Assessment of Capsule Endoscopy Technology as an Imaging Tool for the Equine Small Intestine

by

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ABSTRACT

ASSESSMENT OF CAPSULE ENDOSCOOPY TECHNOLOGY AS AN IMAGING TOOL FOR THE EQUINE SMALL INTESTINE

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Advisor: Professor J. Thomason

Capsule endoscopy (CE), in which an encapsulated camera is ingested, is used in human medicine to image the small intestine (SI) to aid in the diagnosis of intestinal disorders by detecting lesions, inflammation, bleeding, and tumors. The broad objective of this study was to assess the value of using the MiroCam® capsule endoscope in horses. Duration of recording and image quality were assessed in two healthy Standardbred horses that each underwent four CE examinations. Good quality video segments were obtained, demonstrating SI features including distinct villi, mucosal lesions and luminal parasites. However, image transmission was intermittent and of short duration (0.5 to 82.41 minutes) and images were frequently obstructed by residual feed particles. This pilot study demonstrates the potential utility of CE to aid in diagnosis of SI conditions, however further research into signal transmission and equine fasting protocols is required before CE can be effectively used in equine medicine.
ACKNOWLEDGEMENTS

This thesis was possible due to the joint efforts of many individuals who came together to support the project by providing their time, experience and guidance throughout the project and I am grateful for everyone’s contributions.

I would like to firstly and foremost thank my advisor, Dr. Jeff Thomason, for his everlasting support, encouragement and guidance throughout this project; this project was not without its challenges and Dr. Thomason always provided encouragement and steered me in the right direction. I’d also like to thank my committee members Dr. Koenig and Dr. Chalmers for their guidance and valuable comments and suggestions.

Warren Armstrong was instrumental in getting this project off the ground by bringing all parties to the table, and provided the necessary technical expertise in capsule delivery during the project. Warren strongly believed in the success of the project and brought much enthusiasm, always contributing ideas and troubleshooting issues.

I’d also like to thank the Intromedic team for their support and contributions to the project, notably Benjamin Seo for helping to keep the project moving forward and answering my many technical questions! Thank you to Persephone Greco-Otto and Angela Wilson for their help during the research trials. Their enthusiasm and assistance made the long research days very much enjoyable.

I’d like to thank the Ontario Veterinary College for their financial support through the OVC Dean’s fellowship scholarship. I’d also like to acknowledge the financial support of Equine Guelph and Mr. Claude Margue and the support of Halton Equine Veterinary Ser-
vices. I’d also like to thank Vantage Endoscopy for their in-kind donation of the capsule endoscope equipment.

Last but certainly not least, I am extremely grateful for the love and support of Steve and my family and friends through all the highs and lows of the last couple of years! They not only provided continued support and encouragement, but were instrumental in making sure the team was well fed during the long 16 hour research trial days!
DECLARATION OF WORK PERFORMED

I declare that I, Diane Gibbard, performed all the work in this thesis with the exception of the items listed below.

All veterinary procedures during the capsule endoscopy trial, including gastroscopy, administration of drugs and capsule delivery, were performed by staff and veterinarians at Halton Equine Veterinary Services including Dr. Frost, Dr. Armstrong and Warren Armstrong. Two students, Persephone Grecco-Otto and Angela Wilson assisted in data collection, prepping and monitoring horses during CE trials. Intestinal parasites were identified by Dr. Peregrine from the Ontario Veterinary College.

Analysis of signal quality was performed by Benjamin Seo and the team at Intromedic Ltd. Co.
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<td>Bodyweight</td>
</tr>
<tr>
<td>CE</td>
<td>Capsule endoscopy</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CI</td>
<td>Longest bout of largely continuous image transmission</td>
</tr>
<tr>
<td>CI %</td>
<td>Percentage of time with consistent image transmission</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>I%</td>
<td>Percentage time of usable images</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LI</td>
<td>Large intestine</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>MMC</td>
<td>Migrating motor complex</td>
</tr>
<tr>
<td>SI</td>
<td>Small intestine</td>
</tr>
<tr>
<td>T1H1</td>
<td>Trial 1, Horse 1</td>
</tr>
<tr>
<td>T1H2</td>
<td>Trial 1, Horse 2</td>
</tr>
<tr>
<td>T2H1</td>
<td>Trial 2, Horse 1</td>
</tr>
</tbody>
</table>
T2H2  Trial 2, Horse 2

T3H1  Trial 3, Horse 1

T3H2  Trial 3, Horse 2

T4H1  Trial 4, Horse 1

T4H2  Trial 4, Horse 2

TT    Total time of images obtained
INTRODUCTION

Endoscopy, in which a fiber optic camera instrumented to a long flexible cable, is used to visualize internal cavities. It is a powerful diagnostic tool for the gastrointestinal tract (GIT), but is limited to its reach from either oral or rectal entry points due to the length of the endoscope (220-250cm) [1]. In humans, colonoscopy is routinely performed and the entire length of the 1.5 metre (m) colon [2] can be examined, but it is not able to physically reach the small intestine (SI). From the opposite end, enteroscopy is only capable of examining the proximal 10% [1] of the 4-6 m long human SI [2], leaving 90% of the SI inaccessible to this technique. Wireless capsule endoscopy (CE) technology emerged to fill in the gap by using an untethered endoscopic camera to examine the entire SI. In the last decade, CE has been successfully used in human medicine and has been shown to have a high diagnostic yield for detecting SI pathologies linked to inflammatory bowel disease, obscure gastrointestinal bleeding, Crohn’s and celiac diseases [3].

Like humans, horses suffer from a variety of GIT disorders and being able to access sections of the GIT for examination is even more challenging given the much longer length of the intestinal tract (24-30m) [4] and the large size of the animal. Colonoscopy can be performed in the horse but is limited to the distal portion of the colon. Gastroduodenoscopy is often used in foals but can be used to examine the proximal duodenum in the adult horse [5] but, like humans, is limited to the length of the available endoscope (minimum of 3m) and therefore a large portion of the adult equine SI is inaccessible. Moreover, the ability of diagnostic modalities such as ultrasound and radiography to diagnose equine SI disorders is limited by the horse’s large abdomen, generous ribcage and thick body wall [6]. In addition, magnetic resonance imaging and computed tomog-
raphy are not used to assess equine GIT due to the horse’s large size. As a result, CE could serve as a useful imaging tool for the equine SI, which is otherwise inaccessible to the equine practitioner, and could help in diagnosing causes of intestinal disorders in cases of colic and malabsorption syndrome.

The purpose of this thesis is to assess whether current human CE technology can be applied to the horse. The specific objectives of this thesis are to:

1) develop a patient preparation protocol to optimize image quality for CE in horses
2) improve protocols for delivery of the capsule in the horse
3) assess image transmission between dermal and needle wire electrodes in the horse
4) determine the duration, quality, and consistency of CE image transmission in the horse

Regarding the use of CE to image the SI of horses our expectations are that:

1) A reducing ration and a fasting period will be necessary to adequately prepare patients prior to CE
2) the human CE device can be successfully delivered in the horse using an endoscope in combination with a capsule delivery device
3) Subcutaneous needle wire electrodes will improve image transmission compared to dermal electrodes as the horse’s dermis and epidermis layers are thicker than humans, and therefore may act as an insulator and attenuate the electrical signal coming from the capsule
4) duration of image transmission will be lower in the horse compared to the human when using the standard capsule and images will be of similar quality
CHAPTER 1: Review of Literature

1.1 Introduction

A normal GIT is critical for the health, welfare and performance of the horse. Colic is reported to be the number one health issue by horse owners [7] due to high morbidity and mortality rates [7-10]. The ability to diagnose the underlying cause of colic when it occurs improves prognosis by facilitating appropriate treatments. Diagnosing the underlying cause of colic can be challenging given the limitations of diagnostic tools in equine veterinary medicine to access the GIT due to the horse’s large abdomen. Capsule endoscopy, successfully used in human medicine, is being proposed as a diagnostic tool to image the inside of the equine SI to help in the diagnosis of diseases leading to recurrent colic and malabsorption syndrome in the horse. In broad terms, this literature review will highlight the impact of colic to the horse industry and more specifically review recurrent colic and malabsorption syndrome in the horse. This review is by no means a comprehensive review of colic, acute and surgical cases of colic will not be covered in detail as CE would not be useful in these cases. The pathology and diagnosis of SI disorders that are linked to recurrent colic and malabsorption syndrome will be addressed as well as limitations of current diagnostic modalities to image the SI. The potential use of capsule endoscopy will be demonstrated by highlighting its use in human medicine and its ability to cross over to equine medicine. Lastly, equine GIT motility and human preparation protocols will be reviewed in order to develop protocols for emptying the equine SI prior to CE.
1.2 Application of capsule endoscopy

1.2.1 Capsule endoscopy in human medicine

Capsule endoscopy is a procedure in which an encapsulated, disposable camera is ingested to image the inside of the digestive tract as it is propelled forward by peristalsis. It emerged as a diagnostic tool in humans to image segments of the digestive tract that are not visualized using conventional endoscopic investigation due to anatomical (distances from external orifices and length of the GIT) and physiological (peristalsis) constraints. The technique is also less invasive than intraoperative procedures such as exploratory laparoscopy where an endoscope is inserted through the abdomen to visualize the exterior SI surface. Capsule endoscopy has been successfully used in human medicine since the early 2000’s with a high adoption rate, where 91% of gastroenterologists in the United Kingdom reporting that they employed CE for investigating small intestinal diseases [3]. Capsule endoscopy has undergone extensive research and testing of its diagnostic yield and safety. With over 1000 peer-reviewed articles published, capsule endoscopy has broadly been deemed a safe, non-invasive procedure in humans with high diagnostic yield for obscure gastro-intestinal bleeding, detection of lesions in inflammatory bowel disease, Crohn’s disease, celiac disease and detecting tumours [3].

A meta-analysis reviewing 14 controlled CE studies with 396 procedures found that CE was superior for diagnosis pathologies associated with obscure gastro-intestinal bleeding over other modalities including barium radiography and push enteroscopy [11]. CE is also more sensitive in diagnosing obscure gastro-intestinal bleeding compared to computed tomography and magnetic resonance imaging [11]. CE had similar diagnostic yield to double-balloon enteroscopy which is more invasive and usually requires
sedation. That being said, advantages of double-balloon enteroscopy include the ability to directly evaluate and biopsy tissue and deliver targeted drug therapy [3], which is not yet possible using CE.

A second meta-analysis reviewed 9 studies (n=250) comparing the diagnostic yield of CE to small intestinal barium radiography for diagnosis of Crohn’s disease. CE had a diagnostic yield of 63% compared to 23% for barium [12]. It has also been shown to be a useful diagnostic tool to evaluate disease activity and progression [3]

CE is also capable of visualizing changes in mucosal integrity for patients suffering from celiac disease (89% sensitivity) [3]. Although direct duodenal biopsy remains the gold standard for diagnosing celiac disease, CE is able to visualize loss of mucosal folds and villous atrophy which are present in this condition [3].

1.2.2 Use of capsule endoscopy in animals

There have been very few studies completed on the clinical use of CE as a diagnostic tool in veterinary medicine. Dogs and pigs have been used as a model to test advances in CE technology such as the development of self-stabilizing capsules [13], self-propelling capsules [14], and a paddling-based capsule [15]. At this time, CE has been used in dogs as a research tool rather than for diagnostic purposes. This is likely due to the availability of other diagnostic modalities to image the GIT of dogs given their smaller size. CE has been used as an alternative to post mortem investigation for testing the efficacy and safety of veterinary products such as anthelmintics where a strong correlation was found between the worm counts viewed through CE and post-mortem [16].
1.2.3 Use of capsule endoscopy in horses

Pathologies of the equine GIT are similar to those diagnosed by CE in human medicine including gastro-intestinal bleeding from ulcers and lesions [17], inflammation of the mucosal lining [18-20] and tumors [18, 19, 21]; CE has the potential to aid in the diagnosis of these and a variety of other equine SI disorders (Table 1).

<table>
<thead>
<tr>
<th>Gastrointestinal Tract Disorders</th>
<th>Current Diagnostic Tool(s)</th>
<th>Potential Application for CE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulceration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gastric</td>
<td>• Gastroscopy</td>
<td>• image gastrointestinal bleeding and ulcers</td>
<td>[22]</td>
</tr>
<tr>
<td>- Intestinal Ulcers</td>
<td>• Duodenoscopy in foals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Necropsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Exploratory laparotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Duodenitis-proximal jejunitis</td>
<td>• Nasogastric intubation (gastric reflux)</td>
<td>• View reddened, thickening of intestinal lining</td>
<td>[23-25]</td>
</tr>
<tr>
<td></td>
<td>• Ultrasound (distension &amp; wall thickness)</td>
<td>• Visualize hemorrhages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Exploratory laparoscopy/laparotomy (biopsy to confirm)</td>
<td>• View reddened, thickening of intestinal lining</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fecal PCR test (low sensitivity)</td>
<td>• Future applications for diagnosis with biopsy capsule</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Necropsy (biopsy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Proliferative enteropathy (Lawsonia intracellularis bacterial infection)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumors</td>
<td>• Ultrasound</td>
<td>• View tumours within intestinal tract</td>
<td>[26-28]</td>
</tr>
<tr>
<td>- lymphosarcoma</td>
<td>• Exploratory laparotomy/laparoscopy</td>
<td>• Future applications with biopsy capsule to diagnose</td>
<td></td>
</tr>
<tr>
<td>- squamous cell carcinoma</td>
<td>• Gastroscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Necropsy

Table 1: Potential applications of capsule endoscopy in the horse

1.2.3.1 Gastrointestinal disorders in horses: colic and malabsorption syndrome

Colic and malabsorption syndrome are multifactorial disorders [18, 29, 30], where causes are numerous and varied and can present with similar clinical signs [18]. Hence, confirming a diagnosis is often difficult and expensive [7]. Colic is a catch-all term characterizing both acute and chronic “abdominal pain”, triggered most commonly by GIT disorders which can take place anywhere along the GIT as well as from the urogenital system [19]. Diagnosis often requires a thorough abdominal examination to pinpoint causality. On the other hand, malabsorption syndrome is often limited to the SI where the majority of nutrient absorption takes place [31].

Colic

Colic is commonly ranked as a top health issue by horse owners [7, 32] and was cited as the most common cause of death in horses [33]. Perception of colic as a health problem does not always correlate with the true incidence of colic. In an equine survey completed in Michigan, horse owners ranked colic as the most important health problem, although in the same study group, colic was reported as the seventh most common disease complex [7]. The disconnect between perception and incidence of colic demonstrates the impact of colic and its importance to the horse owner, which is most likely linked to the high mortality rate [7, 33] and financial cost [33] attached to colic.

The reported incidence of colic ranges from 3.5-10.6 colic events/100 horses [7, 10, 33, 34] all the way up to the 5-46 episodes/100 horse-years [35], with mortality rates ranging from 6.2% - 13% [7, 9, 10, 34]. The annual cost of colic in the US is estimated at $115
million, 66% ($76 million) of which is linked to mortality. Cost of veterinary services and lost days due to colic accounted for the remaining portion.

The large variation in reported colic incidence is likely due to differences in the population studied where individual horses, farm location and operator-level management all likely play a role. In studies where multiple farm locations were monitored, certain farms had a higher incidence of colic [10]. This highlights the multifactorial nature of colic and the association of risk factors such as feeding practices, parasite burden, stabling and stress that play a role in colic [29]. In addition to the multifactorial nature of colic, diagnosing the cause of a colic episode can be challenging. In many cases of colic, the underlying cause is unknown [29]. As such, “the true incidence of specific intestinal diseases causing colic in the general horse population is not known” [34], with the underlying cause of colic being undiagnosed in an estimated 60-85% of colic cases [9, 29, 36]. The lack of causative diagnosis in colic cases can be attributed to multiple factors including the wide range of causes that present with similar clinical signs [37] and relative inability to identify which segment of the digestive tract is affected [10]. An estimated 90% of colic cases are deemed medical or non-severe [37]. There is often a positive outcome following medical treatment for these cases and further investigation into causality is therefore often not warranted or is not performed due to the additional cost to the horse owner. As a result, a large portion of colic cases are categorized as medical/ spasmodic/ gas or unknown [10, 29, 35] and little is known about these more common types of colic [37]. That being said, owners of horses suffering from repeat episodes of colic, or recurrent colic, are likely to be keener to identify a cause in order to prevent future episodes.
It is well known that horses with a history of colic are at a higher risk of having another episode, especially those that have had abdominal surgery \cite{30, 38-41}. The terms recurrent and chronic colic are often used interchangeably; chronic colic refers to signs of colic observed continuously for multiple days \cite{18}, whereas recurrent colic is the return of signs of colic following a period of absence \cite{19, 41, 42}. In recurrent colic, these episodes can be mild or severe, whereas chronic episodes of colic tend to be milder in nature. For the purpose of this literature review, recurrent and chronic colic are lumped into the same category to differentiate these colic episodes from single acute episodes. Recurrent colic following abdominal surgery will not be covered in this literature review because it is unlikely that these cases can be aided by using CE.

The incidence of recurrent colic in the general horse population is not well reported. It is estimated that 10-19\% of all colic cases are repeat episodes where these horses will experience 2-4 episodes of colic per year \cite{9, 10, 40}. A recurrence rate of 50 colic events per 100 horses was recently reported in the United Kingdom where the first colic episode was a medical colic and had a fatality rate of 10.5\% \cite{42}. The recurrence rate of colic is 5x times higher than the incidence of all colic episodes in the general horse population \cite{7, 10, 33, 34} and fatality rate of the recurring episode is on the high end of the mortality range (6.2-13\%), demonstrating the more serious impact of recurrent colic. In the UK study, there were 54 recurrent episodes in 38 horses, demonstrating that some horses had 2 or more recurrent colic episodes \cite{42}. The study design by Hillyer, 1997, grouped horses based on the frequency of colic episodes within a given timeframe. They found that horses experiencing 3 or more episodes of transient colic within 1 month had the highest mortality rate of 53\%, whereas the group with 3 or more colic episodes within 1
year had the lowest mortality rate of 4%, and if the 3 or more colic episodes were prolonged (chronic) episodes over the 1 year timeframe, the mortality rate was 31% [41].

The difference in mortality rates among the different groups demonstrates that frequency of colic episodes is an important indicator for mortality, but it is still unknown whether the total number of colic episodes is linked to a higher rate of fatalities. It is also reported that 30% of colic cases entering hospitals are repeat colic cases [9], demonstrating the likelihood of recurrent cases resulting in a hospital stay.

The management and behaviour of horses can also impact the rate of recurrence [43]. As is the case with colic [44], the incidence of recurrent colic also varies among individual horses and at the farm level [39, 43]. It has been shown that horses that display wind-sucking and weaving behaviour as well as those with limited access to pasture and dental problems are at increased risk of recurrence [42, 43].

Chronic and recurrent colic can be frustrating in large part due to the low rate of establishing an underlying diagnosis [19]. In the study performed by Mair et al., 1997, up to 78.3% of chronic colic cases were diagnosed by using all available diagnostic tools including exploratory laparotomy [18]. This highlights the difficulty in diagnosing these chronic cases, which the authors attributed to the similarity of clinical signs as well as the limitation of diagnostic procedures. Both acute and chronic colic present with similar clinical signs and the diagnostic approach is very similar. Pinpointing causality in cases of recurrent colic can be difficult, requiring expensive procedures and the use of exploratory surgery with little chance of success [19]. A list of GIT disorders that can lead to repeat episodes of colic and their associated diagnostic procedures is presented in Table 2.
<table>
<thead>
<tr>
<th>GIT Disorders</th>
<th>Available Diagnostic Tools</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation/SI stenosis</td>
<td>- Rectal Palpation (at level of ileum)</td>
<td>[19, 45]</td>
</tr>
<tr>
<td></td>
<td>- Ultrasound</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Biopsy required for confirmation through histology</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>- Ultrasound</td>
<td>[45]</td>
</tr>
<tr>
<td>Intestinal Neoplasia</td>
<td>- Rectal Palpation (abdominal mass)</td>
<td>[19, 21]</td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Ultrasound (gastric)</td>
<td></td>
</tr>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>- Endoscope (stomach only)</td>
<td>[19, 46]</td>
</tr>
<tr>
<td></td>
<td>- Ultrasound</td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>- Explorative Laparotomy</td>
<td>[18, 41]</td>
</tr>
<tr>
<td></td>
<td>- Explorative laparoscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Post-Mortem</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Ultrasound</td>
<td></td>
</tr>
<tr>
<td>Enterolithiasis</td>
<td>- Radiography</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Parasitism</td>
<td>- Fecal Test</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>- Post-Mortem</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Gastroduodenal Ulceration</td>
<td>- Endoscope</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>- Radiography (foals)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Sand Accumulation</td>
<td>- Radiography</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Colonic Impaction</td>
<td>- Rectal Palpation</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Peritonis</td>
<td>- Abdominocentesis (analysis of peritoneal fluid)</td>
<td>[18, 45]</td>
</tr>
<tr>
<td></td>
<td>- Ultrasound</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Explorative Laparotomy</td>
<td></td>
</tr>
<tr>
<td>Chronic Colitis</td>
<td>- Clinical Features (diarrhea, weight loss, ventral edema)</td>
<td>[41, 45]</td>
</tr>
<tr>
<td></td>
<td>- Ultrasound</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Gastrointestinal tract (GIT) disorders linked to recurrent colic

**Malabsorption syndrome**

Similar to colic, unexplained weight loss can be caused by any number of underlying diseases (Table 3) which may be difficult and/or expensive to diagnose [47, 48]. The incidence within the equine population is currently unknown. In malabsorption syndrome,
weight loss can be attributed to a decrease in available nutrient absorption due to changes in the integrity of the mucosal lining of the SI [48, 49]. The intestinal mucosa contains brush border enzymes and absorptive cells that are essential for the digestion and absorption of nutrients [50]. In cases of malabsorption, inflammation and infiltration of immune cells results in a thickened intestinal wall [51], and distortion of normal villous architecture where the villi will appear broader, flattened and shorter [50]. Hemorrhaging may also be visible along the surface of the SI [50]. Horses that present with chronic weight loss may also experience recurrent episodes of colic, with or without diarrhea [31, 48, 52], and may have low levels of plasma albumin [31, 47]. If weight loss is the only clinical sign, the disease is likely limited to the SI [31]. That being said, there could be evidence of large intestinal dysfunction such as an increase in gas production and decrease in intestinal pH due to excessive bacterial fermentation from abnormal digestion of nutrients in the SI [31]. Diagnosing the exact cause behind the malabsorption can be quite challenging given the wide range of possible diseases (Table 3), limited access to the SI [53] and the inability to determine the exact location of the lesion [50]. In a retrospective study, 40% of cases of malabsorption syndrome were categorized as idiopathic due to a lack of diagnosis [48]. Having an underlying diagnosis would allow for targeted treatment and improved prognostication. For a high number of malabsorption cases, the underlying cause is diagnosed at post mortem [47, 48], which may be attributed to the high cost associated with specialized diagnostic tests as well as the invasive nature and surgical complications with exploratory laparotomy. A histological examination of small intestinal tissue is often needed to confirm the type and cause of inflammation which can only be obtained through exploratory laparotomy; however horses suffering from
malabsorption may not be good candidates to undergo this procedure due to complications that may arise during post-operative care due their catabolic and hypoproteinaemic state [31]. Rectal biopsies are used sometimes to diagnose inflammatory bowel disease [54], however these samples may not be representative of pathology in the SI as the histological lesions in malabsorption syndrome are often limited to the SI [31, 47, 54].

<table>
<thead>
<tr>
<th>Causes of malabsorption syndrome</th>
<th>Clinical Presentation *pathological findings are non-specific</th>
<th>Available Diagnostic Tool</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chronic inflammatory bowel diseases</td>
<td>- no diarrhea/colic - macrophages/ epitheliod cells - villous atrophy</td>
<td>biopsy and histopathological examination of affected tissue required for confirmation of IBD and differentiation of disease (biopsy obtained through exploratory laparotomy - gastroduodenal endoscope - rectal biopsy)</td>
<td>[49, 55]</td>
</tr>
<tr>
<td>a. Granulomatous enteritis</td>
<td>- eosinophils in all intestinal layers - mucosal ulceration - SI mucosal necrosis - can cause partial obstruction of lumen (linked to recurrent colic)</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>b. Idiopathic eosinophilic enterocolitis</td>
<td>- infiltration of immune cells in mucosa and submucosa</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>c. Multisystemic eosinophilic enterocolitis</td>
<td>- lymphocytes and plasma cell infiltrate lamina propria - villous atrophy</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>d. Lymphocytic enterocolitis</td>
<td>- focal tumour mass (intestinal wall) - villous atrophy - mucosal ulcers - luminal bleeding</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>2. Alimentary lymphosarcoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Abdominal Neoplasia</td>
<td></td>
<td>- Rectal Palpation - Ultrasound - Radiography - Nuclear Scintigraphy - Gastroscopy</td>
<td>[56]</td>
</tr>
<tr>
<td>4. Enteric Infections e.g. <em>Lawsonia intracellularis</em></td>
<td>-similar clinical infections to inflammatory disease - hypoproteinaemia - thickened sections of small in-</td>
<td>- Ultrasound - detection of <em>L. intracellularis</em> in feces</td>
<td>[55, 57]</td>
</tr>
<tr>
<td></td>
<td>Table 3: Common causes of malabsorption syndrome, clinical and pathogenic presentation and available diagnostic tools [31, 56]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Parasitism</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fecal analysis and recovery following anthelmintics treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**1.3 Diagnosis of equine gastrointestinal tract disorders**

The size and inaccessibility of the equine abdomen makes it a challenge to diagnose GIT disorders. Challenges related to the anatomy of the equine GIT will be described as well as the limitations of current diagnostic tools and the potential role for CE to aid in equine SI imaging.

**1.3.1 Anatomy of the equine gastrointestinal tract**

The horse has a very long digestive tract stretching from 24-30 m in length. It comprises the esophagus (1.2-1.5m), the stomach, the SI (24-26m) and the large intestine (LI) (4m) and ends at the rectum [4, 58]. A large section of the SI, specifically the jejunum, is located in the middle of the horse’s abdomen, which has a width of 80-100cm [4]. As a result, it can be a challenge to access the midsection of the horse’s abdomen to visualize many portions of the SI.

The equine foregut includes the mouth, esophagus, stomach and SI. The horse has a small stomach (10-15 litres) [4, 58] relative to its body size. The stomach is located dorsocranially on the left side of the abdomen [58], caudal to the diaphragm. It is unique in that the cardia (junction between the esophagus and stomach), is near the pylorus (opening to the SI), creating a challenge to reach the duodenum with an endoscope [4]. The SI comprises the duodenum, jejunum and ileum which are attached to the dorsal wall.
body wall by the mesoduodenum, mesojejunum and mesoileum, respectively [4]. The duodenum is 1m in length, starts at the pylorus of the stomach, and is situated dorsally on the right side of the abdomen, positioned near the liver [4, 58]. It is suspended by a short mesoduodenum to the dorsal body wall, limiting its movement. The descending duodenum runs caudally between the liver and right dorsal colon. At the caudal pole of the right kidney, it turns medially where it attaches to the base of the cecum and root of the mesentery [4, 58]. Moving from this position, the duodenum is known as the ascending duodenum as it runs cranially and crosses the medial plane to the left side of the abdomen where it is joined to the jejunum. The jejunum is the longest section of the SI, stretching 25m [4, 58]. It is anchored to the dorsal body wall by the mesojejunum. The mesojejunum can drastically increase its length by up to 50cm, allowing jejunal loops to be highly mobile within the abdominal cavity but can most often be found on left side [58]. The final section of the SI is the ileum which is 30-50cm in length [4, 58] and is secured via the ileocecal fold, also known as the mesoileum. In comparison to other segments of the SI, the ileum has a thicker muscular wall. The duodenum and jejunum cannot be easily differentiated by gross morphology; it is only through histological examination of villus structure that each segment can be identified. In the duodenum, villi will appear blunt and wide, whereas they are long and slender in the jejunum, and club-shaped in the ileum [4].

The hindgut of the horse includes the cecum, large colon (ascending colon), small colon (descending colon) and rectum. The LI is shorter in length than the SI but has a larger diameter that undergoes substantial variation along its length. The cecum is a comma-shaped organ, 1m in length, and lies on the right side of the abdomen [4]. The large colon
wraps around the outside of the abdominal cavity and is separated into 4 segments based on its position. The right ventral colon (diameter of 25cm) [4] starts at the cecum and runs cranially, where it will move medially forming the sternal flexure. From there, coursing caudally along the left side is the left ventral colon, also 25cm in diameter [4]. Caudally, the left ventral colon reaches the pelvic flexure, where it folds back on itself and has a reduced diameter of 8cm [4]. Moving cranially from the pelvic flexure is the left dorsal colon which has a diameter of 50cm [4, 58]. The cranial extent of the left dorsal colon forms the diaphragmatic flexure, from which it turns in the caudal direction and is known as the right dorsal colon. This will terminate at the transverse colon (8cm) [4], which is situated dorsally and connects the large colon with the ascending, or small colon. The small colon is approximately 3-4m in length and 8cm in diameter and terminates at the rectum [4].

1.3.2 Diagnostic tools for the equine small intestine

There are different levels of diagnostic testing that veterinarians will progress through as they proceed towards a diagnosis (Figure 1). As the diagnostic test becomes more specific, often the invasiveness of the procedure as well as the cost increases. The ability to diagnose disorders of the SI is also limited to the availability of diagnostic tools to reach this section of the GIT. Current diagnostic tools for SI include endoscopy, rectal palpation and ultrasound as well as exploratory surgery, each with their own set of limitations that is covered in more detail below.
As you move to the right, diagnostic procedures become more specialized, invasive and higher in cost.

Figure 1: Diagnostic procedure for the equine abdomen (adapted from Tamzli, 2006[47])

1.3.2.1 Physical Examination

The large size, generous rib cage, and thick body wall of horses make abdominal palpation nearly impossible. However, assessment of a horse with colic would always begin with a thorough physical examination. This includes assessment of the horse’s general demeanor, evaluation of the nares and oral cavity, examination of mucous membranes and capillary refill as a measure of hydration [59] as well as taking pulse, respiratory rate and rectal temperature [59]. Auscultation and percussion of the abdominal quadrants is also performed to assess borborygmi (intestinal sounds) as a measure of motility and gastrointestinal function [59].

1.3.2.2 Rectal palpation

Rectal palpation is part of the first line of diagnostic tools available to the general practitioner and is often useful in differentiating between a surgical and non-surgical colic case during an acute colic episode. However, only 30-40% of the horse’s abdomen is within reach from the rectum. The cranial portion of the abdomen cannot be palpated,
including stomach and proximal duodenum [60], therefore a lot of SI abnormalities may be missed [60, 61]. If the SI can be palpated during a rectal palpation examination, this would be an abnormal finding and indicative of an issue along the SI [59].

1.3.2.3 Endoscopy
As previously mentioned, an endoscope is a tiny camera affixed to a long flexible tube containing fibre optic cables that connects to a computer monitor where images can be viewed. The endoscope can be passed into various body cavities to view internal structures and as such, endoscopy has become an invaluable tool in assessing the GIT in human and veterinary applications. Typically, equine endoscopes measure 2.2-2.5m [6], which limits how much of the GIT may be visualized from either oral or rectal entry points. Using longer endoscopes (2.8-3.0m), a small section of caudal duodenum can be viewed and biopsied [60]. From the rectal entry point, endoscopy is limited in horses by the length of the endoscope and larger intestinal diameter, and therefore the colon is not routinely visualized. A smaller diameter endoscope (10mm) can be used in foals; however this equipment may not be widely available and visualization beyond the upstream duodenum is often not possible.

1.3.2.4 Ultrasound
Ultrasound is a non-invasive procedure that utilizes sound waves to generate images of internal structures through the skin. This provides a potential window to evaluate abdominal structures that are inaccessible to other diagnostic techniques [62]. It is commonly used in colic assessment, and is especially useful in cases where rectal palpation is not possible such as with ponies, foals, and miniature horses [63]. It is an integral component of a diagnostic workup to help differentiate between surgical and
non-surgical colic cases [62, 64] and can be more sensitive than rectal palpation [65]. Ultrasound is able to visualize portions of the SI, including the duodenum and some sections of the jejunum and the more peripherally located segments of the LI may also be assessed [62, 63]. Due to its short mesentery, the duodenum is in a fixed location and can be consistently imaged in three locations; however the ascending duodenum cannot be visualized [62, 63, 66]. Luminal contents, intestinal wall thickness, motility patterns and distention of the intestine can be assessed by ultrasound [62, 64]. These changes in intestinal morphology can be an indicator of disease [66] such as proximal enteritis, inflammatory bowel disease, muscular hypertrophy, and ileus. However ultrasonographic findings are often non-specific and therefore these conditions are confirmed through surgery or histological examination. Presumptive diagnosis via ultrasound often relies on improvement following medical therapy [64].

Ultrasound is a useful diagnostic tool to image the GIT of the horse, but it is not without its limitations. The maximum penetration depth depends on the specific probe selection, but for most units is 22-30cm [60]. Only structures that are adjacent to the body wall are imaged, therefore a large portion of the GIT remains invisible [62]. In addition, acoustic impedance of the ribs, lungs, and solid and gaseous large intestinal contents can prevent effective percutaneous imaging in various locations [61, 64].

1.3.2.5 Other imaging modalities

Abdominal radiography is not routinely performed in the adult horse to image the SI given their large body size [60, 65]. Specialized equipment capable of radiographing the abdomen is often only available in referral hospitals. Radiography has been useful to identify abnormal intestinal contents such as enteroliths and sand accumulation given the
sharp contrast between these abnormalities and surrounding tissue [61]. Radiography can be successfully used in the diagnosis of SI distention and enteritis in foals given their smaller size [60].

Three-dimensional imaging techniques such as computed tomography and magnetic resonance imaging are not used in the horse given the large size of the abdomen exceeding the gantry size of commercially available scanners [67].

1.3.2.6 Exploratory laparoscopy and laparotomy
Exploratory laparoscopy is sometimes used in cases of recurrent colic and persistent weight loss when other diagnostic modalities failed to yield a diagnosis [60, 68, 69]. In a retrospective review of 158 abdominal laparoscopies performed, 51% of the cases presented with recurrent colic, 13% with current colic and 17% with weight loss [68]. Gastrointestinal structures that are visible in a standing laparoscopy include the greater curvature of the stomach, duodenum, jejunum and small colon [69]. Biopsies can be collected from the descending duodenum and distal jejunum to undergo histological examination [70], whereby diagnosis can be confirmed in cases of IBD. As with other diagnostic tools, diagnosis can be limited to the accessibility of structures from the incisional entry point [60, 68]. Other limitations to this technology are the expensive cost of equipment and instrumentation, the technical expertise, surgical facilities as well as the risk for accidental penetration of abdominal organs [67, 68].

For a complete examination of the equine abdomen, diagnostic exploratory laparotomy is often necessary; it is still one of the most commonly used diagnostic tools, however, this procedure is much more invasive and does requires three months of recovery.
1.3.2.7 Rise of minimally invasive surgery in veterinary medicine

In veterinary medicine, as with human medicine, there is a rise in minimally invasive surgery due to lower morbidity levels. Consequently, there is an increased demand from clients\(^1\) for these types of procedures and CE could be employed by veterinarians as a minimally invasive diagnostic tool.

1.3.2.8 Capsule endoscopy as a potential diagnostic tool for equine SI

It is evident that despite the range of diagnostic tools to evaluate abdominal pain in cases of colic, there are regions in the equine abdomen that cannot be completely evaluated \[67\]. Capsule endoscopy is a minimally invasive procedure with low capital investment and can foreseeably be performed at the owner’s stable. It could be used as a specialized test (Figure 1) in cases where other diagnostic modalities failed to yield a diagnosis. It could be a useful diagnostic tool to help in the diagnosis of GIT disorders in its early stages by visualizing gross changes in villous structure, identifying lesions or ulcers and gastrointestinal bleeding. Advances in CE technology could include the ability to perform tissue biopsy which could serve as an alternative to laparotomy and laparoscopy. That being said, the tissue biopsy obtained through CE may not be a full SI wall tissue sample, but it may provide some insight into the disease process to help direct treatment.

1.4 Capsule endoscopes

1.4.1 Capsule endoscope technology

There are a wide range of commercially available capsule endoscopes, each with their own specifications, unique features and operating regions (Table 4). PillCam®,

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developed by Given Imaging, was the first capsule available on the market. Other companies soon followed with development of their own capsule including Endocapsule™ by Olympus and MiroCam® from Intromedic Co., Ltd. A new capsule, CapsoCam™, recently developed by CapsoVision has emerged with a unique method of operation.

During CE, the capsule is either swallowed or directly placed into the stomach or proximal duodenum using a specialized capsule delivery system. The capsule is resistant to digestive fluids and will passively move through the digestive tract via naturally occurring peristaltic movement. During its trajectory, the capsule will capture still-frame images and transmit the image data through the body where the ‘signal’ is picked up by an array of sensors that are fixed to the patient’s abdomen. This data is then recorded onto a receiver that is carried by the patient. The recorded data is then downloaded to a specialized software program for image analysis (Figure 2).

![Diagram of capsule endoscopy process]

*Figure 2: Transmission of images from capsule endoscope*

Each capsule contains an ‘imager’ which consists of a video chip, lens and an array of lights (light-emitting diode) to illuminate the intestinal lumen. The video camera is connected to a transmitter that transmits the images to the receiver located outside of the
body. There are a variety of transmission methods, but radiofrequency is the most commonly used in commercial capsules (Table 4).

<table>
<thead>
<tr>
<th>Specifications</th>
<th>PillCam® (Given Imaging)</th>
<th>PillCam Colon2® (Given Imaging)</th>
<th>Endocapsule™ (Olympus)</th>
<th>MiroCam® (Intromedic)</th>
<th>CapsoCam® (CapsoVision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions (mm)</td>
<td>11 x 26</td>
<td>11.6 x 32</td>
<td>11 x 26</td>
<td>10.8 x 24</td>
<td>11 x 31</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3.4</td>
<td>2.9</td>
<td>3.8</td>
<td>3.3</td>
<td>unknown</td>
</tr>
<tr>
<td>Field of View (°)</td>
<td>156</td>
<td>172</td>
<td>160</td>
<td>175</td>
<td>360</td>
</tr>
<tr>
<td>Camera</td>
<td>CMOS</td>
<td>2 x CMOS</td>
<td>CCD</td>
<td>CMOS</td>
<td>4 x CMOS</td>
</tr>
<tr>
<td>Lighting (LED lights)</td>
<td>4</td>
<td>2 x 6</td>
<td>6</td>
<td>6</td>
<td>16 (auto illumination)</td>
</tr>
<tr>
<td>Transmission</td>
<td>RF</td>
<td>RF</td>
<td>RF</td>
<td>HBC or Electrical Field Propagation</td>
<td>N/A (images stored on board on Flash storage device)</td>
</tr>
<tr>
<td>Battery Life (hours)</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Resolution</td>
<td>256 x 256</td>
<td>256 x 256</td>
<td>512x512</td>
<td>320 x 320</td>
<td>unknown</td>
</tr>
<tr>
<td>Image Capture Rate (frames/second)</td>
<td>2</td>
<td>4-35</td>
<td>2</td>
<td>3</td>
<td>12-20 (3-5 frames/second per camera)</td>
</tr>
</tbody>
</table>

*Table 4: Specifications of common commercially capsule endoscopes. CMOS= complementary metal oxide semiconductor; CCD = charged coupled device; RF = radiofrequency; HBC = human body communication CMOS= complementary metal oxide semiconductor; CCD = charged coupled device; RF = radiofrequency; LED = light-emitting diode*
Radiofrequency communication technology, used by Given Imaging and Olympus cameras, utilizes higher levels of energy to cross through body tissues as ionic material and electrons will block the radiofrequency signal [71] and requires the use of antennae. This higher power consumption not only reduces operation time but can lead to lower image resolution [72] and limits the number of lights positioned at the front of the capsule to illuminate the intestinal lumen. As a result, capsules utilizing radiofrequency technology may not be powerful enough for its signal to transmit across the horse’s large abdomen. Additionally, imaging the entire length of the equine SI may not be possible due to limited battery life. Two wireless capsule studies have recently been reported in horses with limited success [73, 74]. Both studies utilized wireless capsules that transmitted signal using radiofrequency technology. PillCam® was deployed with some success and imaged sections of the SI in horses including the duodenum and proximal jejunum; however imaging past the proximal jejunum was not possible due to battery life and lighting limitations when imaging larger luminal diameter [74].

SmartPill®, also manufactured by Given Imaging, communicates via radiofrequency and is not an imaging camera but was used to assess luminal pH, pressure and temperature from the entire GIT including the cecum and large colon. Ponies were used in this study instead of horses given their smaller size [73]. In a previous study, SmartPill® was deployed in horses and issues related to signal transmission were reported despite the addition of a booster antenna [73, 75]. Based on these two equine studies, it appears that capsules utilizing radiofrequency technology are likely not powerful enough to cross through the horse’s large abdomen.
Unlike PillCam® and SmartPill®, MiroCam® uses electric-field propagation, which uses the patient’s body as a conductive medium for data transmission, minimizing power consumption [72]. This should circumvent issues related to signal transmission across the horse’s large abdomen. In addition, MiroCam® has a higher image resolution (320x320 vs. 256x256) and image capture rate (3 frames per second vs. 2 frames per second) which should allow for better quality images. MiroCam® also has a longer battery life (11+ hours vs. 8 hours) which should allow the capsule to image the entire length of the equine SI. It also a wider field of view and two additional lights which will help illuminate sections of the GIT with larger diameters.

A new capsule, CapsoCam®, developed by CapsoVision has emerged with a unique method of operation and has recently received regulatory approval for commercial use. This camera pill has enhanced viewing capabilities allowing for a panoramic 360° view of the SI. A preliminary study in humans has shown promising results using the CapsoCam®, with the duodenal papilla visible in 71% of the patients compared to 18-43% of patients when using Given Imaging capsules [76]. Unlike other capsule cameras, image data is stored on board within the capsule; data is not transmitted across the body which eliminates issues related to transmission, however the capsule must be retrieved for image analysis. This may be difficult to do in the horse given that the capsule may be retained in the horse’s cecum and LI for a long period of time and would require sifting through large amounts of manure. In addition, radiography is often used in humans to locate retained capsules within the GIT and this would be difficult to perform in the horse given limitations of radiography as described above.
MiroCam® capsule has never been investigated in the horse. With its novel method of data transmission, this capsule may be more amenable for use in the horse. It is also expected that imaging the entire length of the equine SI is possible given its longer operation time.

1.4.2 MiroCam®

MiroCam®, manufactured by Intromedic Ltd.Co. in South Korea, is the smallest capsule currently available on the market and has the largest field of view (Table 4). It utilizes electrical field propagation, whereby the capsule generates a low frequency current (1-3 MHz), transmitted through the body tissues that is picked up external sensor electrodes [71, 72]. The CE image sensor converts the optical rays into electrical voltage which is then transmitted by gold plated bands (Figure 3) on the outside of the capsule as a low power electrical field [77]. This electrical signal is picked up by external electrodes placed on the patient’s skin. Electrical field propagation utilizes the body tissues as a semi-conductor and requires direct contact between cellular tissues and/or bodily fluids to transfer the electrical signal. This low-voltage transmission requires less power than radiofrequency transmission technology used by PillCam® and EndoCapsule™ cameras, which allows for longer battery life [71], smaller capsule as well as more free space within the capsule for further modifications.

Figure 3: MiroCam® capsule components [71]. 1. optical dome; 2. lens; 3. LED lights; 4. CMOS Image Sensor; 5. Battery: silver oxide 6. gold plating
The first human pilot trial using MiroCam® showed positive results with a mean recorded time of 9 hours and 51 minutes and a SI transit time of 4 hours and 33 minutes. The transmission rate in the stomach was 99.5%, 99.6% in the SI and 97.2% in the large intestine [71]. There are a limited number of studies conducted to evaluate MiroCam® in a clinical setting. The majority of CE research to date has focused on PillCam® as it was the first capsule available on the market and has set the standard for CE. Most studies evaluate the diagnostic concordance between different capsules and MiroCam® has been shown to have longer duration of recording which translated to a higher rate of examination completion (Table 5). It has a similar diagnostic yield to both PillCam® and EndoCapsule™ and has been to shown to have a higher diagnostic yield for obscure gastrointestinal bleeding [78], demonstrating that MiroCam® capsule is equivalent to other capsules on the market.

<table>
<thead>
<tr>
<th>Specifications</th>
<th>PillCam®</th>
<th>MiroCam®</th>
<th>Endocapsule™</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Recording</td>
<td>7.4hrs ± 28 min</td>
<td>11.7hrs ± 1</td>
<td></td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>11.7hrs ± 56 min</td>
<td>9.63hrs ± 53 min</td>
<td></td>
<td>[80]</td>
</tr>
<tr>
<td>Rate of Completion</td>
<td>58.3%</td>
<td>83.3%</td>
<td></td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>96%</td>
<td>90%</td>
<td></td>
<td>[80]</td>
</tr>
<tr>
<td>Intra-capsule agreement (k= kappa coefficient)</td>
<td>k= 0.74</td>
<td></td>
<td></td>
<td>[77, 79]</td>
</tr>
<tr>
<td></td>
<td>k=0.66</td>
<td></td>
<td></td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>k=0.5</td>
<td></td>
<td>[80]</td>
</tr>
</tbody>
</table>

Table 5: Comparison of commercial capsule endoscopes
1.4.3 Assessment of image quality

Diagnostic yield is used when comparing CE to other diagnostic tools and comparison among different capsule endoscopes on the market [77, 79]. Assessment of image quality in CE is performed during comparison of patient preparation protocols [81, 82]. Images are commonly scored by blinded examiners using different parameters including the proportion of intraluminal fluid, the presence of gas or air bubbles, and the percentage of visible mucosal surface [82]. At the moment, there are no scoring criteria standards for assessing image quality of CE [81-83], and scoring is based on visual observation. Quality is typically assessed as ‘adequate/good’ vs. ‘inadequate/moderate/bad’ based on different scoring categories used by different researchers [81-85], making it difficult to compare results from studies. The lack of a standardized definition for “image quality” may also allow researcher bias.

1.5 Patient preparation protocols

In order to obtain clear images during CE, intestines needs to cleansed and void of any food debris that can obstruct visibility. In humans, different bowel cleansing protocols have been proposed; however due to unique physiological and anatomical features of the equine digestive tract, the human protocol is not suitable for application to horses. As a result, a protocol specific to the horse is being suggested in Chapter 2. The novel protocol is based on a review of the literature on human preparation protocols, equine GIT motility, transit times, fasting and the effect of drugs on GIT as presented below.

1.5.1 Human bowel clearing protocol

Proper bowel preparation is essential for optimal viewing of the mucosal lining by eliminating food debris, bubbles and dark intestinal contents that can obstruct the field of
visibility, whereby reducing diagnostic yield [81, 82]. In people, an overnight fast of 8 - 12 hours, with or without consumption of clear liquids, is recommended to clear the SI. The use of purgatives (laxatives) such as polyethylene glycol (PEG) and sodium phosphate, cathartics such as magnesium sulphate and prokinetic drugs such as metoclopramide are employed to help clear the SI [81, 82]. One drawback to purgatives is that they are not well tolerated by patients [85], likely due to associated side effects including bloating, cramping and gas. Simethicone is a detergent and when provided 20 minutes prior to delivery of CE has been shown to improve visibility by eliminating bubbles from intraluminal gas by disruption their surface tension [86].

To date, all of the studies evaluating preparation protocols use PillCam®, however these protocols are similar regardless of capsule type. That being said, MiroCam®’s communication technology is reliant on proper hydration and patients are encouraged to consume water on a regular basis to increase effectiveness of the procedure [87].

1.5.2 Challenges of clearing the equine digestive tract

1.5.2.1 Equine stomach

The equine stomach is unique in that it is separated into a glandular section, capable of secreting mucus, and a non-glandular section that does not produce mucus and is susceptible to acidic erosion [88]. Unlike humans, the equine stomach constantly secretes acid, which has been proposed to be an evolutionary adaptation due to the horse’s natural trickle feeding system where they are constantly consuming feed [88]. As a result, horses are prone to gastric ulceration, particularly during periods of feed withdrawal. Gastric ulcerations can develop in as little as 24 hours of feed deprivation [89, 90]. The
requirement for longer periods of fasting to empty the longer length of the equine small intestine, coupled with the risk of gastric ulceration poses a challenge to safely clearing the horse’s digestive tract prior to CE. Provision of antiulcer medication, such as omeprazole, is routinely provided to horses for the treatment and prevention of gastric ulcers by decreasing the level of acid production in the stomach [88, 91] and may be used to protect the horse’s stomach during the CE preparation stage.

1.5.2.2 Fasting

Horses are often fasted to improve visualization for diagnostic procedures and to reduce anesthetic risks. An overnight fast of 12 hours is routinely performed prior to gastric endoscopy [60], whereas withholding of feed for 36-48 hours is often required for duodenoscopy and exploratory laparoscopy [60, 67]. In previous wireless capsule studies, horses were fasted for 12-24 hours [73, 74].

Deprivation of feed has been shown to alter GIT motility [92]. A lower number of contractions in conjunction with a decrease in intensity were observed in horses fasted for 20 hours compared to those that were fed [92]. Similarly, a decrease in myoelectric activity at the pelvic flexure was seen in horses fasted for 12 hours compared to fed horses [93]. During fasting, SI motility may be reduced due to the lack of gastric distension that triggers the vagovagal reflex [94] and gastro-ileal complex [95]. The vagovagal reflex controls peristaltic waves in the SI, whereas the gastro-ileal reflex controls ileal motility and increases the rate of passage from the ileum into the cecum. Lack of SI motility will impede clearance of the intestinal tract during the preparation phase as well as peristaltic movement during CE that is necessary to keep the capsule
moving forward during the recording phase. Therefore emptying the horse’s digestive tract, while maintaining motility, is a considerable challenge.

1.5.2.3 Small intestinal motility and transit time

The equine GIT is a highly complex organ where intestinal motility is controlled by myoelectric, neural and humoral factors [92, 96]. Small intestinal motility patterns are quite similar across most species; however the horse has a few unique features. Understanding SI motility and transit times in the horse will help develop an appropriate procedure for safely emptying the horse’s SI prior to CE.

The terms used in the next section are defined as follows: gastric emptying is the process by which solids and liquids are passed from the stomach to the SI through the pylorus. Gastric emptying rate is the time elapsed from the arrival of the food in the stomach to when it exits the stomach. Transit time is the amount of time it takes for food material to travel a specific distance. Mean retention time is the average amount of time the food material is retained within a segment of the GIT.

The proximal duodenum has the highest motility of 30cm/minute and contractions will decrease to 14-15 movements/min in the distal duodenum and 10-11 movements/min in the ileum [95]. Small intestinal contents move aborally through slow and rapid contractions. These contractions are triggered by myoelectrical activities which include slow continuous waves generated by interstitial cells of Cajal [97] and by the cyclical pattern of the migrating motor complex (MMC). Migrating motor complex consists of three phases: Phase 1 when there is little contractile activity (no action potentials), Phase 2 in which there is intermittent action potentials and Phase 3 where there are continuous
intense action potentials [98]. In the horse, one MMC cycle lasts for approximately 2 hours [98] and begins in the upper duodenum [99]. Unlike humans and dogs, Phase 3 in the horse is considerably longer and MMC continues all the time [96, 98, 99] which is likely related to the horses’ continuous feed intake. The continuous MMC motility pattern in the horse may help clear the SI during fasting. However it is important to note that myoelectric activity does not always correlate with mechanical activity and transit time [96].

**Gastric emptying**

Gastric emptying and SI motility are interrelated processes. Gastric emptying is controlled by a number of reflexes including the enteric reflex arc, whereby duodenal receptors will relay information back to the stomach to control gastric emptying [100]. The vagovagal reflex, initiated by stretch receptors in the gastric mucosa, will control gastric emptying and smooth muscle contraction in the SI [94]. Providing enteral fluid may result in gastric distension which can stimulate the vagovagal reflex. Enteral fluid therapy (EFT) will be covered in more detail in the hydration section. Gastric emptying is also influenced by many different factors including feed composition, volume, viscosity and particle size [94] that will be discussed in more detail below.

**Transit time**

Transit times through the equine GIT vary widely depending on the segment of the digestive tract, feed properties as well as inter and intra-horse variation [95, 101]. On average, gastric emptying is 2-6 hours and feed can reach the cecum in 3-5 hours [95], whereas it has a longer retention time (35 hours) in the hindgut [101, 102]. The bulk of research in this field measures transit time along the entire GIT; it does not differentiate
between SI and LI due to the difficulty of performing this type of research in horses [103]. In addition, mathematical models used to estimate passage rates of digesta in the horse do not independently measure SI transit time [104]. As a result research on SI transit time is fairly limited in the horse. Recently, the use of the lactose $^{13}$C-ueirde breath test has been successfully used to measure SI transit time in horses. Mean transit time of a small meal (250g) was 3.24-5.24 hours in horses following a 14 hour fast [105]. The recent study using SmartPill® assessed SI transit time of a wireless capsule in ponies following a 12 hour fast. The mean capsule SI transit time was 4.6 hours, however a large variation in transit times was observed among the ponies (range of 2.4 – 7.6 hours) [73].

**Effect of feed-related factors on transit time**

As mentioned, there are a variety of feed-related factors that influence transit time through the stomach and SI (Table 6 &7). Generally, liquid components of the ration will move more quickly than solid particles through the stomach and SI [95]. Higher moisture feeds, such as grass, will also have a faster passage rate, whereas feeds with a higher water holding capacity such as hay will have a slower passage rate due to its ability to absorb water and retain it within the lumen [106]. Smaller particles have been shown to have slower passage rate in the hindgut, however feed with a reduced fibre length has a faster transit time [107]. In addition, a ration with a high forage:concentrate ratio will have a faster transit time [108]. The effect of meal frequency on transit time is inconclusive; ponies with a restricted diet have the slowest transit time [109], whereas other studies have found no difference in transit time based on feeding frequency [110, 111].
### Gastric Emptying Rate (GER)

<table>
<thead>
<tr>
<th>Feed-related factors</th>
<th>Gastric Emptying Rate (GER)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Solid vs. Liquid Material</td>
<td>- liquid portion will have a higher GER than solid particles (humans)</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td>- liquids will have higher GER than solids</td>
<td></td>
</tr>
<tr>
<td>2. Meal Composition</td>
<td>- higher fat content decreases GER (foals)</td>
<td>[94, 113,</td>
</tr>
<tr>
<td></td>
<td>- higher starch: fibre ratio (higher caloric content) has slower GER</td>
<td>114]</td>
</tr>
<tr>
<td></td>
<td>- no difference in GFR between carbohydrates and fat</td>
<td></td>
</tr>
<tr>
<td>3. Meal Volume</td>
<td>- GER is independent from meal volume (foals)</td>
<td>[113, 114]</td>
</tr>
<tr>
<td></td>
<td>- Larger meals have slower GER</td>
<td></td>
</tr>
</tbody>
</table>

*Table 6: Effect of feed-related factors on gastric emptying rate (GER)*

### Effect on Transit Time

<table>
<thead>
<tr>
<th>Feed-related factors</th>
<th>Effect on Transit Time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Feed Particle Size</td>
<td>- No difference between ground/pelleted hay vs. chopped hay</td>
<td>[101, 115,</td>
</tr>
<tr>
<td></td>
<td>- ground hay or crushed hay had faster transit time than long-stemmed</td>
<td>116]</td>
</tr>
<tr>
<td></td>
<td>- reduction of feed particle size increases hindgut retention time; slower transit time</td>
<td></td>
</tr>
<tr>
<td>2. Fibre Content</td>
<td>- high fibre diets had faster transit time than low fibre diets (based on varying alfalfa:oat straw ratio)</td>
<td>[106, 108]</td>
</tr>
<tr>
<td></td>
<td>- higher hay:grain ratio, faster transit time</td>
<td></td>
</tr>
<tr>
<td>3. Fibre Length</td>
<td>- shorter fibre length has lower mean retention time; faster transit time</td>
<td>[107]</td>
</tr>
</tbody>
</table>
### Table 7: Effect of feed-related factors on transit time

<table>
<thead>
<tr>
<th>Horse-related factors</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Equid Type</td>
<td>- donkeys have longer transit time compared to ponies and thoroughbred horses</td>
<td>[106]</td>
</tr>
<tr>
<td>2. Exercise</td>
<td>- higher transit time of solid phase</td>
<td>[108]</td>
</tr>
</tbody>
</table>
| 3. Water-Holding Capacity of Plant | - fresh grass has faster transit time compared to hay  
- alfalfa has faster transit time than straw | [95]     |
| 4. Feeding Level      | - longer transit times when alfalfa was restricted vs. provided ad lib  
- no change in transit time at different frequencies of feeding | [109, 110] |

It is difficult to draw conclusions on the effect of any one factor since the research that has been done is often contradictory due to differences in study design such as use of different types of feed, feed markers and horses used [101, 117]. In addition, the majority of research measures transit time across the entire GIT where behaviour and passage of feed through the hindgut is quite different than in the SI. Therefore, it is difficult to assess the effect of different feed factors on SI transit time.

Fasted horses have been reported to have a decrease in myoelectric activity at the level of the pelvic flexure [93] as well as a reduction of gastrointestinal sounds which may be indicative of a decrease in contractions [92], suggesting that horses in a fasted state may have altered GIT motility that could impact transit time. It is unknown whether restricting the horse’s ration will influence GIT motility [110]. In addition, given that transit studies are routinely performed in fed horses, we cannot assume that transit of different feed
factors will be the same in the fasted state. This will be important when developing the refeeding protocol following the delivery of the capsule.

1.5.2.4 Maintaining hindgut microbial population

Horses are hind-gut fermenters and rely on a healthy microbial population in the large intestine to properly digest fibrous feedstuffs. Changes in the microbial population can result in GIT dysfunction and higher risk of colic [38]. In order to maintain the microbial population, horses need a consistent supply of fibrous feedstuff (minimum of 1% of the horse’s bodyweight per day) and ration changes must be gradual [118]. These factors also need to be taken into consideration when designing the CE preparation protocol for clearing intestinal contents of the horse.

1.5.2.5 Hydration

Proper water intake is essential when preparing horses for CE to help clear the digestive tract, and to maintain hydration prior to and during the imaging process to ensure optimal signal transmission from the MiroCam® capsule. Humans can be encouraged to drink more water, but horses consume water on a voluntary basis. Water intake in horses is linked to dry matter (DM) intake (2L/kg of DM) [118]. When feed is reduced during fasting, water intake will decrease proportionally. Based on anecdotal evidence, horses will also reduce or stop drinking during fasting, upon moving to a new facility or even exposure to a different water source. As a result, a consistent water source should be provided and intake closely monitored to ensure adequate hydration. Providing electrolytes has been shown to increase water intake in exercising horses [119] and may be useful to encourage drinking.
Enteral fluid therapy, in which fluid is provided via nasogastric intubation directly into the stomach, has been used to maintain hydration in cases of adipsia, or loss of thirst [120] and may be useful during fasting when water intake is expected to decrease. It is often provided in cases of impaction colic in an attempt to hydrate ingesta within the digestive tract due rapid movement of fluid from the stomach to the cecum [121]. Ninety percent of fluid can be emptied from the stomach within 15 minutes [122] and reach the cecum between 30 minutes to 2 hours after administration [123, 124]. This rapid transit time of fluid through the SI may lend enteral fluid therapy as a method to help clear the digestive tract while maintaining hydration. By providing fluid directly into the stomach and causing gastric distention, it might trigger the gastrocolic response and increase colonic and intestinal motility [120]. An increase in GIT motility has been reported following enteral fluid therapy in horses [125]. To date, there has been no research that directly measured SI motility in horses after enteral fluid therapy.

On average, water intake for non-exercising horses will range from 55.1-73.2 ml/kg of body weight/day [120], or 5.5-7.32L per 100kg/bodyweight through consumption of water and moisture content in feed. For an average 500 kg horse, this translates to 27.5-36.6 L/day. To maintain hydration, it has been suggested to provide enteral fluid therapy at a rate of 1L/hr [126] with boluses of 8-10L every 2 hours being well tolerated in horses [124]. Some horses have shown abdominal discomfort when enteral fluid therapy was provided at a rate of 10L/hr for 6 hours [121] which was likely due to excessive gastric distention. Balanced electrolyte solutions are often the fluid choice for enteral fluid therapy [124], however tap water can be used [127]. Given that tap water is hypotonic, it can be given in small volumes over the course of one day [127], provided that the horse is
not dehydrated and does not have any electrolyte imbalances [124]. In addition, gastric emptying following enteral fluid therapy is not affected by temperature, tonicity or glucose content of fluids [122].

1.5.2.6 Effect of exercise on motility

Hand-walking is a common strategy used to promote motility in horses [128] even though there is a lack of supportive evidence. Light exercise, such as hand walking, has been suggested to stimulate intestinal motility in cases of equine grass sickness [129]. It is also commonly part of the post-operative care following SI resection [130] and laparotomy [131].

Stabled horses have been shown to have reduced large intestinal motility compared to those at pasture [132], suggesting that motility may be linked to activity level given that pastured horses are frequently moving, however this may also be linked to higher feed intake as they are constantly grazing. In addition, transit time of solid particles is faster in exercising than non-exercising horses, [108, 133], suggesting that activity level is linked to motility. That being said, one cannot extrapolate results from these studies since the direct effect of exercise on motility was not assessed.

Although there is little evidence supporting the relationship between exercise and motility, hand-walking may be useful to promote the transit of solid feed particles during the SI clearing phase. It may also help stimulate motility, particularly during periods when intestinal motility is suspected to be low.
1.5.3 Effect of drugs on GIT

1.5.3.1 Drugs to promote SI motility for bowel preparation

A variety of drugs are used in human and equine medicine to promote GIT motility in an effort to clear and/or hydrate intestinal contents. Cathartics and prokinetic drugs are commonly used in human bowel preparation prior to CE and colonoscopy. As these procedures are not routinely performed in equine medicine, these drugs have not been employed for this purpose and therefore will be reviewed based on their potential use for CE.

1.5.3.2 Cathartics

In horses, cathartics are often used to treat large intestinal impactions by increasing the amount of water in the digestive tract or promoting transit of digesta [134]. The most commonly used osmotic carthartics are magnesium sulfate and sodium sulfate [134]. Magnesium sulfate, more commonly known as Epsom salts, is believed to osmotically retain and draw water into the digestive tract due the presence of magnesium ion, which is poorly absorbed by the gut [135]. There is some debate whether the increase in transit is a response to the intragastric infusion of fluids used to dissolve the magnesium sulfate that triggered GIT reflexes through distension of the stomach [135] or whether it is the osmotic shift caused by the magnesium ion. High doses of magnesium sulfate can lead to magnesium toxicosis [128] and can cause significant water shifts [134], leading to dehydration. It can also cause enteritis [135-136] and is not recommended for horses suspected to have mucosal injury [136]. Given the low efficacy and potential risk, magnesium sulfate may not be appropriate to clear the SI in horses undergoing CE.
Laxative polyethylene glycol is often used in preparation for human CE, however it is infrequently used in horses and has not been thoroughly researched [128].

1.5.3.3 Prokinetic drugs

In horses, prokinetic drugs are used to promote motility in cases of ileus [96, 137]. There are a variety of prokinetic drugs available (Table 8), each with their own mechanism of action that act on different regions of the digestive tract. Metoclopramide is commonly used to stimulate motility in the upstream GIT [96], including the pylorus, proximal duodenum and mid-jejunum [137]. Metoclopramide is not very specific and can target a wide range of enteric receptors which may lead to other effects [137]. In addition, it has been shown to produce extra-pyramidal effects in horses due to its ability to cross the blood brain barrier and its anti-dopaminergic properties [96, 137]. Cisapride functions similarly to metoclopramide, but has been associated with cardiac arrhythmias in humans [138] and is no longer available on the market. Erythromycin stimulates motilin receptors located throughout the SI, in the cecum and pelvic flexure, causing an increase in the periodicity of the migrating motor complex [137, 138]. Lidocaine has also recently been used to stimulate GIT motility in cases of ileus, however research on its effectiveness and mechanism of action is limited [96, 138].

<table>
<thead>
<tr>
<th>Prokinetic Drug</th>
<th>Target</th>
<th>Mechanism of Action</th>
<th>Effect</th>
<th>Side Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoclopramide</td>
<td>- Stomach (pylorus) - proximal duodenum - mid-jejunum - brain</td>
<td>• 5-HT4 agonist • 5-HT3 antagonist • Dopamine antagonist</td>
<td>↑ gastric and SI motility</td>
<td>extra-pyramidal side effects (transient excitement)</td>
<td>[96, 137, 138]</td>
</tr>
<tr>
<td>Bethanechol</td>
<td>Stomach</td>
<td>• Muscarinic receptor</td>
<td>↑ GER</td>
<td>mild abdominal</td>
<td>[59]</td>
</tr>
</tbody>
</table>
Despite the extensive amount of research that has been done to understand equine motility and the enteric nervous system, the efficacy of prokinetic drugs in horses is still not well understood. Extrapolation is often made from other species, particularly humans, and there could be differences in enteral receptors which may result in altered effects in horses [137].

Given the uncertainty and the associated risks of prokinetic drugs in horses, a natural protocol to promote intestinal motility for CE may be more effective and safer for the horse. The effectiveness of cathartics such as magnesium sulfate to help clear the digestive tract is questionable and may cause further issues in horses undergoing CE, particularly if they have mucosal damage. Stimulating the natural reflexes of the GIT through provision of water boluses may be sufficient to promote intestinal motility while clearing intestinal contents.

**1.5.3.4 Effect of sedatives on motility**

Sedation is often required when performing routine veterinary procedures to improve patient compliance and minimize risk to attending physicians. The use of sedation will be

<table>
<thead>
<tr>
<th>Drug</th>
<th>Antagonist</th>
<th>Effect</th>
<th>Pain</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>- SI Cecum Pelvic Flexure</td>
<td>• Motilin Receptors</td>
<td>↑ GER</td>
<td>clostridial colitis, hyperthermia diarrhea [59, 137]</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>SI</td>
<td>Unknown</td>
<td>↑ motility</td>
<td>Little research [96]</td>
</tr>
<tr>
<td>Cisapride</td>
<td>SI</td>
<td>• 5-HT4 agonist</td>
<td>↑ gastric and SI motility</td>
<td>Cardiac arrhythmias – effects (no longer available in Canada) [96, 138]</td>
</tr>
</tbody>
</table>

Table 8: List of small intestinal prokinetic drugs used in horses. GER = gastric emptying rate; SI = small intestine.
needed to deliver the capsule into the horse’s GIT, however the use of these drugs needs to be done cautiously as they negatively impact GIT motility.

The effect of sedatives on GIT motility has been well researched given the use of these drugs in colic cases and their associated negative effect on GIT motility (Table 9). Xylazine and detomidine are both alpha-2 adrenergic agonists; however detomidine is more potent in suppressing motility, particular at the level of the duodenum [96]. Xylazine has a minimal and transient effect on duodenal motility [96, 139]. In fasted horses, xylazine (0.5mg/kg) appears to reset the duodenal MMC [140], and mildly disrupts GIT motility for 30 minutes following IV administration [140]. When xylazine is combined with butorphanol, there is a more pronounced reduction in duodenal motility [98]. In comparison to xylazine, detomidine is a more effective sedative and analgesic [141], but it also significantly suppresses GIT motility by decreasing the frequency of duodenal contractions for one hour following IV administration (0.0125 mg/kg) [140].

Xylazine increases GER of solid particles [142], whereas it decreases liquid GER [143]. When detomidine and butorphanol are combined, there is a marked decreased of GER of solid particles [142].

Given the suppressive effects of sedation drugs, careful consideration of the type of drug must be taken when using these drugs prior to capsule endoscopy.
### Table 9: Common sedatives used in horses, their mechanism of action and associated side-effects on motility. GER = gastric emptying rate; SI = small intestine.

<table>
<thead>
<tr>
<th>Sedative</th>
<th>Target</th>
<th>Mechanism of Action</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylazine</td>
<td>Stomach SI</td>
<td>α-2 adrenergic agonist</td>
<td>↑ GER (solid particles)</td>
<td>[139, 140, 142, 143]</td>
</tr>
<tr>
<td>(0.05 mg/kg)</td>
<td></td>
<td></td>
<td>↓ GER (liquid)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ SI motility</td>
<td></td>
</tr>
<tr>
<td>Detomidine</td>
<td>Stomach SI</td>
<td>α-2 adrenergic agonist</td>
<td>↓↓ GER (solid)</td>
<td>[139, 140, 142, 143]</td>
</tr>
<tr>
<td>(0.01 mg/kg)</td>
<td></td>
<td></td>
<td>↓↓ SI motility</td>
<td></td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Stomach SI</td>
<td></td>
<td>↓ GER (additive effect with detomidine)</td>
<td>[139, 142]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ SI when combined with xylazine</td>
<td></td>
</tr>
</tbody>
</table>

1.5.4 Previous protocols used in equine wireless capsule studies

A summary of capsule protocols employed in humans and horses are listed in Table 10. In the PillCam® study, horses were fasted for 24 hours with water being withheld 12 hours prior to CE procedure. Magnesium sulfate (Epsom salts) was given 16 hours prior to CE in an attempt to cleanse the SI. Since SmartPill® is not an imaging capsule, clearing the digestive tract is not relevant, however, protocols are included as a reference since it is the only other equine capsule study and provides insight into the behaviour and transit of wireless capsules in horses. In humans, protocols for the MiroCam® include a 12 hour fast and provision of polyethylene glycol and simethicone to improve visibility. The suggested equine protocol for the upcoming equine MiroCam® trial is listed in Table 10, so a direct comparison can be made with other protocols. Details of the protocol will be covered in Chapter 2.
<table>
<thead>
<tr>
<th>CE protocols</th>
<th>Equine - PillCam® [74, 87]</th>
<th>Equine - SmartPill® [73]</th>
<th>Human - MiroCam®</th>
<th>Equine - MiroCam® (current study proposal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Period (hours)</td>
<td>24</td>
<td>3 day reducing ration + 12 hour fast</td>
<td>12 (light lunch the day before CE)</td>
<td>7 day reducing ration + 12 hour fast</td>
</tr>
<tr>
<td>Water Fasting</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Purgatives</td>
<td>N/A</td>
<td>N/A</td>
<td>2L PEG (12 hours prior to CE)</td>
<td>N/A</td>
</tr>
<tr>
<td>Cathartics</td>
<td>1 g/kg magnesium sulfate in 4L water (16 hours prior to CE)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Detergents</td>
<td>N/A</td>
<td>N/A</td>
<td>Simethicone (20 mins prior to CE)</td>
<td>N/A</td>
</tr>
<tr>
<td>Water reintroduction</td>
<td>2</td>
<td>N/A</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>(hours after capsule delivery)</td>
<td></td>
<td></td>
<td></td>
<td>(always have access to water)</td>
</tr>
<tr>
<td>Feed reintroduction</td>
<td>4</td>
<td>Immediately following capsule delivery</td>
<td>4 (light meal)</td>
<td>3 (light meal)</td>
</tr>
<tr>
<td>(hours after capsule delivery)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteral Fluid Therapy</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>5L water bolus every 2 hours during recording stage</td>
</tr>
</tbody>
</table>

*Table 10: Comparison of protocols used in equine wireless capsule studies and suggested protocols for MiroCam® in horses*
1.6 Research Rationale

There is a need for a practical, minimally invasive imaging tool to view the inside of the horse’s SI. Capsule endoscopy has the potential to help in the diagnosis of SI disorders by imaging the gastrointestinal mucosa to detect findings such as bleeding, ulceration and inflammation. The MiroCam® capsule offers a novel method of data transmission that is more amenable to the horse’s large abdomen and has not previously been assessed in horses.
CHAPTER 2: Assessment of capsule-endoscopy technology as an imaging tool for the equine small intestine

2.1 Introduction

Malabsorption syndrome, causing persistent and unexplained weight loss, as well as recurrent and chronic colic can be caused by any number of underlying diseases associated with the SI [19, 31]. These disorders are multifactorial in nature [18, 29], where causes are numerous and varied [144] and present with similar clinical signs [18]. As such, confirming a diagnosis can be difficult and expensive [7, 18]. Moreover, current diagnostic tools such as ultrasound, endoscopy, radiography and rectal palpation are not able to reach portions of the small intestine (SI) due to the horse’s large abdomen [60, 62, 67, 68]. Therefore, more invasive procedures such as exploratory surgery may be necessary to access sections of the SI [67, 68] for diagnostic purposes.

Capsule endoscopy (CE), successfully used in human medicine, is being proposed as a novel, minimally invasive tool to image the inside of the equine SI to aid in the diagnosis of SI diseases. A capsule endoscope is an ingestible, disposable camera that images the inside of the digestive tract as it is propelled forward by peristalsis [3]. In humans, CE has a high diagnostic yield for investigating pathologies associated with SI diseases such as obscure gastro-intestinal bleeding, inflammatory bowel disease, Crohn’s and celiac disease as well as detecting tumours [3], and has been shown to be superior to other SI diagnostic imaging modalities [11, 12]. Several diseases of the equine SI are similar to those diagnosed by CE in human medicine including bleeding from gastro-intestinal ulcers [17], mucosal inflammation [18-20], and in rare cases intestinal tumors [18, 19, 21].

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Two wireless capsule studies were performed in horses with limited success due to battery and signal transmission issues [73, 74]. Previous studies deployed capsules that used radiofrequency communication to transmit data from the capsule to external sensors. Radiofrequency communication technology requires high levels of energy to cross through body tissues [71], and may not be powerful enough to get across the horse’s large abdomen. This high energy requirement also limits the capsule’s operation time and shorter battery life which may not allow imaging of the entire length of the equine SI.

The aim of this research is to investigate the application of the MiroCam® capsule endoscope for imaging the SI of the horse. The MiroCam® capsule was chosen for multiple reasons. It communicates via electric-field propagation, which uses the patient’s body as a conductive medium for data transmission [71]. This could circumvent issues related to transmission through the horse’s large abdomen. It also has a longer battery life (11+ hours) which could allow the capsule to image the entire length of the equine SI. In addition, MiroCam® has higher image resolution (320x320 pixels) and image capture rate (3 frames per second) compared to other commercially available capsules, which may allow for better image quality. It also has two additional LED lights and a wider field of view (170°) which may help view sections of the equine gastrointestinal tract (GIT) that have larger luminal diameters.
The objectives of this study are to:

1) develop a patient preparation protocol to optimize image quality for CE in horses
2) improve protocols for delivery of the capsule in the horse
3) assess image transmission between dermal and needle wire electrodes in the horse
4) determine the duration, quality, and consistency of CE image transmission in the horse

2.2 Materials and Methods

2.2.1 Animals

Two Standardbred mares (Table 11) were each used in four CE trials. A physical exam, biochemistry profile and complete blood cell count were performed. Results were normal and both horses were deemed healthy. All experimental procedures were approved by the University of Guelph’s Animal Care Committee (Animal Use Protocol 1476). Prior to the start of the study, horses were maintained on pasture 24 hours/day with ad libitum hay and concentrates fed twice daily.

<table>
<thead>
<tr>
<th></th>
<th>Horse 1</th>
<th>Horse 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Height (hands)</td>
<td>15</td>
<td>16.1</td>
</tr>
<tr>
<td>Body Condition Score (1-9 Henneke Scoring System)[145]</td>
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<td>5.1</td>
</tr>
<tr>
<td>Heart Girth (cm)</td>
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<td>193</td>
</tr>
<tr>
<td>Shoulder to Rump (cm)</td>
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<td>170.2</td>
</tr>
<tr>
<td>Widest Abdominal Girth (cm)</td>
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<td>215.9</td>
</tr>
<tr>
<td>Estimated Weight (kg)</td>
<td>409</td>
<td>533</td>
</tr>
</tbody>
</table>

*Table 11: Horse details*
2.2.2 Procedures

Four sequential trials were conducted, each trial including both horses (T1H1, T1H2, T2H1, T2H2, etc.). The protocol for each trial had the following procedural stages: 1) preparation, during which the horse’s GIT was emptied, 2) instrumentation, in which sensor electrodes were applied, 3) capsule deployment into the stomach, 4) image recording, and 5) assessment of images obtained.

Details of the protocol for Trials 2-4 were altered based on the results of the preceding trial, in an attempt to progressively improve image results.

Health Status: A physical examination was performed twice daily (am/pm) during the preparation stage and every 4 hours during the image recording stage. Respiratory rate, heart rate, hydration status, and general demeanor were assessed based on horse health guidelines and recorded in a log book for each horse (Appendix A-C). Blood was collected prior to capsule delivery, 6 hours into, and at the end of image recording. Complete blood count and blood chemistry was performed for each blood collection.

Animal Water and Feed Intake: Feed and water intake were monitored throughout the preparation and image recording stages (Appendix D). Feed (dry) was weighed prior to feeding; unconsumed feed was weighed before the next feeding. Water intake was estimated in quarter increments of a 19L water bucket.

2.2.2.1 Common features of all protocols

Preparation stage: Horses were housed in individual stalls and hand-walked twice daily for 20 minutes during the preparation stage. Before each trial, horses were adapted to hay
cubes or fibre nuggets\textsuperscript{a} for 10 days to prepare them for a reducing ration which was given for 3-7 days, which was itself in preparation for a 12-hour fast. The goal of the fasting period was to minimize SI contents and thereby facilitate imaging of the SI mucosa.

Horses were provided free access to water at all times. During housing at the veterinary facility, water from the home farm was supplied to prevent changes in water palatability. One to three days before each trial (during the preparation stage), both horses were transported to a veterinary facility and housed in individual stalls and hand-walked twice daily for 20 minutes, for 4-6 days. Two days before the end of this period, CE for one horse was performed, and on the next day for the other horse, after which both were returned to the home farm. A minimum washout period of 2 weeks was given between trials.

\textit{Instrumentation stage:} Nine external electrode sensors were used. Prior to electrode placement, positions were marked and surrounding hair was clipped, shaved, and washed with antiseptic soap and alcohol. Electrode sensor configuration (Figure 4) was modified from anatomical positions developed for humans \cite{87}. Modifications were made due to limitations of cable length and avoiding placement on the ventral abdomen to minimize irritation to the horse. Two cranial and two caudal abdominal electrode sensors were placed on both the right and left side and were positioned as to optimize the likelihood of detecting SI signal based on anatomic landmarks (Appendix E). The ninth electrode was used as an electronic reference point and placed on the right side.
**Figure 4**: Electrode sensor placement

**4A: RIGHT ABDOMEN**
- #1) Stifle line at the 9th intercostal space, directly opposite sensor #4;
- #2) Intersection of point of shoulder and xiphoid process lines (at the 8th intercostal space).
- #6) Intersection of umbilical and stifle lines. Directly opposite sensor #7;
- #5) Point of buttock line, midway between pelvic and umbilical lines, directly opposite #8.
- R) Reference Sensor at the 7th intercostal space, dorsal to sensor #2

**4B: LEFT ABDOMEN**
- #3) Intersection of point of shoulder and xiphoid process lines (at the 8th intercostal space). Directly opposite sensor #2:
- #4) Stifle line at the 9th intercostal space, directly opposite sensor #1;
- #7) Intersection of umbilical and stifle lines, directly opposite sensor #6;
- #8) Point of buttock line, midway between pelvic and umbilical lines, directly opposite #5
Local analgesia was used for the needle electrodes\(^b\), either lidocaine cream\(^c\), 30 minutes prior to needle insertion in Trial 1, or subcutaneous injected mepivacaine hydrochloride\(^d\), immediately prior to needle insertion in subsequent trials. Needles were inserted at a 45° angle into the subcutaneous tissue layer. Once in position, the needle was pulled back, leaving the wire in place. The uninsulated exposed end of the wire was then wrapped around the button-snap dermal electrode (SureTrace 1800-030)\(^e\), clipped to the cable lead and taped to the horse’s skin (Appendix F). The electrodes were held in place by adhesive tape (#1362)\(^f\) in Trial 1, and by a fenestrated blanket and stronger adhesive film (Opsite)\(^g\), for better stability, in subsequent trials. Cable leads connected the sensor electrodes to a receiver (MR1000 or MR1100)\(^h\), depending on Trial, which was secured to a surcingle around the horse’s abdomen (Appendix E).

![Figure 5: External view of MiroCam® capsule. 1. optical dome; 2. lens; 3.LED lights; 4.gold plated bands. Scale: 1cm=3.5mm](image)

**Capsule deployment stage:** Two capsules were used: a standard capsule (MiroCam® MC1000-W)\(^h\) for the first three trials, and a modified version with double the signal strength in the last trial. Prior to deployment, signal transmission between capsule and receiver were verified, following instructions in the user guide [87]. An intravenous 14 gauge Teflon® catheter was placed in the left jugular vein of the horse. Horses were se-
dated using either xylazine\textsuperscript{i} (0.02 mg/kg) or detomidine\textsuperscript{j} (0.01 mg/kg) and placed into stocks. A 2.3-meter endoscope (Fujinon EV-450LP5/23) was fed into a customized, flexible overtube\textsuperscript{k} (200cm long, outer diameter, 16.5mm; Figure 6; Appendix G), and both were introduced into the horse’s stomach via the right naris, at which time a visual inspection of the stomach was performed. Once the overtube\textsuperscript{k} was in place within the stomach, the scope was withdrawn and a custom capsule delivery device (AdvanCE)\textsuperscript{l}, 350cm in length, was inserted in the endoscope’s biopsy channel, and the capsule was placed into the capsule holder (Figure 6). The endoscope with the capsule delivery device was passed through the overtube\textsuperscript{k} into the stomach. Once inside the stomach, receipt of signal was confirmed by the signal indicator light on the receiver, then the capsule was deployed near the pylorus after which the overtube\textsuperscript{k} and endoscope were removed.

\textit{Figure 6}: Instrumentation for capsule delivery. Instrumentation includes an endoscope, an overtube\textsuperscript{k} and a capsule delivery device\textsuperscript{l} (capsule holder and cable attached to a release trigger). The endoscope was placed within the overtube\textsuperscript{k}. Cable from the capsule delivery device was inserted into the endoscope’s biopsy channel and screwed into the back of the capsule holder. L= length; D= diameter; OD=outer diameter; ID= inner diameter; dotted line indicate cable running the length of the endoscope.
A nasogastric feeding tube was endoscopically guided via the nostril into the stomach, and was kept in place for the duration of the image recording stage by being taped to the halter and/or by two sutures in the nostril following administration of local topical analgesic (mepivicaine hydrochloride\textsuperscript{d}).

*Image recording stage:* The horse was led from the stocks to a stall and lightly restrained (with a halter or cross ties, as necessary). The receiver was monitored for the 12-hour battery life of the camera (which was turned on as soon as it was taken out of its case prior to deployment).

Five-liter boluses of tap water were provided via the nasogastric feeding tube every 2 hours (as per protocol in Appendix H/I) using a suspended fluid bag (Figure 7) to maintain hydration and stimulate motility by increasing gastric distention \cite{120}. A small soaked meal consisting of 500g of hay cubes/fibre nuggets\textsuperscript{a} was provided and consumed orally 3 hours after deployment of the capsule and every 2 hours thereafter (Appendix H/I). Horses were hand-walked for 15 minutes every 2 hours to simulate motility \cite{128} and keep the capsule moving forward.
Figure 7: View of horse during image recording stage. Horses were housed in individual stalls during the image recording stage. Five-liter boluses of tap water were provided every 2 hours via an indwelling nasogastric feeding tube using a suspended fluid bag.

At the end of the 12-hour session, all of the recording equipment, feeding tube and blanket were removed from the horse. Image data was downloaded to the MiroView™ software for analysis.

Image assessment stage: Two data sets were assessed—graphs of the signal strength versus time, and videos of the images recorded at 3Hz for ~12 hours—using MiroView™ commercial software. A typical signal-quality graph (Figure 8) shows periods above and below a preset 85% threshold. A filter in the MiroView™ software erases images in the video below that threshold (as being of insufficient quality).
Figure 8: Example of signal quality graph. Graph was generated by Intromedic Ltd. Co. Red line designates 85% signal quality. If signal quality is above the 85% threshold line, images will appear in the MiroView™ software. If signal quality is below 85%, images are filtered out by the software.

From the graph, a qualitative assessment was made of the number and duration of periods when the signal quality exceeded 85%. These were quantified from the videos which reported the total time of images obtained (TT), which was divided by the recording session duration (12 hours) to give the percentage time of usable images (I%). Capsule operation time was defined as the elapsed time between the first and last confirmation of signal receipt as determined by the MiroView™ software. The longest bout of largely continuous images (CI) was assessed by counting frames, and included bouts that had no internal gaps longer than 15s. CI was divided by TT to give the percentage of time with consistent image transmission (CI%).

Assessment of image quality (in terms of lighting, resolution and obstruction) was performed after all trials and is described below.
2.2.2.2 Differences in protocols among trials

*Trial 1:* During the preparation stage, the reducing ration (RR1) maintained a fibre level of 1.5% of the horse’s BW per day while reducing the percentage of long-stemmed fibre (hay) and replacing it with a source of short fibre length feed (soaked hay cube) to increase transit through the SI [95]. Hay was slowly replaced (0.2-0.3%/day) with soaked hay cubes over 7 days and fed in two meals (Appendix H). Both horses were transported to the clinic 3 days prior to CE. Dermal electrodes were used in Horse 1 and needle electrodes in Horse 2. Thirty minutes after the capsule was deployed, Horse 2 shook off the needle electrodes and it was decided to continue the trial using dermal electrodes.

*Trial 2:* On the basis of the results of Trial 1, the following parameters were changed (Figure 9). Reducing Ration 2 (RR2) took place over 3 days rather than 7 days and hay cubes were replaced with a fibre source of smaller particle size. Ration was reduced from an initial feeding level of 1.0% BW to 0.6% over 3 days (reduction of 0.2%/day) in conjunction with replacing hay with soaked fibre nuggets (Equiline Fibre Nugget) (Appendix I). It was noted in Trial 1, horses showed signs of stress and a drop in water intake at the clinic compared to the home farm. As such, horses were transported to the clinic the day before CE to limit the number of days at the clinic and minimize stress during the preparation stage. Prior to placement of the feeding tube and following deployment of the CE, a 6L water bolus was delivered through the reach overtube to promote gastric emptying and passage of the capsule into the duodenum.

*Trial 3:* Modifications to protocols in Trial 3 were influenced by the results from Trial 1 and 2 (Figure 9). Omeprazole (2.28 g/day) (Gastrogard®) was provided during the
preparation and image recording stages to protect the horses’ stomachs [91]. A five-liter bolus of water was provided via a nasogastric tube at the start of the fasting period to promote SI clearance. A new receiver (MR1100\textsuperscript{h}) was used given its enhance feature that provides additional information on signal receipt in relation to recording time.

\textit{Trial 4:} On the basis of the results of all preceding trials, a modified capsule (MC1000-W\textsuperscript{h}) with double the signal strength was used in both horses. MR1000\textsuperscript{h} and MR1100\textsuperscript{h} receivers were used and tested during periods of positive signal receipt.
<table>
<thead>
<tr>
<th>Preparation Stage</th>
<th>Instrumentation Stage</th>
<th>Imaging Recording Stage</th>
<th>Results (Trial 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing Ration</td>
<td>Configuration I</td>
<td>3 hr after delivery</td>
<td>↑ Obstruction (feed)</td>
</tr>
<tr>
<td>Fasting Period (hrs)</td>
<td>Dermal</td>
<td>+ every 2 hrs</td>
<td>↓ Image Transmission</td>
</tr>
<tr>
<td>Water Bolus</td>
<td>Standard Capsule</td>
<td>5L bolus/ 2 hrs</td>
<td>↓ Signal Quality (&lt;85%)</td>
</tr>
<tr>
<td>(at the start of fast)</td>
<td>MR1000</td>
<td>15min/2 hrs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standard Protocol</td>
<td></td>
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<td>none</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RR1</td>
<td>RR2+ Omep.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>nc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>5L</td>
<td></td>
</tr>
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<table>
<thead>
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<th>Imaging Recording Stage</th>
<th>Preparation Stage</th>
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<td>Electrode Placement</td>
<td></td>
<td>RR1</td>
<td>Gastric Ulceration</td>
</tr>
<tr>
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<td>12</td>
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</tr>
<tr>
<td>Capsule</td>
<td></td>
<td>none</td>
<td>↓ Image Transmission</td>
</tr>
<tr>
<td>Receiver</td>
<td></td>
<td>nc</td>
<td>↓ Signal Quality (&lt;85%)</td>
</tr>
<tr>
<td>Capsule Introduction</td>
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<td></td>
</tr>
<tr>
<td>Water Bolus</td>
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<td>nc</td>
<td></td>
</tr>
<tr>
<td>(after capsule)</td>
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<td>nc</td>
<td></td>
</tr>
<tr>
<td>3 hr after delivery</td>
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</tr>
<tr>
<td>+ every 2 hrs</td>
<td></td>
<td>nc</td>
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</tr>
<tr>
<td>5L bolus/ 2 hrs</td>
<td></td>
<td>nc</td>
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</tr>
<tr>
<td>15min/2 hrs</td>
<td></td>
<td>nc</td>
<td></td>
</tr>
</tbody>
</table>

Figure 9: Flowchart of CE trial protocols. Variations to the protocol were made based on results from previous trial (denoted by coloured lines). RR1= reducing ration 1; RR2= reducing ration 2; Omeprazole; nc = no change.
2.2.2.3 Procedures following all trials

Assessment of Image Quality: Adequacy of lighting was qualitatively assessed based on the presence or absence of light in recorded images. Resolution was quantified in terms of the smallest structures clearly visible in any of the images. The degree of obstruction of the intestinal lumen by feed particles was scored using a 4-point scale: 0-25%; 25-50%; 50-75%; 75-100% obstruction [83]. A graticule (grid) was placed over one frame per second of recording and the number of intersections occupied by feed particles were counted and expressed as a percentage over the total number of intersections [147].

Capsule retrieval: To determine if the capsule was expelled, manure was collected for 7 days after CE and sifted to look for the capsule. Horse 2 was euthanized 3 months after completion of the last trial (T4H2) for unrelated reasons. A post-mortem capsule search was performed to determine if capsules were retained within the GIT. The day following euthanasia, the SI, cecum and LI was extracted and laid out from duodenum to rectum. The SI was severed at the point of entry into the cecum. Starting from the duodenum, contents of the SI were manually advanced downstream until evacuated at the end of the SI, where a manually search for the capsule was performed. The cecum and LI were opened sequentially and contents were sifted through manually.
2.3 Results

2.3.1 Results from each trial

Trial 1

*Preparation stage:* Both horses consumed all feed provided. Water intake was low at the farm with Horse 1 consuming approximately 0.75 buckets of water (12.25L) per day and Horse 2 drinking 0.5 buckets of water (9.5L). A decrease in water intake was observed at the clinic with Horse 1 and 2 drinking approximately 0.25-0.5 buckets of water/each per day (4.75 - 9.5L). Horse 2 displayed signs of stress including pawing and stall-walking behaviour at the clinic.

*Instrumentation stage:* Capsule was successfully deployed into the stomach in both T1H1 and T1H2. Dermal electrodes adhered well to the horse’s skin for the duration of the image recording stage; however both horses developed mild swelling at the electrode site from the dermal electrode which disappeared the following day.

*Duration of Recording:* Total time of images obtained in T1H1 was 42 minutes (I%=5.86%) (Table 12) and a capsule operation time of 11hr 46min 57s. In T1H2, TT was 13 minutes (I%=1.81%) and the capsule operation time was 34min 44s. Transmission of images obtained was inconsistent in T1H1 where small and large time gaps were observed in the capsule operation time as highlighted by the discrepancy between TT and the capsule operation time (Table 12). CI for T1H1 was 4.75min (CI%=11.26%), whereas CI for T1H2 was 7.17min (CI%=55.01%) (Table 12).
<table>
<thead>
<tr>
<th>Trial #</th>
<th>Capsule Operation Time (hh.mm.ss)</th>
<th>TT (hh.mm.ss)</th>
<th>I%</th>
<th>CI min</th>
<th>CI%</th>
<th>Other outcomes</th>
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<td>4.75</td>
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<td>7.17</td>
<td>55.01 MF</td>
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<td>4.90</td>
<td>7.50</td>
<td>21.26 U</td>
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<td></td>
<td>T2H2</td>
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<td>00.00.31</td>
<td>0.04</td>
<td>ND</td>
<td>MF + U</td>
</tr>
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<td>T3H1</td>
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<td>ND</td>
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<td>ND</td>
<td>ND</td>
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<td>00.17.17</td>
<td>2.40</td>
<td>3.42</td>
<td>19.77</td>
</tr>
</tbody>
</table>

Table 12: Duration of recording per trial. TT= total time of obtained images; I%= percent of usable images calculated by dividing TT by the 12 hour recording session; CI= longest continuous image recording bout that had no internal gaps longer than 15s; CI% = percentage of time with longest bout of continuous image transmission calculated by dividing TT by CI; MF= muscle fasciculation; U= gastric ulcers; T1H1= Trial 1, Horse 1; T1H2 = Trial, Horse 2; T2H1 = Trial 2, Horse 1; T2H2= Trial 2, Horse 2; T3H1 = Trial 3, Horse 1; T3H2 = Trial 3, Horse 2; T4H1= Trial 4.

Signal Transmission: In T1H1, signal transmission was consistent (Figure 10A) throughout the recording session although a large portion of signal was below the 85% signal quality threshold, whereas signal transmission in T1H2 was of high quality but only for a short duration during the ninth hour of recording (Figure 10B).
Figure 10: Signal quality from Trial 1. Figure A represents data from T1H1 (Trial 1, Horse 1) and Figure B represents data from T1H2 (Trial 1, Horse 2). Graphs were generated by Intromedic Ltd. Co. from raw recorded data. Red line designates 85% signal quality. If signal quality is below 85%, images are removed by the MiroView™ software.

Recorded Images: Images were captured from both T1H1 and T1H2. Examples of still-frames images extracted from video are shown in Figure 11. In both T1H1 and T1H2, residual food particles in the intestines obstructed visibility of the mucosal wall (Figure 11A/B), however this obstruction was more pronounced in T1H1 with all images being obstructed. In T1H2, there was less obstruction by feed particles and it was possible to view mucosal wall (Figure 11B/C). A parasite, identified as a tapeworm (anoplocephala perfoliata) by Dr. Peregrine from the Ontario Veterinary College, was observed in T2H2 (Figure 11D).
Figure 11: Still-frame images captured from Trial 1 (T1H1; T1H2); A) complete obstruction of mucosal wall by residual feed particles; B) partial obstruction from residual feed particles in T1H2 with limited visibility of mucosal wall; C) no obstruction from feed particles with observable mucosal wall where distinct villi can be seen; D) tapeworm parasite (anoplocephala perfoliata), identified by Dr. Peregrine at the Ontario Veterinary College.

Horse Health: Horse 2 experienced muscle fasciculations between the second and third hour of recording. These tremors started in her shoulders and slowly progressed to her rump and continued for approximately one hour. The horse did not appear distressed; a complete physical examination was performed and was otherwise within normal range. Serum biochemical profile, including creatine kinase levels, collected at the 6th hour of recording and at the end of CE recording, was within normal range.
Trial 2

*Preparation stage:* Both horses consumed all feed provided. Water intake at the farm was ¾ bucket/day (14.25L) for Horse 1 and ¾-1 bucket/day (14.25-19L) in Horse 2. At the clinic, Horse 1 did not consume any water on Day 3, whereas Horse 2 consumed ¼ bucket/day (4.75L). Horse 2 continued to display signs of stress as described in Trial 1, however these signs were not as pronounced. Reduced GIT sounds were observed during physical examinations in both Horse 1 and Horse 2 on Day 3 of the preparation stage and on the morning of the CE trial.

*Instrumentation stage:* Signal receipt was confirmed within the stomach in both T2H1 and T2H2, after which the capsule was successfully released. Needle electrodes were secured in place for the duration of the image recording stage in T2H1 and for the first six hours of T2H2. Due to the absence of signal receipt and image transmission in T2H2 after the capsule was deployed, needle electrodes were replaced with dermal electrodes at the 6th hour of recording to determine whether image transmission would be improved using dermal electrodes.

*Duration of Recording:* Capsule operation time was 9hr 41min 13sec and 29min for T2H1 and T2H2, respectively (Table 12). In T2H1, TT was 35min (I%=4.90%) and CI was 7.5min (CI%=21.26%). In T2H2, TT was 31sec (I%=0.04%) where 22sec was recorded during the first six hours and 9sec from the last six hours of the recording session. CI and CI% was not calculated for T2H2 due to the short duration of recording (Table 12).
**Signal Transmission:** The output style of the graphs for Trial 2 (Figure 12) is different than graphs from Trial 1 (Figure 10) due to modifications in Intromedic’s in-house analysis software and compatibility issues with the MR1000 receiver. In T2H1, signal transmission was consistent for the 9 hour duration of the capsule operation time, with a greater proportion of signal transmission above the 85% signal quality threshold (Figure 12A) compared to Trial 1 (Figure 10A). In T2H2, signal transmission was short and below the 85% signal during the 6\textsuperscript{th}-12\textsuperscript{th} hour of recording. There was no signal quality data from hour 0 to 6 due to raw data being erased during re-initializing of the receiver prior to placement of dermal electrodes.

![Graph A: T2H1](image)

**Figure 12:** Signal quality from Trial 2; A) Quality of signal from standard capsule using needle electrodes in T2H1 and B) dermal electrodes in T2H2. In T2H2, raw data from hour 0-6 was erased during receiver re-initialization prior to converting to dermal electrodes; therefore no signal quality data was analyzed from hour 0-6. Data was analyzed by Intromedic Ltd. Co. from raw recorded data. Red line designates 85% signal quality. If signal quality is below 85%, images are erased by MiroView™ software.
Recorded Images: In T2H1, it was possible to visualize mucosal wall (Figure 13A), however residual food debris continued to obstruct visibility (Figure 13B). In the ninth hour of T2H1, there was a change in mucosal appearance, decreased lighting, a more fluid environment and increased obstruction from gas bubbles as well as an apparent change in motility pattern (Figure 13C-F), suggesting the transition from SI into LI. A snapshot of a tapeworm (*anoplocephala perfoliata*) (Figure 13G) and the swimming of a pinworm (*Oxyuris equi*), as identified by Dr. Peregrine from the Ontario Veterinary College, was visualized from 09.15.20 to 09.16.04 (Figure 13H). In addition, a region of mild inflammation was visible and identified based on the change in mucosal wall colour (Figure 13I). In T2H2, images were only transmitted from the stomach when needle electrodes were used (Figure 14A) while the capsule was in the capsule holder. A couple of intestinal images (Figure 14B) were captured in T2H2 shortly after dermal electrodes were applied; however these images were obstructed by residual food debris (Figure 14B).
Figure 13: Still-frame images captured from T2H1; A) view of unobstructed mucosal wall (02.56.22); B) partial obstruction of mucosal wall by residual food debris (03.47.11); C-F) image captured from 08.48.04-09.05.04 where a change in mucosal appearance, (C), decreased illumination (D), and a more fluid environment* (D-F) with interference from gas bubbles (E-F) is observed; D) A reflection of the capsule (indicated by red circle) is observed in the fluid environment; E) gas bubble; F) entire image appears to be capture through a large gas bubble; G) tapeworm parasite (anoplocephala perfoliata) in upper left hand corner (03.14.56); H) pinworm (oxyurus equi) parasite in red circle, viewed ‘swimming’ from 09.15.20 to 09.16.04; I) region of mild inflammation in mucosal wall, indicated by red arrow (09.15.02)
Figure 14: Still-frame images captured from T2H2. A) stomach image (00.02.44) while the capsule was in the capsule holder (indicated by the four white arched lines on the perimeter of the image); B) complete obstruction of mucosal wall from residual food debris (06.27.16)

Horse Health: During the gastroscopy prior to introduction of the capsule, Grade II gastric ulcerations were detected in Horse 1 and 2; both horses were placed on a 28 day treatment (2.28g/day) of omeprazole (Gastroguard®). A gastroscopic exam was performed following omeprazole treatment and both horses were deemed ulcer free prior to entry into Trial 3.

Horse 2 experienced muscle fasciculation (as described in Trial 1) during the second and third hour of recording. All vitals and results from serum biochemical profile for blood collected at the 6th hour and at the end of CE recording were within normal range.

Trial 3:

Preparation stage: Both horses consumed all feed provided. Water intake for Horse 1 was ¾ bucket/day (14.25L) at the home farm and ½ bucket/day (9.5L) at the veterinary facility. Horse 2 consumed ¾-1 bucket (14.25-19L) of water at the home farm and ½- ¾ bucket (9.5-14.25L) at the veterinary facility. A decrease in gut sounds was observed on
Day 3 of the preparation stage and on the morning of CE. Both horses had normal attitude and did not display pawing or stall walking behaviour.

**Instrumentation stage:** The capsule was successfully delivered to the stomach in both T3H1 and T3H2. In T3H2, the adhesive film used to secure the needle wire was replaced on the ventral caudal electrodes on the right and left abdomen due to sweating from the warmer temperature during this trial.

**Duration of Recording:** Images were captured when the capsule was in the capsule holder in the stomach. There was very little transmission of images or receipt of signal once the capsule was released. Data from this trial was removed given suspected equipment malfunction with the MR1100 receiver as per discussion with Intromedic Ltd. Co.

**Signal Transmission:**
Signal transmission was highest within the first 30 minutes of recording in both T3H1 and T3H2 with a couple of spikes above the 85% threshold (Figure 15). In T3H1, transmission of signal briefly took place between the first and second hour of recording, after which there was no signal transmission. The quality of signal during this period was below 85% and no images were captured. In T3H2, signal transmission occurred between the eight and eleventh hour of recording, however no images were captured as signal quality was below the threshold (Figure 15).
Health Status: Horse 2 experienced muscle fasciculation (as described in Trial 1) that lasted 45 minutes. All vitals and results from serum biochemical profile were within normal range.

Trial 4:

Preparation stage: All feed was consumed by both horses in T4H1 and T4H2. Water intake was approximately ¼-½ bucket/day (4.75-9.5L) and ¾ bucket/day for Horse 1 and 2, respectively. At the veterinary facility, Horse 1 consumed no water and Horse 2 consumed ½ bucket/day (9.5L). Horses did not display any stall-walking or pawing behaviour at the veterinary clinic.

Figure 15: Signal quality from Trial 3. A) Quality of signal from standard capsule using needle electrodes. Data was analyzed by Intromedic Ltd. Co. from raw recorded data. Red line designates 85% signal quality. If signal quality is below 85%, images are erased by MiroView™ software.
**Instrumentation stage:** The capsule was successfully released in the stomach in both T4H1 and T4H2. MR1000 and MR1100 receivers were switched and used during periods of positive signal receipt and were deemed to be functioning well given the continuity of positive signal receipt and transmission of images as viewed through the MicroView™ Real Time Viewer.

**Duration of Recording:** In T4H1, TT was 1hr 22min 41sec (I%=11.49%) and CI was 20.67 min (CI%=24.99). In T4H2, TT was 17min 17 sec (I%=2.40%) and CI was 3.42min (CI%=19.77%) (Table 12).

**Signal Transmission:** Signal quality was only analyzed from MR1100 receivers and only covered segments of the 12 hours of recording. For T4H1, signal quality was analyzed from the start of recording to 05:50.38 and 08.52.00 to end of recording. In T4H1, signal transmission was consistent for the first six hours of recording with a few segments above the 85% threshold within the first hour and between the fourth and sixth hour of recording. Quality of signal was slightly below 85% between the first and fourth hour. During the ninth hour of recording, there was signal transmission; however it was of low quality, below the 85% threshold (Figure 16A). For T4H2, signal quality was analyzed from the start of recording to 03.15.00 and from 04.21.30 to end of recording for T4H2. In T4H2, signal transmission was not as consistent as T4H1 with a few periods of signal spiking above 85% within the first hour and around the sixth hour of recording. Signal transmission was sporadically observed, however the signal quality was below 85% (Figure 16B).
Recorded Images: In T4H1, it was possible to observe the capsule attempting to pass through the pylorus (Figure 17A) into the duodenum from 2.10.10 – 2.39.09 hour of recording by viewing alternating images of duodenal and gastric mucosal wall (Figure 17B). The first duodenal image when the capsule was moving in an aboral direction appeared at 2.52.08 (Figure 17C). Contraction (Figure 17D) and relaxation phases (Figure 17E) of peristalsis could also be viewed. Residual food debris continued to obstruct visibility of mucosal wall in the intestines starting at 03.34.37 (Figure 17F). Food particles were also observed in the stomach via endoscope during capsule introduction.

Figure 16: Signal quality from Trial 4. Data was analyzed by Intromedic Ltd.Co from MR1100 receivers. Both MR1000 and MR1100 receivers were used in Trial 4; signal quality was only analyzed from MR1100 receivers and covered the following capsule operation times (T4H1: Start of recording to 05:50.38 & 08.52.00 to end of recording; T4H2: Start of recording to 03.15.00 & from 04.21.30 to end of record-

<table>
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<tr>
<td>B: T4H2</td>
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intestinal image captured in T4H2 appeared at 04.30.23 (Figure 18A). During peristaltic contraction, movement of fluids and residual particles darkened and obstructed visibility of mucosal wall (Figure 18B). In segments prior to relaxation, mucosal wall was visible (Figure 18C); there was continuous stream of obstructed and non-obstructed images during the relaxation and contraction phases of peristalsis. Tapeworm parasites were also visualized on multiple instances (Figure 18D-F)

*Health Status:* Horse 2 did not have any muscle fasciculations in Trial 4 when using the modified capsule. Results from physical examination and blood analysis were within normal range.

*Figure 17:* Still-frame images captured from T4H1. A) view of pylorus (02.10.10) where capsule was attempting to pass into the duodenum; B) view of gastric mucosa (01.01.09); C) first duodenal image (02.52.08) once capsule was moving in an aboral direction; D) image during a contraction phase of peristalsis (02.53.01); E) image during a relaxation phase of peristalsis (2.52.33); F) partial obstruction of mucosal wall by residual food debris (04.02.37)
Figure 18: Still-frame images captured from T4H2. A) First intestinal image (04.30.23) where distinct villi can be seen; B) obstruction of mucosal wall by feed particles and fluid during a relaxation phase of peristalsis (04.30.24); C) view of mucosal wall during a contraction phase of peristalsis (04.56.26); D-F tapeworm parasites

2.3.2 Summary of results across all trials

Throughout all trials, image transmission was inconsistent where small and large time gaps were observed in the capsule operation time as highlighted by the discrepancy between TT and capsule operation time (Table 12). Horse 1 had higher I% than Horse 2 across all trials (Table 12; Figure 19). In trials using the standard capsule (Trial 1 and 2), I% was longer in both horses when using dermal electrodes (T1H1, T1H2) compared to needle electrodes (T2H1, T2H2) (Table 12; Figure 19A). When a modified capsule was used in Trial 4, both horses had an increase in I% compared to the standard capsule (Figure 19B). In T4H1, there was an increase of 5.63% and 6.59% from T1H1 and T2H1 re-
respectively. In T4H2, there was an increase of 0.59\% and 2.36\% from T1H2 and T2H2, respectively.

\[ \begin{align*}
\text{Table} & \\
\text{I\%} & \text{Dermal} & \text{Needle} & \text{Standard Capsule} & \text{Modified Capsule} & \text{Horse 1} & \text{Horse 2} \\
\text{Trial 1 vs. 2} & & & & & & \\
\text{Modified Capsule} & & & & & & \\
\text{I\%} & & & & & & \\
\end{align*} \]

*Figure 19:* Percent recorded time of usable images between dermal and needle electrodes and between standard and modified capsule. A) I\% between dermal and needle electrodes using standard capsules (Trial 1 vs. 2); B) I\% between standard and modified capsules using needle electrodes (Trial 2 vs. 4). Horse 1 consistently had higher I\% than Horse 2 across all trials. Both horses had an increase in I\% with modified capsule and slightly higher I\% with dermal electrodes.

**2.3.3 Results following all trials**

*Assessment of Image Quality:* Light was consistently present in the majority of recorded images and was adequate to visualize mucosal wall and luminal contents. As the diameter of the lumen increased and presence of fluid and gas bubbles were greater (Figure 13D), light was present but insufficient to visualize the entire lumen. The smallest visible structures observed were pinworms (*oxyuris equi*) (Figure 13H) that measure 5-8cm in length and 5mm thick in actual size [148], tapeworms (*anoplocephala perfoliata*) (Figure 11D;13G;18D-F) that are 4-8 cm in length and 1-2cm wide [148] and villi that are 2.5-3.0 mm in length [149]. Differentiating individual villi relied on the proximity of the camera to the mucosal wall; as the camera was further away distinct villi could not be observed.
Obstruction by feed particles was observed across all trials regardless of preparation protocols (Figure 20). Horse 2 had a higher percentage of images scoring in the 0-25% obstruction category at 67.02% and 61.87% in T1H2 and T4H2, respectively, compared to Horse 1 that scored 0.17% and 6.43% in the same trials (T1H1, T4H1, respectively). Reduced visibility by intraluminal bubbles (Figure 17C-D) was occasionally observed and partially obstructed visibility of the mucosal wall. In T2H1, when the capsule was suspected to be in LI, the presence of large gas bubbles obstructed visibility of mucosal wall (Figure 13E-F).

![Graph showing percentage of images obstructed by feed particles across all trials](image)

**Figure 20:** Percentage of images obstructed by feed particles across all trials once capsule was released from delivery device into the stomach. Images were scored in four categories based on percent of obstructed visible field (0-25%, 25-50%, 50-75% and 75-100%). Data from Trial 3 was omitted due to low TT.

**Capsule Retrieval:** None of the capsules were retrieved during the 7 day fecal collection period. Capsules were also not found in the post-mortem search in Horse 2.
2.4 Discussion

As CE technology is new to equine medicine, this research was conducted as a proof of principle that the equine SI can be imaged using the standard MiroCam® human CE equipment. The capsule was successfully deployed in all trials and SI images were obtained. The total time of images recorded using the standard MiroCam® capsule ranged from 0.45-42.11min (Table 12), compared to an average of 704± 56min [71, 80, 150, 151] in humans. Duration of recording increased in both horses (TT = 17.17 - 82.41min) when the modified capsule was used. In the only other equine capsule study that deployed PillCam®, total time of images obtained was not reported and recording was terminated at the 6th hour [74], therefore a comparison cannot be made between the two types of capsule endoscopes used in horses.

Horse 2 had shorter periods of image transmission than Horse 1 in Trial 1 (Table 12), but signal quality appeared to be slightly better (Figure 10B) where 55% of TT was continuous (Table 12). In other words, if signal was being received in Horse 2, it was of high quality. These brief recorded segments in T1H2 suggest that the capsule may have been closer to a set of electrodes at the time of recording, providing better image transmission. As the distance between the capsule and the sensor increases, signal strength and hence image transmission will decrease (personal communication with B.Seo, Intromedic Ltd. Co.). The disparity in image transmission between Horse 1 and 2 could also be caused by differences in body size and composition. Horse 1, being smaller in size and abdominal width and lower in body condition score than Horse 2 (Table 11) consistently had higher image transmission across all trials.
Based on initial results, the current CE system is not ready for clinical deployment in the horse but has potential to be further developed into a useful imaging tool based on the following positive outcomes of the study. High quality images were obtained, where mucosal colour could be seen, distinct villi detected and parasites identified. Images in the horse are comparable to humans [71], which can likely be attributed to the filtering of images by the software, where only high quality images are retained. Lighting was adequate to visualize the intestinal lumen and its contents. This is in contrast to the Pill-Cam® capsule study which encountered darkened images in the aboral portion of the SI due to lighting limitations and smaller field of view [74]. Battery life of the MiroCam® capsule appears to be sufficient to image the entire length of the SI given that the capsule reached the colon or cecum in T2H1. In the study using the SmartPill® capsule, the mean capsule SI transit time in ponies, following a 12 hour fast, was 4.6 hours (range of 2.4 – 7.6 hours) [73]. Taking into account the longer length of the horse’s SI, we estimated that the MiroCam® capsule SI transit time should be between 4.25-13.25 hrs, with an average of 8.18hrs, which falls within the range of the 12 hour capsule battery life.

Drawbacks of the research include short bouts of images obtained, obstruction from residual feed particles and lack of capsule positional information.

Image transmission: Image transmission was intermittent in all CE trials where large time gaps (hours) and small time jumps (seconds) were observed, demonstrating inconsistent signal receipt. Image transmission may have been influenced by the capsule’s position relative to the body surface; as the capsule becomes more superficial, or closer to surface electrodes, this may allow for better image transmission. It was not possible to determine
the capsule’s location and therefore we cannot correlate image transmission with capsule position. Location of the capsule was likely influenced by gut motility and transit time, with can have large inter and intra-horse variability [73, 95] and could have been impacted by study protocols, which is covered in more detail below. Due to MiroView™ built-in filter, a portion of images with lower signal quality may be lost, resulting in lower TT, however there was no method of quantifying signal quality and the number of filtered images. Lowering the 85% threshold may improve TT, but the impact on image quality is unknown. Furthermore, this criteria is currently not under operator control and is inherent in the software provided by the manufacturer. Quality of signal, as analyzed by Intromedic Co., Ltd., provided a qualitative assessment of signal quality. Data on signal quality and TT were not congruent in Trial 1 and 2; signal quality appeared higher in T2H1 compared to T1H1 (Figure 12A/Fig10A) but TT was higher T1H1 (Table 12). The disparity between signal quality and TT brings into question the validity of the graph provided by Intromedic Ltd. Co. This was brought to their attention and it was revealed that the software used to analyze signal quality was not designed for the MR1000 receiver and required modification to their software programming. In addition, the software used to generate the signal quality graph selects certain ranges within a collection of images and averages the quality of the images within that range (personal communication with B.Seo, Intromedic Ltd. Co.). More detailed information regarding the selected ‘range’ was confidential and therefore was not provided. Given that the software appeared to group signal quality and average it over time, the graph may not represent an accurate depiction of signal quality across the capsule’s operation time. As a result, the
signal quality graph was used as a qualitative assessment of signal quality but it could not be used to correlate signal quality with TT.

Factors that likely affect signal transmission include 1) distance between the capsule and the external sensor electrodes, 2) tissue resistance, and 3) frequency generated by the capsule.

*Influence of body size and tissue composition:* Horse 1 who was smaller in size and lower in body condition score than Horse 2 (Table 11) consistently had higher image transmission across all trials. Horse 2 was larger in size with a higher percentage of body fat and consistently had lower TT, suggesting that body composition (i.e. fat distribution), and size contributed to poor signal transmission. As the distance travelled by the signal increases, signal strength will decrease (personal communication with B.Seo, Intromedic Ltd. Co.) as it encounters resistance through body tissues. Therefore it is possible that image transmission would be better in smaller equids such as ponies and foals. It is also highlights the importance of the placement of electrode sensor relative to the capsule’s trajectory to minimize the distance between the capsule and sensors.

*Tissue resistance:* MiroCam® communication technology relies on tissue conductivity to transmit data from the capsule to the outside of the body. Conductivity will vary by tissue type and is greatest in tissues with high water content [152], such as muscle tissue (75.5%), and lowest in skin (68%) and fat (12.5%) [153]. In humans, bone, fat and the stratum corneum (the most outer layer portion of the epidermis), have the highest levels of resistance, respectively [153]. Based on the frequency generated by MiroCam® (1-3Mhz) [72], conductivity in humans is greatest in muscle, followed by wet skin, bone
marrow, fat, bone and dry skin (personal communication with B.Seo, Intromedic Ltd. Co.). No such research has been done in horses and it was assumed that tissue conductivity is similar between horses and humans despite differences in tissue thickness. In humans, abdominal skin thickness ranges from 1.42-1.68 mm [154], whereas skin in the horse’s abdomen is thicker, measuring on average 1.94mm [155], suggesting that horse’s skin may pose a higher resistance to capsule signal. Initial data from Trial 1 and 2 suggested that dermal electrodes provided better signal transmission than subcutaneous wire electrodes. This contradicts our expectation that the skin’s high resistivity would attenuate signal transmission in the horse. There could have been other factors such as the capsule’s location relative to the body’s surface, proximity to the electrode sensors as well as the surrounding intestinal fluid environment influencing image transmission; therefore it is not possible in this study to determine the effect of needle and dermal electrodes on image transmission. Needle wires were placed subcutaneously in an effort to minimize loss of signal through skin; however the signal still needed to cross through subcutaneous fat which is also lower in conductivity. Conductivity will remain the same within the same type of tissue, but will drop when the signal is being transferred from one type of tissue to another (personal communication with B.Seo, Intromedic Ltd. Co.); the fewer tissue layers the signal needs to cross, the higher the conductivity. Therefore, it is arguable that placing electrodes directly into muscle tissue would not only minimize loss of conductivity through subcutaneous tissues and skin but may enhance image transmission given muscle’s higher conductivity.

Unlike humans, the ribcage encloses a large portion of the SI in the horse and interferes with the placement of sensor electrodes. Electrodes were strategically placed in intercos-
tal spaces to minimize signal attenuation from the ribcage; nevertheless the presence of bone may have contributed to signal attenuation. In humans, electrodes are placed below the ribcage [87] which likely minimizes impedance from bone.

*Image Obstruction:* Obstruction by feed particles negatively affected visibility of the mucosa throughout all trials. Horse 2 had a lower percentage of images obstructed by feed particles across all trials except for Trial 2. Differences in obstruction could be linked to differences in motility and transit time between individual animals [102] and changes in protocols between trials. In addition, given the lower I% in Horse 2, the difference in obstruction could be linked to the capsule’s location and randomly being in a section of intestine with fewer residual feed particles. Given that image transmission was inconsistent, we only received a random snapshot, so it was not possible to assess the extent of residual feed debris within the entire length of the intestinal tract. Obstruction was more apparent as the capsule operation time increased, suggesting that obstruction was higher in aboral sections of the SI, however this cannot be confirmed as the capsule’s location was unknown. Residual feed was also occasionally observed in the stomach at the start of CE, suggesting that it would be very difficult to completely empty the digestive tract of food debris.

*Capsule location:* Once the capsule is released, there was no means of knowing the capsule’s location within the intestinal tract and abdomen and whether the capsule is moving in an aboral direction. In humans, a positional map has been developed based on a tracking algorithm using sensor signal strength data (personal communication with B.Seo, Intromedic Ltd. Co.), however, this was not available for the horse since it
requires significant technical modification and research by Intromedic Ltd.Co. Being able
to identify sensors that are receiving signal and the strength of that signal would help in
tracking the capsule’s location as the signal would theoretically be stronger the closer the
capsule is to a sensor. Ultrasound was initially considered as a method of tracking the
capsule given that the capsule was echogenic. However, equine SI can only be
consistently visualized in three locations [66] and would require performing ultrasound
for the duration of the CE image recording with very little chance of viewing the capsule.
Radiographic abdominal examination could have been possible; however specialized
equipment capable of penetrating the equine abdomen was not available at the clinic and
this procedure would require horses to be exposed to multiple events of radiation.
Furthermore, the relative small size of the capsule would likely limit its visibility,
especially when superimposed on thicker segments of colon which are generally very
opaque on radiographs [156].

Preparation: A longer period of fasting (24-36 hours), as is done prior to duodenoscopy
and exploratory surgery [60, 67], may be necessary to sufficiently clear the SI. The
equine trial using PillCam® used a 24 hour fast and magnesium sulfate (1g/kg, dissolved
in 4L) 16 hours prior to CE, however, feed-related obstruction was not assessed and re-
ported in the study, but the capsule did not image downstream from the proximal jejunum
[74] where a considerable portion of feed obstruction is suspected to have occurred in our
study. The stress of a new environment may have also altered GIT motility [157] and im-
peded clearance of the SI. During the study, both horses had a decrease in water intake
during the preparation stage which was likely due to a decrease in feed intake [118] and
change of environment. Providing electrolytes in future trials may be useful to encourage
drinking. Administering enteral fluid therapy during the fasting period using intermittent water bolus may enhance clearance of the SI by stimulating motility [120, 125] and flushing out residual food debris. In humans, various medications including purgatives, prokinetic drugs and cathartics, are provided to clear the digestive tract prior to CE [81, 83] and has been show to improve visibility of the mucosa [81]. Given the lack of research on the use of these drugs in horses and their potential negative effects [138], a protocol that stimulated the natural reflexes was employed in this study to avoid causing gastrointestinal disturbances.

**Instrumentation:** Placement of electrodes was the same across all trials to minimize variation. Positions were chosen based on human electrode placement, equine SI location and length of cable lead. There was no means within the available software to identify which electrode sensors are receiving signal at any given time and the strength of that signal. This type of information would have been valuable to not only determine optimal placement of sensors but would be a useful guide in tracking the capsule’s location within the intestinal tract.

At this point, it is difficult to determine whether the type of electrode exerts an influence on image transmission or whether it is simply a size effect of the horse’s large abdomen and the longer distance the signal needs to travel in the horse compared to the human. Data comparing needle to dermal electrodes is inconclusive at this time given that dermal electrodes were only used once in each horse in Trial 1, the effect of other study variables as well as the discrepancy between signal quality graphs and TT in Trial 2. That said, it is likely that signal strength, body size and composition of the horse are the primary factors
affecting image transmission and electrode type may only be secondary in attenuating signal transmission.

Modified capsules with double the signal strength theoretically generated a higher frequency electrical current. Tissue resistance is dependent on frequency [153] and therefore tissue conductivity may have been altered in these trials resulting in better image transmission. It is possible that an even higher increase in signal frequency may result in further enhanced signal transmission; however, research will be needed to determine the upper safe frequency limit of the capsule within equine biological tissues.

Capsule Deployment: In all eight CE trials, the capsule was successfully released in the stomach. Providing that a longer endoscope of 3m were available, it is likely that the capsule could be delivered directly into the duodenum [5]. This would have the advantage of shortening the overall transit time, hence avoiding wasting capsule battery life in the stomach. Gastric emptying may have been altered by the indwelling nasogastric tube [158] and the use of sedation, particularly detomidine [96], which likely suppressed intestinal motility during the image recording stage. Xylazine was the sedation of choice due to its minimal and transient effect [139]; however detomidine was sometimes used in CE trials based on the attending veterinarian’s preference given it is a more effective sedative and analgesic [141].

Horse Health: Horse 2 displayed pawing and stall-walking behaviour at the veterinary facility during the preparation stage in Trial 1 and 2 which may have been attributed to the stress of a new environment. These behaviours were less pronounced in subsequent trials suggesting an adaptation to this environment. Horse 2 also experienced skeletal
muscle fasciculation in Trials 1-3 using the standard capsule. To the best of our
knowledge, no such muscle tremors have been reported in humans during CE. Similar
muscle tremors in horses have been reported in cases of lidocaine toxicity, electrolyte
imbalance or when horses are brought out of anesthesia [159]. Slight muscle tremors
have also been reported shortly following administration of detomidine [160]. Given that
muscle fasciculations began two hours following sedation, it is unlikely that detomidine
was the trigger. Serum creatine kinase levels were within normal range at the time of
blood collection. Measuring serum creatine kinase levels, during or shortly after muscle
fasciculation, would be beneficial to determine presence of transient muscle injury.
Interestingly, Horse 2 did not experience muscle tremors in Trial 4 when a modified
capsule was used with double the signal strength, suggesting that the electrical current
generated by the capsule may not be triggering muscle fasciculation. Horse 1 did not
experience any muscle fasciculations suggesting that the reaction could be horse specific.

Capsule Retrieval: None of the capsules were recovered during the fecal collection
period and post-mortem search in Horse 2. It is likely that the capsule was retained for a
period of time in the cecum and expelled following the 7 day fecal collection period.
Horses were not placed in collection stalls and it is possible that some manure may have
been missed during collection. In humans, capsule retention is low at 2% and in most
cases is asymptomatic and rarely causes obstruction unless the patient has known
strictures [3]. Given the larger diameter of equine intestines, it is unlikely that the capsule
would cause an obstruction.
Study Limitations: Two horses were used in this study to minimize horse variation during the protocol testing phase of the research. The low number of horses and trials conducted did not allow for statistical analysis and results were qualitative by nature.

Troubleshooting CE protocols was challenging due to proprietary restrictions on the technical information about the MiroCam® equipment and constraints of commercial equipment developed for the end-user which resulted in lack of information on positional data and being able to identify leads receiving capsule signal.

2.5 Conclusion

In this preliminary study, quality images of the equine SI were obtained with useful clinical information on mucosal lesions and parasites but the segments lacked positional information and were of short duration. Providing that issues related to signal transmission and feed obstruction are resolved, CE has the potential to be useful as an imaging tool, however, further research and development from the manufacturer is needed before it can be effectively used in horses.
2.6 Manufacturers’ details

a Nutreco Canada Inc. Guelph, Ontario, Canada
b Life Technologies™, distributed by Laborie Canada, Brossard, Quebec, Canada
c EMLA™ cream, AstraZeneca Canada Inc., Mississauga, Ontario, Canada
d EquiCaine™, Zoetis, Kirkland, Quebec, Canada
e ConMed Canada, distributed by Stevens Medical, Brampton, Ontario, Canada
f Elastic Adhesive Tape, #1362, 3M™, London, Ontario, Canada
g Smith & Nephew, distributed by Cardinal Health Canada, Vaughan, Ontario, Canada
h Intromedic Co., Ltd., Guro-Gu, Seoul, Korea
i Rompun™, Bayer Healthcare LLC, Animal Health Division, Germany
j Dormosedan®, Orion Corporation, Espoo, Finland
k Reach® overtube, US endoscopy, Mentor, Ohio, USA
l AdvanCE®, US endoscopy, Mentor, Ohio, USA
m Merial Canada Inc., Baie-d'Urfé, Quebec, Canada

2.7 Conflict of Interest

No conflict of interest exists between the manufacturer of the endoscopic camera (Intromedic Co., Ltd.) and the author of this paper. The manufacturer provided technical support and donation of equipment, but did not influence results or outcome of the study.
CHAPTER 3: General Discussion

3.1 Project discussion
Given that CE is new to equine medicine, protocols for clearing the SI, electrode placement and capsule delivery needed to be developed prior to being able to assess its usability in the horse. Development of protocols was initially based on a literature review and required substantial troubleshooting prior to, and during CE trials to maximize image transmission and quality. These alterations led to the procedures employed in Trial 4, however additional refinements to protocols are needed to further advance CE’s usability in the horse.

3.2 Limitations of capsule endoscopy
Drawbacks of CE includes the absence of positional information in the horse, lack of control over the movement of the capsule, orientation of the camera as well as the inability to remove any bubbles or debris obstructing the camera lens [3]. At the moment diagnosis through CE is purely visual as there is no means to evaluate tissues through biopsy or intervene at the site through targeted therapy [3]. The review of video footage (over 8+ hours) can be time consuming and requires the experience of a gastroenterologist [3]. Manufacturers are attempting to resolve this issue through improved software that is capable of identifying potential pathologies (e.g. areas of gastrointestinal bleeding, tumour identification) through automated image analysis that can detect changes in colour, texture and shapes [161]. In addition, training is required to develop the ability to interpret images captured by CE.
3.3 Advances in capsule endoscopy

Advances in CE technology are aimed at improving diagnostic yield and broaden the use of CE. The next step in research is the development of ‘steerable’ capsules, allowing controlled movement [3]. Currently, there are a few capsules available on the market that can be steered using magnets; however this is limited to movement down the esophagus and guiding the capsule from the stomach into the duodenum through the pyloric sphincter. There is also interest in self-propelling capsules which are aimed at maintaining a forward direction and enhancing capsule maneuverability [14]. Ability to direct the capsule while it is in the digestive tract would greatly enhance the usability of CE and allow the development of capsules capable of taking tissue samples for biopsy and targeted drug delivery [162].

3.4 Future direction

Further research into the use of CE is warranted given the high quality of images obtained from the equine SI, which is a portion of the digestive tract that is relatively inaccessible to other imaging modalities. Further research on CE technology in the horse should investigate the effect of body size on image transmission by performing CE in ponies using a standard capsule. Patient protocols should focus on minimizing residual feed in the SI by increasing fasting period from 12 to 24 hours and providing additional water boluses throughout fasting. Further research on methods of identifying electrode sensors that are picking up signal and the strength of the signal would also be valuable in advancing CE research in the horse.
LITERATURE CITED

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APPENDICES

Appendix A: Horse health assessment sheet

Horse Health Assessment Sheet

*Horse Health Check Results*

Name of Horse:

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Inquisitive to visual /noise (Y/N)</th>
<th>Eating (Y/N)</th>
<th>Drinking (Y/N)</th>
<th>Eyes*</th>
<th>Capillary Refill (s)</th>
<th>Mucous Membranes</th>
<th>Jugular Refill (s)</th>
<th>Skin Pinch (s)</th>
<th>Heart Rate (bpm)</th>
<th>Respiratory Rate*</th>
<th>Gut Sound *(4 quadrants)</th>
<th>Attitude (description if abnormal)</th>
<th>Other</th>
<th>Recorded by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Key Actions*

In the presence of one of the Horse Health Check parameters that is within the **B category**, notify Halton Equine Veterinary Services at **905-659-4387**, during work hours and at 905-659-4387 after hours.

In the presence of one of the Horse Health Check parameters that is within the **C category**, IMMEDIATELY contact Dr. Frost at **289-260-1057**.

*See Horse Health Check Parameters and Scoring System for further details*
### Horse Health Assessment Sheet

*Horse Health Check Parameter Guideline*

<table>
<thead>
<tr>
<th>Parameter</th>
<th><strong>Green</strong> (normal range)</th>
<th><strong>Yellow</strong> (notify veterinarian)</th>
<th><strong>Red</strong> (call vet immediately)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>Bright, clear</td>
<td>Glassy</td>
<td>Fixed Stare/sunken eye</td>
</tr>
<tr>
<td>Capillary Refill</td>
<td>0 -1 seconds</td>
<td>2-3 seconds</td>
<td>4+ seconds</td>
</tr>
<tr>
<td>Mucous Membranes</td>
<td>Pink, moist</td>
<td>Pale, tacky</td>
<td>Dry, purple, blie</td>
</tr>
<tr>
<td>Jugular Refill</td>
<td>1-2 seconds</td>
<td>2-3 seconds</td>
<td>4+ seconds</td>
</tr>
<tr>
<td>Skin Pinch</td>
<td>0-1.5 seconds</td>
<td>2-3 seconds</td>
<td>4+ seconds</td>
</tr>
<tr>
<td>Heart Rate (beats per minute)</td>
<td>32-48</td>
<td>48-68 for &gt; 10 minutes</td>
<td>&gt; 68 for &gt; 10 minutes</td>
</tr>
<tr>
<td>Respiratory Rate (breaths per minute)</td>
<td>Relaxed/Regular (12-15 bpm)</td>
<td>Panting/Inversion (15+)</td>
<td>Laboured, abnormal (40+)</td>
</tr>
<tr>
<td>Gut Sounds (4 quadrants)</td>
<td>Normal</td>
<td>Reduced/Increased</td>
<td>Absent/Abnormal</td>
</tr>
<tr>
<td>Rectal Temperature</td>
<td>&lt; 38.6°C</td>
<td>38.6-40.4°C</td>
<td>&gt; 40.5°C</td>
</tr>
<tr>
<td>Attitude</td>
<td>Bright, eating, drinking</td>
<td>Depressed, lethargic</td>
<td>Dull, uninterested, absence of thirst, appetite, urination/defecation</td>
</tr>
</tbody>
</table>
### Appendix C: Horse health assessment scoring system

<table>
<thead>
<tr>
<th>Eyes</th>
<th>Symbol</th>
<th>Capillary Refill</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright, clear</td>
<td>A</td>
<td>0-1 seconds</td>
<td>A</td>
</tr>
<tr>
<td>Glassy</td>
<td>B</td>
<td>2-3 seconds</td>
<td>B</td>
</tr>
<tr>
<td>Fixed Stare/sunken eye</td>
<td>C</td>
<td>4+ seconds</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mucous Membranes</th>
<th>Symbol</th>
<th>Jugular Refill</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink, moist</td>
<td>A</td>
<td>0-1 seconds</td>
<td>A</td>
</tr>
<tr>
<td>2-3 seconds</td>
<td>B</td>
<td>2-3 seconds</td>
<td>B</td>
</tr>
<tr>
<td>4+ seconds</td>
<td>C</td>
<td>4+ seconds</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin Pin</th>
<th>Symbol</th>
<th>Heart Rate (beats per minute = bpm)</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1.5 seconds</td>
<td>A</td>
<td>32-48 bpm</td>
<td>A/Record Heart Rate</td>
</tr>
<tr>
<td>2-3 seconds</td>
<td>B</td>
<td>48-68 bpm for &gt; 10 minutes</td>
<td>B/Record Heart Rate</td>
</tr>
<tr>
<td>4+ seconds</td>
<td>C</td>
<td>&gt; 68 bpm for &gt; 10 minutes</td>
<td>C/Record Heart Rate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gut Sounds (for each quadrant)</th>
<th>Symbol</th>
<th>Respiratory Rate (beats per minute = bpm)</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal gut sounds</td>
<td>+</td>
<td>12-15 bpm</td>
<td>A</td>
</tr>
<tr>
<td>Reduced gut sounds</td>
<td>-</td>
<td>15+ bpm</td>
<td>B/Record Respiratory Rate</td>
</tr>
<tr>
<td>Absent gut sounds</td>
<td>0</td>
<td>40+</td>
<td>C/Record Respiratory Rate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rectal Temperature</th>
<th>Symbol</th>
<th>Attitude</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 38.6°C</td>
<td>A/Record Temperature</td>
<td>Bright, eating, drinking</td>
<td>A</td>
</tr>
<tr>
<td>38.6-40.4°C</td>
<td>B/Record Temperature</td>
<td>Depressed, lethargic</td>
<td>B</td>
</tr>
<tr>
<td>&gt; 40.5°C</td>
<td>C/Record Temperature</td>
<td>Dull, uninterested, absence of thirst, appetite, urination/defecation</td>
<td>C</td>
</tr>
</tbody>
</table>
Appendix D: Horse feed and water intake

Name of horse: ________________

<table>
<thead>
<tr>
<th>Trial Day</th>
<th>Date</th>
<th>Hay FED</th>
<th>Residual</th>
<th>Hay Cubes FED</th>
<th>Residual</th>
<th>Water Intake</th>
<th>Enteric Fluid Time (5L bolus)</th>
<th>Notes</th>
<th>Recorded by</th>
</tr>
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<tbody>
<tr>
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</tbody>
</table>

Start:__________________________
Finish:__________________________
Appendix E: Electrode arrangements for dermal and needle wire

E-I. Arrangement of electrode sensors (dermal) on right abdomen. Skin was clipped, shaved and washed with antiseptic soap and alcohol to remove oil residue. Sensors leads are connected to the receiver that is secured to a surcingle around the horse’s abdomen.

E-II. Arrangement of electrode sensors (needle wire) on right abdomen using a fenestrated blanket. Dermal electrodes were placed on blanket to avoid signal interference between needle wire and dermal sensors.
Appendix F: Protocol for insertion of needle wire electrodes

F-I. Administration of local subcutaneous anesthetic (mepivicaine)

F-II. Appearance of anesthetized region (indicated by red arrow) prior to needle insertion

F-III. Needle wire (indicated by red arrow), wrapped around button snap electrode

F-IV. Exposed wire secured to abdomen using adhesive film (indicated by red arrow)
Appendix G: Customized endoscope overtube and capsule delivery device

G-I. Custom, flexible endoscope reach overtube (200cm in length, 16.5mm outer diameter, 13.5mm inner diameter) placed in the horse stomach in order to protect the capsule during placement and allows for multiple endoscope entries causing minimal esophageal tissue damage.

G-II. Capsule Delivery Device was inserted into the endoscope’s biopsy channel. The capsule holder (indicated by red arrow) is threaded onto the end of cable wire of the capsule delivery device. Endoscope with the capsule delivery device was inserted into the overtube and capsule released into the stomach by the rigger release of the capsule delivery device.
### Appendix H: Reducing ration 1 (Trial 1)

**Reduction Level**

(Total Daily Feed Intake* (kg) and Intake per % Body Weight (BW) (*based on a 500kg horse)

<table>
<thead>
<tr>
<th>Reduction Level</th>
<th>Hay (kg)</th>
<th>Hay Cubes</th>
<th>Time</th>
<th>Feeding</th>
<th>Water</th>
<th>Bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td>1.5% BW (7.5 kg/day)</td>
<td>0.0% BW (0 kg/day)</td>
<td>8:00</td>
<td>3.75</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>3.75</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 1</strong></td>
<td>1.3% BW (6.5 kg/day)</td>
<td>0.2% BW (1 kg/day)</td>
<td>8:00</td>
<td>3.25</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>3.25</td>
<td>2.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td>1.1% BW (5.5 kg/day)</td>
<td>0.4% BW (2 kg/day)</td>
<td>8:00</td>
<td>2.75</td>
<td>2.25</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>2.75</td>
<td>2.25</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td>0.9% BW (4.5 kg/day)</td>
<td>0.6% BW (3 kg/day)</td>
<td>8:00</td>
<td>2.25</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>2.25</td>
<td>2</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td>0.7% BW (3.5 kg/day)</td>
<td>0.8% BW (4 kg/day)</td>
<td>8:00</td>
<td>1.75</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>1.75</td>
<td>1.5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td>0.5% BW (2.5 kg/day)</td>
<td>1.0% BW (5 kg/day)</td>
<td>8:00</td>
<td>1.25</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>1.25</td>
<td>1</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 6</strong></td>
<td>0.3% BW (1.5 kg/day)</td>
<td>1.2% BW (6 kg/day)</td>
<td>8:00</td>
<td>0.75</td>
<td>½</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>0.75</td>
<td>½</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>0.0% BW (0 kg/day)</td>
<td>1.5% BW (7.5 kg/day)</td>
<td>8:00</td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20:00</td>
<td>3.75</td>
<td></td>
<td></td>
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</tbody>
</table>

**Preparation Stage**

Day 8

**End of Recording**

<table>
<thead>
<tr>
<th>Electrode Placement</th>
<th>Capsule Introduction</th>
<th>Imaging Recording Stage</th>
<th>FASTING (12 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>10:00</td>
<td>12:00</td>
<td>5L</td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td>13:00</td>
<td>0.25</td>
</tr>
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<td>14:00</td>
<td></td>
<td>15:00</td>
<td>0.25</td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td>17:00</td>
<td>0.25</td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td>19:00</td>
<td>0.25</td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td>21:00</td>
<td>0.25</td>
</tr>
<tr>
<td>22:00</td>
<td></td>
<td>22:00</td>
<td>2.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Bucket</th>
<th>Bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75</td>
<td>2</td>
</tr>
<tr>
<td>1.25</td>
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</table>
Appendix I: Reducing ration 2 (Trial 2-4)

<table>
<thead>
<tr>
<th>Reduction Level</th>
<th>Feeding</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>Hay (kg)</td>
</tr>
<tr>
<td>Total Daily Feed Intake* (kg) and Intake per % Body Weight (BW) (*based on a 500kg horse)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 1</strong></td>
<td>8:00</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>12:00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16:00</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>20:00</td>
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</tr>
<tr>
<td></td>
<td>22:00</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td>8:00</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>12:00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16:00</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>19:00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22:00</td>
<td></td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td>8:00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12:00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16:00</td>
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<tr>
<td></td>
<td>19:00</td>
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</tr>
<tr>
<td></td>
<td>22:00</td>
<td>FASTING (12 hours)</td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
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<td>Electrode Placement</td>
</tr>
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<td></td>
<td>10:00</td>
<td>Capsule Introduction</td>
</tr>
<tr>
<td></td>
<td>12:00</td>
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<td>13:00</td>
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<td>14:00</td>
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<td>15:00</td>
<td>Imaging Recording Stage</td>
</tr>
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<td>18:00</td>
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<tr>
<td></td>
<td>20:00</td>
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<tr>
<td></td>
<td>22:00</td>
<td>End of Recording</td>
</tr>
</tbody>
</table>