1. Introduction

Colic has a high incidence in horses and represents a major cause of mortality. Advancements in gastrointestinal surgery have significantly improved its management and prognosis. However, there remains a need for continued research in this area, and this work seeks to contribute to the evidence-based diagnosis and treatment of intestinal ischaemia in horses. The pathophysiology of ischaemia-reperfusion injury (IRI) in the equine intestine is complex, with many different intestinal lesions that elicit intestinal IRI. Understanding the underlying pathophysiology of colic can lead to new treatment strategies and improved outcomes for affected horses. In regard to intraoperative diagnostics, there are hardly any options for diagnostic tools that can reliably assess the viability of intestinal tissue during colic surgery.

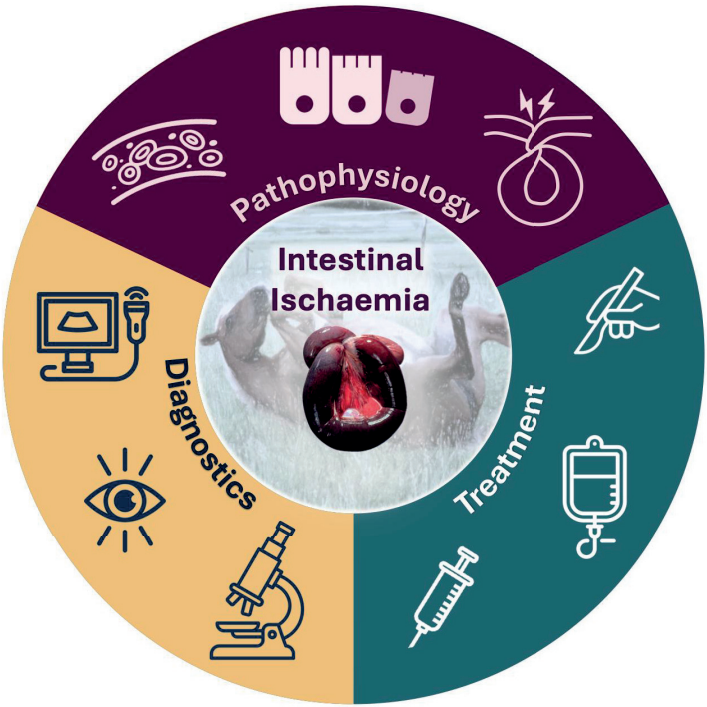

Figure 1. Overview of contents.

This needs to be addressed to enable better treatment and prognostication of ischaemic intestinal injury. Treatment strategies for equine colic must also adapt to ongoing research findings in other species. Pharmacological options, such as the systemic administration of alpha-2-adrenoreceptor agonists, show promise in mitigating the effects of IRI, yet their application in clinical settings requires further investigation. By exploring new approaches in pathophysiology, diagnostics and treatments, this research aims to address the gaps in current knowledge and, by doing so, to improve the outcomes for horses with colic.

2. List of Publications

1. Grages, A. M.*, **Verhaar, N.***, Pfarrer, C., Breves, G., Burmester, M., Neudeck, S., & Kästner, S. (2022). Low Flow versus No Flow: Ischaemia Reperfusion Injury Following Different Experimental Models in the Equine Small Intestine. *Animals*, *12*(16), 2158.

2. Dengler, F., Sternberg, F., Grages, M., Kästner, S. B.*, & **Verhaar, N.*** (2022). Adaptive mechanisms in no flow vs. low flow ischemia in equine jejunum epithelium: Different paths to the same destination. *Frontiers in Veterinary Science*, *9*, 947482.

3. **Verhaar, N.**, Geburek, F., Cuevas Ramos, G., & Skov Hansen, S. (2025). Mesenteric rents in the ascending mesocolon as a cause of colic—A retrospective case series. *Equine Veterinary Education*, *37*(11), e275-e282.

4. **Verhaar, N.**, & Geburek, F. (2025). Real-time ancillary diagnostics for intraoperative assessment of intestinal viability in horses-looking for answers across species. *Veterinary Surgery, 54*(4), 648–664.

5. **Verhaar, N.**, Grages, A. M., Bienert-Zeit, A., Schwieder, A., Reineking, W., Hewicker-Trautwein, M., Kästner, S.*, & Geburek, F.* (2024). Flowmetry and spectrophotometry for the assessment of intestinal viability in horses with naturally occurring strangulating small intestinal lesions. *Equine Veterinary Journal*, *56*(6), 1138–1148.

6. **Verhaar, N.**, Grages, A. M., Sauer, F. J., Geiger, T., Reineking, W., Hewicker-Trautwein, M., Geburek, F.*, & Kästner, S. B. R.* (2024). Measuring tissue oxygen saturation in the orad intestinal segment during equine colic surgery may aid in predicting the occurrence of postoperative ileus. *American Journal of Veterinary Research*, *85*(7), ajvr.23.12.0286.

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7. **Verhaar, N.**, Reineking, W., Hewicker-Trautwein, M., Grages, A. M., Kästner, S. B. R.*, & Geburek, F.* (2024). Flowmetry and spectrophotometry can detect reduced intestinal microperfusion in nonsurvivors during equine colic surgery for large intestinal strangulation. *American Journal of Veterinary Research*, *85*(10), ajvr.24.05.0142.

8. **Verhaar, N.**, Hammer, E., Reineking, W., Hewicker-Trautwein, M., & Geburek, F. (2025). Ex vivo comparison of full-thickness biopsy techniques in the equine small intestine. *Veterinary Surgery, 54*(1), 208–218.

9. **Verhaar, N.**, Hoppe, S., Grages, A. M., Hansen, K., Neudeck, S., Kästner, S.*, & Mazzuoli-Weber, G.* (2023). Dexmedetomidine Has Differential Effects on the Contractility of Equine Jejunal Smooth Muscle Layers In Vitro. *Animals*, *13*(6), 1021.

10. König, K. S.*, **Verhaar, N.***, Hopster, K.*, Pfarrer, C., Neudeck, S., Rohn, K., & Kästner, S. B. R. (2020). Ischaemic preconditioning and pharmacological preconditioning with dexmedetomidine in an equine model of small intestinal ischaemia-reperfusion. *PloS one*, *15*(4), e0224720.

11. VanderBroek, A. R., Engiles, J. B., Kästner, S. B. R., Kopp, V., **Verhaar, N.**, & Hopster, K. (2021). Protective effects of dexmedetomidine on small intestinal ischaemia-reperfusion injury in horses. *Equine Veterinary Journal*, *53*(3), 569–578.

12. **Verhaar, N.**, Kopp, V., Pfarrer, C., Neudeck, S., König, K., Rohn, K., & Kästner, S. (2023). Alpha$_2$ Antagonist Vatinoxan Does Not Abolish the Preconditioning Effect of Dexmedetomidine on Experimental Ischaemia-Reperfusion Injury in the Equine Small Intestine. *Animals, 13*(17), 2755.

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3. List of Abbreviations

BF	Biopsy Forceps
BP	Biopsy Punch
CMA	Cranial Mesenteric artery
cm	Centimeter
CRI	Continuous rate infusion
CSM	Circular Smooth Muscle
DXM	Dexmedetomidine
DIS	Disseminated Intravascular Coagulation
DMSO	Dimethyl-Sulfoxide
ECA	Electrical Control Activity
ENS	Enteric Nervous System
ERA	Electrical Response Activity
FA	Fluorescence Angiography
GALT	Gut-Associated Lymphoid Tissue
HIF	Hypoxia Inducible Factor
ICG	Indocyanine Green
IPreC	Ischaemic Preconditioning
IPoC	Ischaemic Postconditioning
IRI	Ischaemia-Reperfusion Injury
LDF	Laser Doppler Flowmetry
LDFS	Laser Doppler Flowmetry and Spectrophotometry
LSM	Longitudinal Smooth Muscle
LW	Longitudinal Wedge
m	Meter

MIS	Mucosal Injury Score
MMC	Migrating Myoelectrical Complex
NIRS	Near-infrared Spectrophotometry
POR	Postoperative Reflux
PPreC	Pharmacological Preconditioning
PPoC	Pharmacological Postconditioning
ROS	Reactive Oxygen Species
SIRS	Systemic Inflammatory Response Syndrome
StO_2	Tissue Oxygen Saturation
tBF	Tissue Blood Flow
tHB	Tissue Haemoglobin
TW	Transverse Wedge

4. Scientific Background

4.1. Intestinal anatomy and function

4.1.1. Macroscopic anatomy

The small intestine is the longest section of the gastrointestinal tract in the horse, measuring approximately 25 m in length. It consists of three parts: the duodenum, the jejunum, and the ileum, attached to the dorsal abdominal wall by the mesentery. The mesentery becomes progressively longer going aborad, thereby allowing greater mobility of the jejunum within the abdominal cavity. The aboral boundary of the duodenum is indicated by the duodenocolic fold, and the transition from the jejunum to the ileum is marked by the presence of the ileocaecal fold, which attaches the antimesenteric side of the ileum to the caecum. The end of the ileum is formed by the ileal papilla at the ileocaecal ostium, which is located on the axial side of the base of the caecum (Krunkosky et al. 2017; Nickel et al. 2005; Budras et al. 2003; Freeman 2019)

The large intestine includes the caecum, colon, and rectum. All large intestinal segments have sacculations (*Haustrae*) and a varying number of longitudinal bands (*Taeniae*). The blind-ending caecum is approximately 1 metre in length and consists of a base, body, and apex. Dorsally, the caecum is attached to the right kidney, the pancreas, and the abdominal wall. At the caecocolic ostium, located at the base of the caecum, the ascending colon originates with the right ventral colon. This colon section is attached to the cecum by the ceacocolic ligament. The ascending colon consists of a dorsal and ventral segments, that are interconnected by the ascending mesocolon. The ascending colon's only attachments are at its origin and termination, making it relatively mobile and prone to displacements. At the level of the base of the caecum, the right dorsal colon forms the junction with the transverse colon. The transverse colon passes from right to left cranial to the root of the mesentery, fixed in position by its short mesenteric attachment to the dorsal abdominal wall. It continues as the descending colon, followed by the rectum, with a combined length of 3 to 4 m. The

descending colon is attached by a longer mesentery, the descending mesocolon (Krunkosky et al. 2017; Nickel et al. 2005; Budras et al. 2003; Prange et al. 2019).

4.1.2. Small and large intestinal vascularisation

The cranial mesenteric artery (CMA, *Arteria mesenterica cranialis*) supplies most intestinal segments. The small intestine's arterial supply consists of multiple jejunal arteries and the ileal artery, a branch from the ileocaecocolic artery. The major jejunal arteries continue as arcuate vessels that form a loop with the next jejunal vessel. From the arcuate vessels, several vasa recta pass to the intestinal wall (Dart et al. 1992a). In all intestinal segments, the venous system closely follows the arterial supply and enters the portal vein (Snyder et al. 1989; Dart et al. 1991; Dart et al. 1992a; Dart et al. 1992b).

Continuing with the large intestine, the caecum and the ventral ascending colon are supplied by the caecal and colic arteries that arise from the ileocaecocolic artery. The dorsal ascending colon is supplied by the right colic artery, also a branch of the CMA. In the caecum and ascending colon, many smaller arteries branch from the main vessels to anastomose with adjacent vessels, creating a colonic or caecal rete before continuing into the intestinal wall (Snyder et al. 1989; Dart et al. 1991).

The middle colic artery is the final branch of the CMA and supplies the transverse and cranial descending colon. The caudal mesenteric artery supplies the caudal descending colon via anastomosing branches of the left colic artery and the cranial rectal artery. The rectum is supplied by the rectal arteries (*A. rectalis cranialis*, *media* and *caudalis*). Similar to the jejunum, the arteries of the descending colon continue as an arcuate artery that subsequently splits into a cranial and caudal marginal artery. These run parallel to the small colon and anastomose with the adjacent arcuate arteries. A secondary anastomosing arcade originates from the marginal arteries and penetrates the wall of the small colon (Dart, Snyder, and Harmon 1992).

4.1.3. Microscopic anatomy

The intestinal wall of the small and large intestine has a similar structure and consists of four layers: the *Tunica mucosa, Tela submucosa, Tunica muscularis* and the *Tunica serosa*.

Beginning with the *Tunica mucosa*, this layer forms the barrier between the lumen and the tissues. The epithelium is situated on a basal membrane, and the epithelial cells are held together by tight junctions, resulting in a closed paracellular space. Besides the absorptive enterocytes, the mucosa also contains the intestinal glands (*Glandulae intestinales*) with goblet, paneth and enteroendocrine cells. The surface area is increased by luminal folds, that are circular in the small intestine and longitudinal in the large intestine. In the small intestine only, the surface area is further increased by villi (*Villi intestinales*). Underneath the epithelium, the *Lamina propria mucosae* contains the blood supply and immune cells, such as neutrophilic granulocytes, eosinophilic granulocytes, plasma cells and macrophages. Furthermore, the gut-associated lymphoid tissue (GALT) extends from the submucosa into the lamina propria of the mucosa. The *Lamina muscularis mucosa* forms the border to the submucosa (Young et al. 2009; Liebich 2010; Bacha Jr and Bacha 2012; Krunkosky et al. 2017).

The *Tela submucosa* consists of connective tissue and contains the intestinal blood vessels and the submucosal plexus of the enteric nervous system (Meissner's plexus). Furthermore, it houses the lymphoid tissue, with solitary lymphatic nodules and the previously mentioned GALT. Submucosal glands are present in the duodenum and the jejunum, and they empty into the fundus of the intestinal glands of the mucosa (Young et al. 2009; Liebich 2010; Bacha Jr and Bacha 2012; Krunkosky et al. 2017).

The *Tunica muscularis* consists of two layers of smooth muscle: the more prominent inner circular smooth muscle (CSM) layer (*Stratum circulare*) and the outer longitudinal smooth muscle (LSM) layer (*Stratum longitudinale*). These layers are separated by a fascia of connective tissue, that contains the second plexus of the enteric nervous system (myenteric or Auerbach's plexus), accompanied by blood and lymph vessels. There is some variation in the *Tunica muscularis* between the different intestinal segments. In the ileum, it is clearly thicker than in the other small intestinal segments. In the colon, the longitudinal muscle layer is locally thickened and enforced with elastic fibers to form the *Taeniae* (Young et al. 2009; Liebich 2010; Bacha Jr and Bacha 2012; Krunkosky et al. 2017).

The *Tunica serosa* forms the outermost layer. It consists of the mesothelium and its associated *Lamina propria serosae*, which is connected to the *Tunica muscularis* by a layer of connective tissue, the *Tela subserosa*. The serosa

transitions into the mesentery at the level of the mesenteric attachment (Young et al. 2009; Liebich 2010; Bacha Jr and Bacha 2012; Krunkosky et al. 2017).

Looking at the microvascularisation within the intestinal wall, this is similar in the small and large intestine. The arteries enter the submucosa through the *Tunica muscularis*, and form a submucosal plexus that supplies all layers of the intestinal wall. Arterioles extend from here into the mucosa where extensive capillary networks form around the crypts (Snyder et al. 1989; Dart et al. 1991; Dart, Snyder, and Harmon 1992; Dart et al. 1992). Specifically in the small intestine, the villi are supplied by an arteriole that spirals to the tip, where it forms another capillary network (Dart et al. 1992).

4.1.4. Intestinal motility

The *Tunica muscularis*, with its two smooth muscle layers, is responsible for the propagation of the intestinal contents. Contraction of the circular muscle narrows the intestinal lumen and lengthens the segment, whereas the longitudinal muscle shortens the intestinal segment and widens the lumen (Nieto and Rakestraw 2017). Because of these antagonistic functions, the contractility must be temporally and spatially coordinated. This starts at the level of the myocytes, which exhibit pacemaker activity or electrical control activity (ECA), also known as slow waves. In the absence of neural or chemical stimulation, ECA remains under the threshold for an action potential, and electrical response activity (ERA) cannot occur. When the myocytes are subjected to neurochemical excitation, ECA depolarization exceeds the excitation threshold potential, leading to an ERA burst and smooth muscle contraction. The adjacent myocytes are connected, and the electrical current is passed on through gap junctions. Since ERA and the resulting contractile activity are superimposed on the slow waves, the slow waves determine the maximal frequency of contractions. This frequency is higher in the proximal segment of each intestinal region and decreases moving distally, facilitating aboral transit (Szurszewski 1969; Sarna et al. 1983). The pacemaker activity is initiated by the interstitial cells of Cajal (Ward et al. 2000). For the activation of ERA, the enteric nervous system (ENS) comes into play through a rhythmic activity called the migrating myoelectrical complex (MMC) (Szurszewski 1969; Bueno et al. 1975; Code and Marlett 1975). Depending on the stage of digestion, different phases in the MMC exhibit