. Horses fed a high energy diet have remarkably lower oral xylose absorption curves than horses fed a low energy diet (Jacobs et al. 1982b). It is possible that changing the diet may have resulted in an alteration in the bacterial flora and this has finally led to altered D-xylose absorption, however. It has also been reported that mucosal blood flow and also the integrity of mucosal and submucosal architecture can affect the rate of D-xylose assimilation (Roberts et al. 1979). Thus pathologic lesions of *Strongylus vulgaris* in the cranial mesenteric artery and its branches may limit xylose absorption (Duncan et al. 1972). Freeman (1993) discovered that xylose concentrations in plasma are much lower in horses compared with other species after oral administration of D-xylose. This is most probably due to the fact that the active transport system for xylose becomes saturated at very low intraluminal concentrations, hence there is a strong possibility that abnormal D-xylose absorption test results in horses are caused by abnormalities in mucosal surface area and mucosal permeability rather than by abnormalities of nutrient carbohydrate absorption per se (Freeman 1993).

In conclusion, neither the oral glucose tolerance test nor D-xylose absorption test are specific for the diagnosis of IBD, however Kemper et al. (2000) observed that the results of above-mentioned tests were abnormal in 75% of horses diagnosed with lympho-plasmacytic enteritis, so despite their limitations, these tests are still valuable for diagnostic of IBD at the present time. It would however, be advantageous to use the oral glucose tolerance test and D-xylose absorption test in combination to gain more accurate information of the location of the lesion because D-xylose absorption test alone doesn't preclude the existence of pathophysiological changes in the lower small intestine (Roberts et al. 1979).

Gastroscopy and an endoscopic assisted proximal intestinal biopsy can be useful diagnostic methods of IBD if the small intestine is involved. With gastroscopy it is possible to confirm or exclude gastric ulceration as a cause of the hypoproteinemia (Kalck 2009), however, endoscopic biopsy specimens yield an incorrect or inconclusive diagnosis in many cases (Evans et al. 2006). Disadvantages of endoscopy include inability to obtain full-thickness biopsy specimens and there is no access to the jejunum and ileum (Evans et al. 2006). During intestinal biopsy, care should be taken to avoid the opening of the common bile duct in the proximal duodenum (Kalck 2009). In gastroscopy horses need to be fasted and sedated which make the procedure slightly more impractical.

A full-thickness biopsy is a definite way to achieve the diagnosis of IBD (Kalck 2009). A full-thickness proximal intestinal biopsy can be obtained via a ventral midline incision under general anesthesia or via a standing flank laparotomy under local anesthesia (Mair 2002). These biopsies are definitely invasive and usually not performed for the diagnosis of IBD because hypoproteinemia delays wound healing and catabolic state of the patient may add the risk of complications (Roberts 1983). Exploratory laparotomy and biopsy are necessary in most cases to achieve a confirmation of diagnosis, however (Roberts 1983).

Rectal biopsies can be performed easily with minor risks if circumstances are appropriate (Lindberg et al. 1996). There are several disadvantages in rectal biopsy, nonetheless. It is important to remember that the histopathologic findings at this level of the GI system may

not represent the entire GI tract, and the findings provide only suggestive data of the GI tract condition. Lindberg et al. (1996) investigated rectal biopsy specimens of horses with equine granulomatous enteritis and eosinophilic gastroenteritis. The diagnosis was confirmed on rectal biopsy in 50 and 44% of the cases, respectively. Also Kemper et al. (2000) inspected rectal mucosal biopsies of horses with lymphocytic-plasmacytic enteritis and the biopsies were abnormal in only 43% of cases. Interpretation of specimens is sometimes difficult and definitely requires experience and attainment. Lindberg et al. (1996) investigated 131 rectal biopsy specimens and they diagnosed the majority of cases as non-specific proctitis without determining a specific diagnosis. There is also a small risk of rectal perforation; however the risk is minimized with appropriate restraint. After the procedure, the horse should be carefully monitored for complications, e.g. rectal perforation or bacterial translocation (Plummer 2006).

In certain cases it is advantageous to use some additional methods in order to achieve a definitive diagnosis. For instance, transabdominal ultrasound can be used to estimate small intestinal wall thickness (Kalck 2009). Even though there is no need to use all the above-mentioned methods to achieve a diagnosis, a number of different tests may be required when diagnosing IBD and it is rather laborious and time-consuming work.

### 2.2.2 Other methods for estimation of intestinal permeability in the horse

In addition to the OGTT and xylose absorption test, there have been many other studies done in horses to evaluate intestinal permeability in a non-invasive manner. Unfortunately most of these tests are non-specific and offer little advantage over the OGTT and xylose absorption test.

Weiss et al. (1998) used technetium Tc 99m pentetate to evaluate changes in intestinal permeability in ponies with carbohydrate- induced laminitis. Even though technetium Tc 99m pentetate was suitable for detecting the increased intestinal permeability, the continuous collection of urine made the procedure impractical, however. Escala et al. (2006) assessed the potential of the $^{51}$Cr-EDTA absorption test for the estimation of intestinal permeability in the horse. They discovered increased urinary recovery of $^{51}$Cr-

EDTA in ponies with cyathostome infection which suggests that $^{51}$Cr-EDTA may be suitable for evaluation of intestinal permeability in the horse. Also $^{51}$Cr-chloride has been used in horses to demonstrate excessive enteric protein loss (Meuten et al. 1978). In comparison to the sugar permeability tests however, information relating to intestinal permeability is very crude when using radio-labelled markers, since there are many physiological routes for permeation of these probes (Escala et al. 2006). Even though the measurement of radio-labelled molecules is easy, their radioactivity makes the methods impractical.

On the whole, better diagnostic methods for IBD and other diseases altering intestinal permeability are needed and therefore we decided to estimate intestinal permeability in horses using the non-radioactive contrast medium iohexol (Omnipaque-350).

### 2.2.3 Iohexol

Iohexol, sold under the trade name Omnipaque™, has been used in human and veterinary medicine for many years. Iohexol has been used in myelography (Rose et al. 2007), urography (Punto et al. 1983) and angiography (Tragardh 1980). Nagy et al. (2009) injected radiopaque contrast medium iohexol subcutaneously over the palmar nerves and evaluated potential diffusion and distribution of local anaesthetic solution after perineural analgesia in the distal limb. Besides utilizing the radiopaque characteristic of iohexol, there are several studies that have been done to evaluate iohexol as a marker of intestinal permeability (Klenner et al. 2009) and glomerular filtration rate (GFR) (Gleadhill et al. 1996). These studies have been done in a variety of species, including cat (Goy-Thollot et al. 2006), dog (Klenner et al. 2009), pig (Skalpe et al. 1995), sheep (Nesje et al. 1997), horse (Wilson et al. 2009), rat (Andersen et al. 2001), and human (Halme et al. 1997).

Iohexol fulfills the criteria of an ideal intestinal permeability probe: it is easy and safe to handle and does not require special equipment (Andersen et al. 2001). Iohexol is an inert substance and is not metabolized and it is also rapidly excreted in the urine after glomerular filtration (Andersen et al. 2001). Because iohexol is not degraded in the gut, it may be possible to estimate permeability of the whole intestine in contrast to other markers of

gastrointestinal permeability like sucrose that is already rapidly degraded in the beginning of the small intestine (Meddings et al. 1998). Secondly, Andersen et al. (2001) noticed that small intestinal permeability in rats for the other water-soluble contrast medium iodixanol is notably high compared to colonic permeability, about 10:1. This is presumably valid also for iohexol.

Iohexol has been discovered to be well tolerated and safe (Gomi et al. 2009). Some adverse reactions during myelography, e.g. headache, nausea and mental confusion are reported in humans; regardless, seizure is the most severe adverse effects that has been reported (Omnipaque$^{TM}$ instructions). Iohexol was associated with lower risk of adverse effects and seizures than metrizamide (Fedutes et al. 2003). Widmer et al. (1998) compared metrizamide and iohexol in horses and they concluded that iohexol is safer and better suited for equine myelography than metrizamide. IV (Wilson et al. 2009) and per oral (Klenner et al. 2009) administration of iohexol is appeared to be safe and with minimal side effects as well, just watery diarrhea was observed in the high dosages of iohexol assigned per orally to dogs (Klenner et al. 2009).

Because iohexol is water soluble, iodinated contrast medium permeates the normal intestinal wall to a minor degree and thus it may be utilized as an intestinal marker under various clinical conditions of intestinal epithelial changes (Andersen et al. 2001). It is presumed that iohexol shares a similar paracellular permeate pathway as $^{51}$Cr-EDTA (Frias et al. 2005), the gold standard marker at the moment (Frias et al. 2004). In humans, iohexol has been noticed to be a better marker of inflammatory bowel disease than lactulose-mannitol ratio test (Halme et al. 2000).

On the other hand, it remains difficult to recognize mild disease with iohexol, as it is difficult with other earlier mentioned markers as well. It has been speculated that iodixanol (dimer, 15 Å) is more sensitive in detecting minimal alterations in intestinal permeability, because of its bigger size when compared to iohexol (monomer, 12 Å), hence a normal intestinal wall seems to be more permeable for iohexol than the larger iodixanol molecule (Andersen et al. 2001). However, ulcerations of the intestinal mucosa seem necessary to gain a reliably measurable and substantial increase in intestinal permeation of both

iodixanol and iohexol (Andersen et al. 2001). Despite this fact, Andersen et al. (2001) demonstrated that orally ingested iohexol and iodixanol were detectable in urine even though the mucosal wall was non-ulcerated.

The use of iohexol as an intestinal permeability marker has been investigated for several years. As early as 1989, Stordahl investigated urinary excretion of iohexol after intestinal administration in rats with bowel ischemia. He discovered that the degree of morphologic changes in the ischemic bowel can be estimated by measuring the serum and urinary levels of iohexol. Whereupon according to Stordahl (1989), iohexol is suitable for discriminating mesenteric venous occlusion from normal bowel. Later, Halme et al. (1997) noticed that intestinal permeability of iohexol is significantly increased in patients with active inflammatory bowel disease.

In 2009, Klenner et al. used orally administered iohexol (Omnipaque-350) for estimation of intestinal permeability in healthy dogs. It was demonstrated that using the optimal iohexol dosage, an intestinal permeability serum test was easy to perform as a marker for intestinal permeability in dogs. Iohexol has been administered orally to horses as well. In order to assess intestinal permeability iohexol (Omnipaque-350) was assigned to healthy horses orally with food (Strube 2007). Strube (2007) noticed that iohexol should be administered to fasting animals to gain reliable and usable results, but was able to demonstrate that iohexol was detectable in serum. A major limitation of Strube's study was that the iohexol was not administered via a nasogastric tube in fasting animals, and thus the dose and effect of food on gastric emptying could not be controlled. Our study was undertaken to evaluate iohexol as an intestinal permeability marker in fasting healthy horses by administering a fixed dose of iohexol via a nasogastric tube.

# 3 MATERIALS AND METHODS

## 3.1 Study animals

Ten clinically healthy horses owned by Equine College Ypäjä Finland were used in the study. The mean age of the horses was 14.0 year (range 10.0 – 19.1 years). The body weight ranged from 485 to 616 kg (mean 542 kg). The body condition of the horses was evaluated on a scale of 1 to 9 (Henneke et al. 1983), 1 being poor and 9 being extremely obese. The mean body condition score was 6. Eight of the horses were Finnhorses and the rest were Warmbloods. Gender distribution was one mare and nine geldings. The horses were housed separately in stalls. Except for the fast periods before gastroscopy (16 hours) and time before and during the iohexol administration (total 22 hours), horses were provided free choice hay and water. The periods of food deprivation were achieved by keeping horses in a stable. A full clinical examination was performed on each horse. The study protocol was approved by the National Animal Experiment Board of Finland (Eläinkoelautakunta ELLA) (Request for Animal Experiments, ref. no. ESAVI-2010-06567/Ym-23).

## 3.2 Exclusion of gastrointestinal diseases

The horses were clinically healthy and there was no previous history of gastrointestinal disease. In order to confirm the absence of gastrointestinal disease, each horse underwent a thorough clinical examination 2 days prior to testing (Appendix I) and at the same time blood samples (20 ml) were collected with an 18-gauge vacutainer needle from the left jugular vein. A complete blood count (CBC[a]), serum biochemistry[b] and fibrinogen (Millar et al. 1971) were measured. Additionally, gastroscopy* was performed on each horse using a 3-m equine videoendoscope[c]. Before endoscopy horses were sedated with the combination of detomidine hydrochloride[d] and butorphanol tartrate[e]. The stomach was distended by insufflation with air through the biopsy channel of the endoscope until the luminal surface of the stomach was properly visible. In order to ensure proper visibility of the entire squamous and glandular mucosae, gastric contents were rinsed from the mucosa

with tap water flushed through the biopsy channel. The severity and location of gastric ulcers were graded by means of a published four-point gastric ulcer scoring system (Andrews et al. 1999) (Table 1). All endoscopic examinations were recorded. At the end of the gastric examination, the stomach was deflated by suctioning air through the biopsy channel. Once the horses had been determined to be disease free, they were included in the research.

**Table 1.** Four-point gastric ulcer scoring system.

| Score | Description |
|---|---|
| 0 | Mucosal epithelium is intact; there may be hyperemia (reddening) or hyperkeratosis (yellow appearance to the squamous mucosa) |
| 1 | Small single or multiple ulcers |
| 2 | Large single or multiple ulcers |
| 3 | Extensive (often coalescing) ulcers with areas of apparent deep ulceration |

* Due to financial and practical reasons, gastroscopy was done retrospectively; 19 days after the horses had undergone iohexol permeability testing.

### 3.3 Administration of iohexol and collection of samples

After a 16-hour period of food deprivation, each horse was restrained in stocks and a 12-gauge; 80-mm teflon catheter[f] was inserted into the left jugular vein and secured with a nylon suture. A pre-iohexol administration blood sample was collected and then 1.0 mL/kg of iohexol[g] was administered as a 10 % solution (100mg/ml) in tap water via a nasogastric tube. Blood samples (20 ml) were taken 30 minutes after iohexol application, and then hourly for 6 hours, and placed into vacuumed clot tubes. After each use, the IV catheter was flushed with 5 ml of heparinized saline[h] (3 U of heparin/mL) and removed after the last blood sample had been collected.

### 3.4 Sample processing and analysis

Approximately 3 hours after the last collection, all samples were centrifuged 10 minutes at 3660g and the serum was frozen at -80 °C. Time until analysis was 13 days. Samples were analyzed for iohexol using a modified rapid high-performance liquid chromatography

(HPLC)-UV[i] method (Pöytäkangas et al. 2010). Before HPLC analysis all samples were prepared; 30 µl of internal standard (para-aminobenzoic acid, 100 µg/ml in MeOH) was added to empty tubes and the solvent was vaporized in a block heater at 45 °C under a stream of nitrogen. 200 µl of thawed serum and 400 µl of 10 % trifluoroacetic acid (TFA) were added to the tubes. Additionally, 100 µl of deionized milli-Q water was added to the tubes. The samples were mixed in a multitube vortexer for 1 minute, and centrifuged (14,000 × g for 10 minutes at 4 °C). The supernatants were filtered through Acrodisc 0.2 µm syringe filters to autosampler vials for analysis by high-performance liquid chromatography.

The analytical column was Rapid Resolution Zorbax SB-C18 600 bar, 4.6 x 150 mm, pore size 1.8 µm. The mobile phase consisted of water (pH 3.0, adjusted with TFA) and methanol. The mobile phase gradient was linear with a time course as follows: 95:5 water:methanol at 0 min, 50:50 water:methanol at 4.5 min and 0:100 water:methanol at 7 min. The flow rate was 1 mL/min and column oven temperature of 50˚C. Injection volume was 20 µL. Component detection was made at a wavelength of 246 nm. Iohexol consists of two isomers; exo-iohexol and endo-iohexol (Klenner et al. 2007). The calibration was performed according to exo-iohexol.

The assay was linear at the measurement range (from 1 to 20 µg/mL). The analytical recovery after spiking serum samples ranged from 93.1 to 99.2 %. Inter- and intra-assay precision were evaluated at 4 different iohexol concentrations (1.0, 2.0, 5.0, and 20.0 µg/mL) measured in triplicate. The relative SD for the repeated interassay and intra-assay measurements of iohexol in serum varied from 1.6 to 3.0 % and from 1.2 to 2.6 %, respectively.

3.5 Statistical analysis

For the 10 healthy horses in the study, the iohexol concentration at each time point was expressed as the mean ± 1.69 SD, and upper and lower 95% percentiles (i.e. 2.5th and 97.5th percentiles), where the upper 97.5th percentile is defined as the value such that 97.5% percent of the values lie below it and the lower 2.5th percentile is defined as the

value such that 97.5% percent of the values lie above it. This allows for an estimation of variance in the data.

Normality of the distribution was tested by using Kolmogorov-Smirnov and Shapiro-Wilk. The data was then plotted on a line graph to create an iohexol permeability curve for normal horses.

Statistical analyses were performed by means of a computer software package[j].

# 4 RESULTS

## 4.1 Clinical examination, blood sample analysis and gastroscopy

Clinical examination revealed no abnormalities and the results for all horses were unremarkable (Appendix II). There was no diarrhea or a history of weight loss among the horses. CBC and blood chemistry results were within reference values, except for a few clinically insignificant alterations (Appendix III). Horse 003 had slightly increased serum concentrations of CK and AST (903 and 692 U/l, respectively) in comparison with the reference interval in the laboratory (<350 and <604 U/l, respectively). Horse 010 had slightly increased serum fibrinogen (4.6 g/l) compared with the reference range (<4.0 g/l). On gastroscopy, 8/10 (80%) of the horses had endoscopically visible gastric ulceration. Ulcer scores varied from 0 to 2.

## 4.2 Estimation of intestinal permeability

All horses tolerated the iohexol well when administered as a 10% solution via nasogastric intubation and there were no adverse effects. At a dose of 1.0 mL/kg, iohexol was detected in serum within 30 minutes of nasogastric administration in 9/10 (90%) of horses, and was detected in serum within 60 minutes in 10/10 (100%) of horses (Table 2). The concentration of iohexol in serum remained above the limit of detection for HPLC-(UV)

throughout the period of blood sampling and was still detectable in serum at 360 minutes post-iohexol administration in all horses. Iohexol concentrations peaked for all horses between 30 and 180 minutes (7 horses peaked at 60 minutes, and one at each of 30, 120 and 180 minutes) and then gradually decreased over the subsequent 30 to 240 minutes, at which time there appeared to be a second trend upwards that was still increasing at 360 minutes in all cases (Fig.1). When the upper and lower 95th percentiles for iohexol serum concentration were plotted against time, the upper 95th percentile appeared to represent a bimodal distribution over time, confirming the presence of a double peak (Fig.2). Data weren't normally distributed according to Kolmogorov-Smirnov and Shapiro-Wilk tests.

**Table 2.** Iohexol serum concentration [µg/mL] measured 0-360 minutes after administration of 1,0 mL/kg Omnipaque-350 to 10 clinically healthy horses

| Time after administration | Horse No. | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 008 | 009 | 010 | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | **30** | 0,00 | 3,93 | 3,71 | 2,41 | 3,41 | 3,90 | 5,94 | 11,05 | 4,37 | 2,37 | 4,11 | 2,89 |
| | **60** | 1,34 | 4,82 | 5,34 | 4,08 | 3,49 | 4,08 | 11,08 | 13,20 | 4,25 | 5,63 | 5,73 | 3,61 |
| | **120** | 3,23 | 4,67 | 5,13 | 3,49 | 2,75 | 2,85 | 6,69 | 9,28 | 5,03 | 7,02 | 5,01 | 2,12 |
| | **180** | 3,69 | 4,21 | 4,05 | 6,47 | 2,52 | 3,25 | 5,17 | 7,45 | 6,10 | 5,62 | 4,85 | 1,56 |
| | **240** | 3,41 | 5,03 | 2,95 | 8,91 | 2,21 | 3,44 | 5,05 | 6,46 | 7,67 | 4,79 | 4,99 | 2,15 |
| | **300** | 3,11 | 5,18 | 2,46 | 10,34 | 2,61 | 3,39 | 5,22 | 6,47 | 8,97 | 5,25 | 5,30 | 2,67 |
| | **360** | 3,61 | 6,23 | 2,65 | 15,18 | 2,66 | 3,99 | 5,86 | 6,90 | 10,81 | 5,37 | 6,33 | 3,94 |

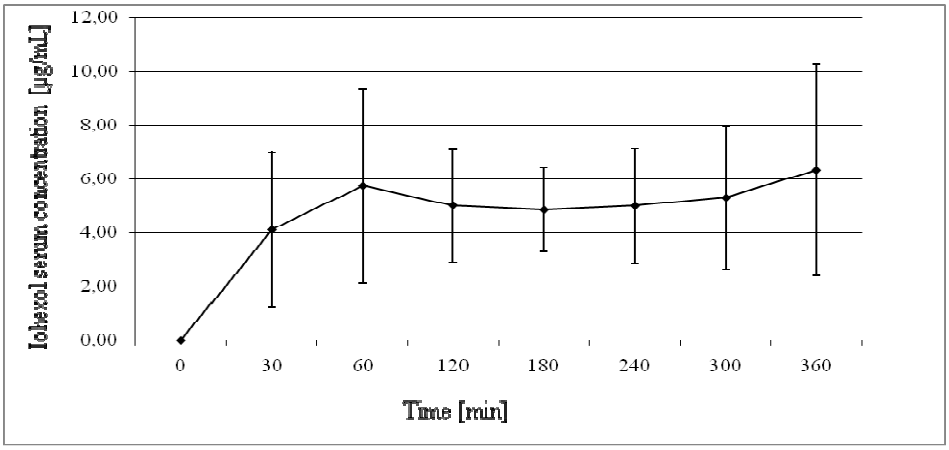


**Figure 1.** Iohexol serum concentrations (mean ±1.69 SD) in 10 clinically healthy horses after oral administration of 1mL iohexol/kg.

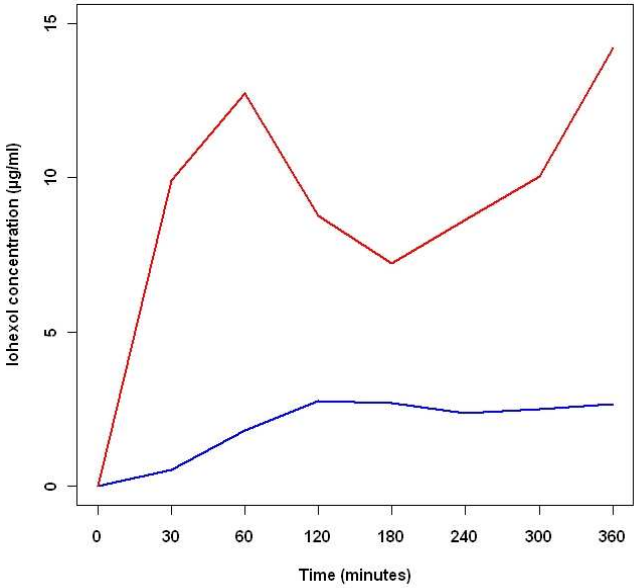


**Figure 2**. Iohexol serum concentrations (upper and lower 95% percentiles) in 10 clinically healthy horses after oral administration of 1mL iohexol/kg.

## 5 DISCUSSION

The nonionic radiographic contrast medium iohexol was evaluated as an intestinal permeability marker in fasting healthy horses. Inflammatory bowel disease (IBD) causes an increase in intestinal permeability and thus higher amounts of iohexol cross the intestinal wall and enter the circulation. Under these circumstances, the serum concentration of iohexol in horses with IBD should be above the reference ranges for healthy horses following oral administration of a fixed quantity of iohexol.

Before administration of iohexol each horse underwent a thorough clinical examination in order to confirm the absence of gastrointestinal diseases. Clinical examination, blood sample analysis and gastroscopy were considered to be sufficient for ruling out gastrointestinal diseases which can affect iohexol intestinal permeability in this study. Typically, IBD causes progressive weight loss because of malabsorption of nutrients and protein losing enteropathy (Schumacher et al. 2000), and it is reasonable to assume that a

clinically healthy horse in good body condition with a normal serum protein is highly unlikely to have any significant alterations in intestinal permeability. A similar approach was used by Escala et al. (2006) when assessing intestinal permeability in healthy ponies vs. ponies with cyathostome infection using the $^{51}$Cr-EDTA absorption test, and in fact, no ancillary diagnostics tests were used to rule out existing altered intestinal permeability in their study.

In this study, a thorough clinical examination, CBC, and serum biochemistry did not reveal any significant abnormalities and no horses had diarrhea or a history of weight loss. The horses were also regularly treated with anthelmintics ruling out the likelihood of cyathostomiasis. The serum biochemistry indicated that serum protein was normal in all the horses studied, ruling out protein losing enteropathy. Gastroscopy was done in order to exclude gastric ulceration as a cause of increased iohexol permeability. Unfortunately a large proportion of the horses had evidence of mild gastric ulceration (80%), and it is possible that this may have had an influence on iohexol permeability in this study. The degree of ulceration was mild however, and thus its effect on the overall permeability curve is unlikely to have been significant.

The objective of this study was to investigate if iohexol could be detected in the serum of healthy fasted horses following administration of iohexol (Omnipaque-350) at a dose of 1 mL/kg via a nasogastric tube. Detection of iohexol in blood rather than in urine would be a major advantage in horses as urine collection is time consuming and technically difficult to perform. The dose of iohexol selected for this study was based upon preliminary research done in dogs and horses. Strube (2007) assigned a dose of 2.0 mL/kg of Omnipaque-350 (iohexol) orally with food to horses and had managed to detect iohexol in serum at this dose, however, when dosing iohexol with food there were some difficulties with ascertaining if all the iohexol had in fact been consumed, and consequently reliable and definite conclusions regarding the optimal dose of iohexol could not be made. Klenner et al. (2009) used dosages of 0.25, 0.5, 1.0, 2.0, and 4.0 mL/kg of Omnipaque-350 (iohexol) for evaluation of intestinal permeability in healthy dogs. Because of the absence of side effects and appropriate radiopacity after administration of the dosage of 2.0 mL/kg, they concluded that 2.0 mL/kg was the best choice for the intestinal permeability serum test in

fasting dogs. Since higher dosages of iohexol are associated with increased cost for the marker substance and differentiation of the gut segments in X-ray is not relevant in the horse; 1.0 mL/kg was selected to be optimal for determining intestinal permeability in healthy horses. Our hypothesis was that a dose of 2 mL/kg of Omnipaque-350 was enough for iohexol to be detected in serum when fed to horses, so administration of 1mL/kg Omnipaque-350 administered directly into the stomach via nasogastric intubation would probably be enough to be detected in serum. Detection of iohexol in serum at doses lower than 1 mL/kg (e.g. 0.25 and 0.5 mL/kg) would be challenging due to interfering peaks in the HPLC chromatogram (Klenner et al. 2009) and thus a smaller dose was decided to exclude, despite the fact that a smaller dose would have been less costly. Because administration of a hyperosmolar mixture may bring about osmotic diarrhea (Klenner et al. 2009), iohexol was administered as a 10 % solution in tap water in this study. The calculated concentration of the mixture was 117 mg I/mL which is isotonic with the blood and tissue fluids. Besides preventing osmotic diarrhea, there are also other reasons for promoting the use of physiological isotonic solutions. The osmolarity of a solution affects the permeability of several substances, possibly including iohexol (Laker et al. 1977, Wheeler et al. 1978).

In order to estimate intestinal permeability, serum blood samples were obtained hourly for 6 hours. A remarkably significant finding was that the iohexol permeability curve demonstrated a double peaking phenomenon which has not been reported in other species (Klenner et al. 2009). Iohexol concentrations peaked for all horses between 30 and 180 minutes and then gradually decreased over the subsequent 30 to 240 minutes, at which time there appeared to be a second upward trend that was still increasing at 360 minutes in all cases. Multiple peaking in the concentration-time curve is occasionally encountered in pharmacokinetics and can occur as a consequence of a number of different mechanisms (Davies et al. 2010). These include the physiological makeup of the gastrointestinal tract and factors related to the formulation, for instance the drug chemical entity itself and the excipients incorporated into the drug. Because this kind of double peak phenomenon has not existed in other species even though the solution has been the same, a fundamental reason for the double peak phenomenon may be related to species differences, including differences in the structure and composition of the gastrointestinal tract.

There are multiple factors related to the gastrointestinal tract which may have an influence on the shape of the concentration-time curve, including secretion and components of bile, enterohepatic recycling and pH. Biochemical differences in the regional areas of the gastrointestinal tract may also play a role. For example, regiospecificity in the bile concentration and transport proteins can lead to a multiple peaking phenomenon (Davies et al. 2010), and it is possible that differences in secretion and components of bile may lead to alterations in intestinal permeability of iohexol in horses. Equids do not have a gall bladder and bile, a fat emulsifying agent, is secreted constantly, as an adaption to grazing and several small feedings per day. This is a significant difference in comparison with dogs and humans, and thus can be a part of the reason for multiple peaking in the concentration-time curve. On the other hand, iohexol is a water-soluble marker, and hence it could be argued that bile does not influence iohexol intestinal permeability.

Studies indicate that the most common cause of multiple peaking in the blood concentration-time curve is enterohepatic recycling (Davies et al. 2010). Enterohepatic recycling is related to the physiological processes involved in bile acid and bile salt removal and retention. Typically substances undergo extensive first-pass metabolism, for instance glucuronidation, after which they are eliminated via the biliary elimination pathway (Marier et al. 2002). In all likelihood, enterohepatic recycling is not related to the double peaking phenomenon of iohexol, because in addition to the water-solubility of iohexol, iohexol is an inert substance and not metabolized. Furthermore, iohexol is almost completely excreted in urine after glomerular filtration which rules out the possibility of enterohepatic recycling (Andersen et al. 2001). There was no bimodal distribution in the serum concentration-time curve after intravenous administration of iohexol which also excludes enterohepatic recycling as an explanation for a multiple peaking phenomenon (Goy-Thollot et al. 2006, Wilson et al. 2009).

In addition to secretion of bile, there are also differences in intraluminal pH of the equine gastrointestinal tract when compared with dogs or humans. Changes in local pH environments affect the solubility of weak acids and bases which ultimately leads to site-specific absorption (Davies et al. 2010). However, iohexol is a non-ionic contrast agent and

thus it could be argued that pH does not affect iohexol and cannot be used as an explanation for the double peak phenomenon that has been observed. Regiospecificity in transport proteins is another potential cause of a double peaking phenomenon (Davies et al. 2010). Studies suggest that iohexol permeates the intestinal wall through paracellular permeate pathways (Frias et al. 2005), and hence the distribution of transport proteins does not play an important role in the permeability of iohexol either.

Another possible explanation for the bimodal distribution of iohexol is the gastric emptying rate and intestinal transit time (Davies et al. 2010). Usually absorption of substances is marginal from the stomach in comparison with the small intestine, because of the decreased transit time and the smaller surface area of the stomach. According to Davies et al. (2010) gastric emptying may be a significant determining factor in absorption of water-soluble substances, like iohexol for instance. There are multiple pharmaceutical non-functional substances which are often considered to be inert and without remarkable effect within the gastrointestinal tract (Davies et al. 2010). Despite this fact, polyethylene glycol molecular weight 400 (PEG 400), which is considered to be an inert substance, can influence small intestinal transit time and thus provoke a multiple peaking phenomenon in a concentration-time curve (Basit et al. 2002), and it is reasonable to assume that iohexol may behave in a similar manner.

Interestingly, feeding conditions and the route of administration may affect the presence of double peaking as well, and this is possibly related to the gastric emptying rate and intestinal motility. Bergstrom et al. (1981a) administered penicillamine orally during a period of fasting and then 30 minutes after ingestion of food, and they discovered a double peak in the fasting plasma levels of penicillamine. That finding is congruent with previous findigs regarding absorption of iohexol in the horse. Strube (2007) administered iohexol per orally with food to horses and there was no bimodal distribution in the concentration-time curve in contrast to this study in which iohexol was administered per orally to fasting horses, and it does suggest that the feeding conditions may have a significant effect on the shape of the iohexol concentration-time curve.

In the case of penicillamine, a multiple peaking phenomenon was only detectable after an oral not an intravenous administration to dogs and humans (Bergstrom et al. 1981b). It may be possible that the route of administration of iohexol affects the shape of the concentration-time curve in the horse as well. The iohexol concentration-time curve revealed no signs of bimodal distribution after an intravenous administration in contrast to an oral administration (Wilson et al. 2009). Even though the upper 95% percentile in this study supports the presence of a bimodal distribution of iohexol after nasogatric administration, it has to take into consideration that only 10 horses were used, and further work with a larger number of horses is needed before definitive conclusions about the apparent double peak phenomenon observed in this study can be made.

In this study, there was a clear upward trend in serum concentrations of iohexol after 360 minutes. There also appeared to be a similar trend in the iohexol concentration-time curve for up to 12 hours after feeding horses 2.0 mL/kg of iohexol (Strube 2007). In Strube's study, it is possible that the persistent elevation in iohexol concentrations could be due to delayed consumption of iohexol that was offered in food. However the form of the concentration-time curve of this study indicates that an upward trend is real and iohexol concentrations do continue to rise for many hours after oral administration in horses. Andersen et al. (2001) discovered that small intestinal permeability for iodixanol is the proportion of 10 to 1 in comparison with colonic permeability, and it is likely that this is similar for iohexol. Decreased and protracted colonic permeability may be one reason for a continuous elevation in iohexol over time. Furthermore, the same factors giving rise to a double peaking phenomenon, e.g. gastric emptying rate and intestinal motility (Davies et al. 2010), might also be reasons for an upward trend in the iohexol concentration-time curve. Further studies are needed to determine at what point iohexol begins to decline when administered as a fixed dose via nasogastric intubation, as this will have important implications regarding the optimal time to take blood samples if the iohexol permeability test is validated as a diagnostic test in the future.

# 6 CONCLUSION

In this study, 1 ml/kg of iohexol was administered to healthy horses as a 10% solution via a nasogastric tube and the results indicate that (1) iohexol can be detected in equine serum and (2) no adverse effects were related to oral administration of iohexol. Consequently according to this preliminary study, an iohexol permeability serum test may be a useful method for estimation of equine intestinal permeability, and a larger study to investigate iohexol permeability in diseased vs. non-diseased horses is being planned. Horses with IBD will be recruited via the University of Helsinki web pages and advertisements in trotting and riding magazines (Appendix IV) and will be tested in an identical manner to the 10 healthy horses reported in this preliminary study. If there is a statistical difference in the serum concentration of iohexol between normal horses and horses with IBD, then the next phase will be to validate an iohexol permeability serum test against a gold standard (most probably intestinal histopathology) and assess the sensitivity and specificity of an iohexol permeability serum test for the diagnosis of IBD in horses.

In future, it would be beneficial to modify the current iohexol testing protocol to make it more practical for veterinarians that will be using the test in a clinical practice. Iohexol was easy to administer via a nasogastric tube, however the dose was based upon the weight of the horse. It would be more practical if a fixed dose (e.g. 500 mls) could be used for all horses, as assessing a horse weight is often problematic in practice. Furthermore, repeat blood sampling over a six-hour period is impractical, and it would be necessary to limit the number of taken samples to certain time points. Lastly, from a client's standpoint, the cost of the procedure plays an important role in compliance to the performance of the test. Iohexol is a relatively expensive intestinal permeability marker in comparison with e.g. glucose. In order to limit costs, a lower dose of iohexol should be tested on horses. Additionally, it would be worthwhile to market a cheaper non-sterile formulation for per oral administration.

## 7 FOOTNOTES

[a] An automated multiparameter analyzer with software for animal samples, CELL-DYN 3700 System, ABBOTT Diagnostics Division, ABBOTT Park, IL, USA

[b] Konelab 30i Clinical chemistry analyzer, ThermoFisher Scientific, Vantaa, Finland

[c] Olympus GIF-100, Olympus America Inc, Melville, NY, USA

[d] Domosedan, Orion oyj, Espoo, Finland

[e] Butordol, Intervet international B.V, Boxmeer, Netherlands

[f] Intraflown 2 Vycon laboratories, Ecouen, France

[g] Omnipaque-350, GE Healthcare AS, Oslo, Norway

[h] Heparin, LEO Pharma A/S, Ballerup, Denmark

[i] Agilent model 1200 series rapid resolution LC system, Agilent Technologies, Böblingen, Germany

[j] R Development Core Team (2008), R: A Language and Environment for Statistical Computing, Vienna, Austria

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# Appendix I

UNIVERSITY OF HELSINKI

# IOHEXOL STUDY DATA SHEET

**Date:** **Identification number:**

| | | | | |
|---|---|---|---|---|
| **Body condition:** | **Sex:** | | **Weight:** **(kg)** | |
| **Breed:** | **Age:** | | | |
| **General status:** | Locomotion: | | | |
| Behaviour: | Coat: | | | |
| **Management** | Stable vs. pasture? | | Change: yes / no | |
| **Feed** | What is fed and how much? How often? | | Change: yes / no | |
| **De-worming** | Interval | Last de-wormed | What product was used | |
| | | | | |
| **Drugs** | Name | Amount | Start date? | End date? |
| | | | | |
| | | | | |
| | | | | |
| **Physical examination** | Resp. rate/min: | | CRT / sec: | |
| | Pulse rate/min: | | MM colour: | |
| | Temperature: | | | |
| **Gastrointestinal function** | Teeth: | | Abdominal auscultation | |
| | Abdominal appearance: | | LU | RU |
| | | | LL | RL |

| | | | | | |
|---|---|---|---|---|---|
| **Gastroscopy findings** | | | | | |
| **Ulcer score** | **0** | **1** | **2** | **3** | **4** |
| **Defecation** | **Amount** | | **Consistency** | **Last passed** | |
| | | | | | |
| **Notes:** | | | | | |

| **Time** | **Study Activity (Week 2), Iohexol blood samples** | **Actual time and notes** |
|---|---|---|
| T0 | Place temporary catheter | |
| T0 | Iohexol T0 samples | |
| **T0** | Dose horses with 1ml/kg iohexol via nasogastric tube | |
| T30 | Blood sample | |
| T60 | Blood sample | |
| T120 | Blood sample | |
| T180 | Blood sample | |
| T240 | Blood Sample | |
| T300 | Blood sample | |
| T360 | Blood sample | |

# Appendix II

Clinical examination results. G= gelding, M= mare, Wb= Warmblood, Fh= Finnhorse

| Horse No. | Age (yrs) | Sex | Breed | Body condition | Weight (kg) | Pulse rate/min | Resp. rate/min | CRT/sec | MM color | Temp |
|---|---|---|---|---|---|---|---|---|---|---|
| 001 | 17.2 | G | Wb | 7 | 570 | 40 | 16 | <2 | Pink | 36.8 |
| 002 | 14.2 | G | Fh | 6 | 616 | 38 | 22 | <2 | Pale pink | 37.4 |
| 003 | 10.0 | M | Wb | 5 | 590 | 38 | 16 | <2 | Pale pink | 37.0 |
| 004 | 17.3 | G | Fh | 7 | 558 | 40 | 20 | <2 | Pale pink | 37.3 |
| 005 | 12.1 | G | Fh | 6 | 515 | 38 | 16 | <2 | Pink | 37.3 |
| 006 | 11.1 | G | Fh | 7 | 530 | 32 | 20 | <2 | Pale pink | 37.5 |
| 007 | 15.2 | G | Fh | 7 | 495 | 30 | 14 | <2 | Pale pink | 37.6 |
| 008 | 19.1 | G | Fh | 6 | 514 | 30 | 12 | <2 | Pale pink | 37.6 |
| 009 | 13.1 | G | Fh | 6 | 485 | 48 | 16 | <2 | Pale pink | 37.4 |
| 010 | 11.1 | G | Fh | 7 | 550 | 33 | 16 | <2 | Pale pink | 37.6 |

# Appendix III

Biochemistry and complete blood count (CBC) results. ND, not determined

| Horse No. | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 008 | 009 | 010 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cl (mmol/l) | 98.4 | 94.1 | 96.2 | 96.1 | 97.2 | 100.4 | 98.4 | 100.8 | 99.0 | 98.0 |
| K (mmol/l) | 4.3 | 4.0 | 4.1 | 4.0 | 4.0 | 4.3 | 3.5 | 3.9 | 3.9 | 4.0 |
| Na (mmol/l) | 135 | 133 | 137 | 136 | 138 | 136 | 141 | 142 | 138 | 139 |
| ASAT (U/l) | 296 | 395 | 692 | 376 | 399 | 552 | 377 | 364 | 357 | 339 |
| CK (U/l) | 192 | 234 | 903 | 292 | 260 | 279 | 289 | 294 | 198 | 428 |
| GT (U/l) | 13 | 30 | 27 | 21 | 21 | 25 | 19 | 21 | 20 | 20 |
| Mg (mmol/l) | 0.81 | 0.71 | 0.87 | 0.79 | 0.75 | 0.80 | 0.88 | 0.81 | 0.83 | 0.74 |
| SDH (U/l) | 0.5 | 6.4 | 34.4 | 13.6 | 5.7 | 18.1 | 8.4 | 15.5 | 7.4 | 3.2 |
| ALP (U/l) | 283 | 415 | 527 | 376 | 313 | 367 | 306 | 592 | 316 | 549 |
| Alb (g/l) | 34.1 | 29.2 | 35.4 | 33.2 | 30.6 | 33.6 | 33.5 | 34.1 | 36.4 | 32.1 |
| Bil. Tot (µmol/l) | 29.3 | 23.6 | 23.8 | 27.1 | 20.0 | 19.3 | 23.3 | 29.2 | 27.9 | 24.1 |
| Glu (mmol/l) | 3.9 | 4.1 | 5.5 | 4.5 | 4.4 | 4.7 | 4.2 | 4.2 | 4.7 | 4.6 |
| Ca (mmol/l) | 3.22 | 3.13 | 3.18 | 3.27 | 3.13 | 3.29 | 3.33 | 3.36 | 3.30 | 3.26 |
| Crea (µmol/l) | 122 | 111 | 97 | 104 | 102 | 97 | 91 | 114 | 113 | 100 |
| Prot (g/l) | 60 | 69 | 61 | 70 | 70 | 67 | 73 | 74 | 59 | 68 |

| Horse No. | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 008 | 009 | 010 |
|---|---|---|---|---|---|---|---|---|---|---|
| FB (g/l) | 4.4 | 3.6 | 3.6 | 4.3 | 3.3 | 4.4 | 4.0 | 4.4 | 3.7 | 4.6 |
| WBC ($\times10^9$/l) | 6.31 | 7.38 | 4.20 | 7.29 | 4.64 | 5.01 | 9.52 | 7.19 | 5.92 | 7.25 |
| NEU($\times10^9$/l) | 4.14 | 5.06 | 2.36 | 4.38 | 2.47 | 3.28 | 5.95 | 4.45 | 3.95 | 4.39 |
| LYM($\times10^9$/l) | 1.69 | 1.55 | 1.12 | 1.74 | 1.75 | 1.26 | 2.94 | 1.88 | 1.55 | 2.08 |
| MONO($\times10^9$/l) | 0.310 | 0.370 | 0.209 | 0.638 | 0.189 | 0.278 | 0.418 | 0.337 | 0.224 | 0.251 |
| EOS ($\times10^9$/l) | 0.130 | 0.358 | 0.495 | 0.483 | 0.204 | 0.165 | 0.146 | 0.498 | 0.147 | 0.516 |
| BASO ($\times10^9$/l) | 0.040 | 0.036 | 0.014 | 0.053 | 0.022 | 0.021 | 0.059 | 0.023 | 0.054 | 0.015 |
| RBC ($\times10^{12}$/l) | 8.01 | 6.63 | 7.04 | 7.93 | 6.67 | 7.28 | 7.79 | 7.30 | 9.30 | 6.82 |
| HGB (g/l) | 132 | 112 | 118 | 131 | 112 | 119 | 130 | 135 | 158 | 116 |
| HCT (%) | 38.2 | 30.7 | 33.6 | 36.7 | 31.3 | 33.2 | 37.1 | 38.2 | 44.2 | 32.8 |
| MCV (fl) | 47.7 | 46.3 | 47.8 | 46.2 | 47.0 | 45.6 | 47.6 | 52.4 | 47.5 | 48.1 |
| MCH (pg) | 16.5 | 16.8 | 16.8 | 16.5 | 16.8 | 16.3 | 16.6 | 18.5 | 17.0 | 17.0 |
| MCHC (g/l) | 346 | 363 | 352 | 358 | 359 | 358 | 349 | 352 | 357 | 353 |
| RDW (%CV) | 28.2 | 25.0 | 22.9 | 25.0 | 26.5 | 25.0 | 27.2 | 24.4 | 24.4 | 24.3 |
| PLT ($\times10^9$/l) | 137 | 152 | 104 | 149 | 124 | 122 | 160 | 158 | 160 | 145 |
| MPV (fl) | 10.2 | 9.35 | 10.3 | ND | 6.90 | 7.03 | 6.45 | 10.3 | 7.14 | 8.50 |

# Appendix IV

UUSI TUTKIMUS HEVOSEN LAIHTUMISEN DIAGNOSTIIKASTA

Iohexolin käyttö suoliston läpäisevyyden arvioimisessa tulehduksellisissa suolistosairauksissa

Hevosen suoliston läpäisevyys muuttuu eri tautitiloissa kuten tulehduksellisissa suolistosairauksissa. Tästä esimerkkinä on hevosen tulehduksellinen suolistosairaus eli ’inflammatory bowel disease’ (IBD), jonka syytä ei tunneta. Suoliston läpäisevyyden muutoksia on havaittu hevosilla myös erilaisissa hapenpuutteesta johtuvissa kudosta vaurioittavissa tiloissa, esim. suolikierteen yhteydessä ja tietyissä matoinfektioissa. Nykymenetelmin suolistosairauksien diagnostiikka on erittäin haastavaa ja vaatii jopa vatsaontelon tähystyksen, koepalojen oton muuttuneesta suolesta ja niiden kudostutkimuksen. Tällä hetkellä on hevosen suoliston läpäisevyyttä mitataan glukoosi- ja ksyloosi-absorptiotesteillä. Ksyloosin analyysi on kallis, tuloksiltaan epäluotettava eikä sitä ole myöskään aina saatavilla. Glukoosiin perustuva menetelmä ei ole tarpeeksi luotettava, sillä hevosen aineenvaihdunta vaikuttaa tuloksiin. Siksi tarvitaan uusia ja luotettavampia menetelmiä.

Yliopistollisessa eläinsairaalassa Helsingin Viikissä on käynnistetty tutkimus, jonka tarkoituksena on kehittää hevosen suoliston läpäisevyyttä muuttavien suolistosairauksien diagnostiikkaa entistä helpompaan, luotettavampaan ja turvallisempaan suuntaan. Tutkimuksessamme hevosille annetaan suun kautta iohexolia, vaaratonta varjoainekuvauksissa käytettyä merkkiainetta, joka imeytyy suolesta vereen. Mittaamalla iohexolin pitoisuus hevosen seerumissa voisimme arvioida suoliston läpäisevyyttä ja mahdollisesti diagnosoida suoliston läpäisevyyteen vaikuttavia em. tautitiloja. Koirilla ja ihmisillä iohexol on jo osoittautunut käyttökelpoiseksi aineeksi suoliston läpäisevyyden arvioimisessa. Tämä voisi toimia myös hevosella vaihtoehtona glukoosi- ja ksyloosi-absorptiotesteille ja tarjoaisi siten lisää mahdollisuuksia ja keinoja diagnosoida hevosen suoliston läpäisevyyttä muuttavia tautitiloja.

Tästä syystä haemme Yliopistollisessa eläinsairaalassa tehtävään tutkimuksemme kroonisesta tulehduksellisesta suolistosairaudesta kärsiviä yli kahden vuoden ikäisiä hevosia. Tyypillistä IBD:tä sairastaville hevosille on laihtuminen, joka aiheutuu suoliston häiriötilasta. Hevonen saattaa olla yleisolemukseltaan vaisu ja sen ruokahalu voi olla heikentynyt. Myös pitkään jatkunut ripuli on mahdollinen. Osalla hevosista on toistuvia ähkyjä, joiden vakavuus vaihtelee lievästä jopa kirurgista hoitoa vaativiin. Edellä mainittujen oireiden lisäksi hevosella saattaa esiintyä turvotuksia, johtuen seerumin valkuaisaineiden menetyksestä suoliston häiriötilan yhteydessä.

Tutkimuksessa hevoselle annostellaan iohexolia suun kautta ja mitataan sen pitoisuuksia seerumista tietyillä ajanhetkillä. Ennen iohexolin antamista varmistetaan, ettei hevosella ole muita tuloksiin vaikuttavia mahasuolikanavan sairauksia kuten mahahaavaa. Hevoselle tehdään kliininen tutkimus ja siltä otetaan verinäyte. Verinäytteestä tutkitaan mm. veren soluarvoja ja seerumin entsyymipitoisuuksia. Tämän lisäksi tehdään mahalaukun tähystys ja glukoosi-absorptiotesti. Tapauskohtaisesti hevoselle tehdään myös muita tutkimuksia, mikäli ne koetaan aiheellisiksi. Jos hevosellanne on todettu edellä mainittuja oireita tai jos hevosellanne on diagnosoitu IBD, otattehan yhteyttä. Tutkimukseen valituille ilmoitetaan henkilökohtaisesti. Kaikki meille lähetetyt tiedot ovat luottamuksellisia.