

# The Evaluation of Small Intestinal Volvulus Caused by Pathogenic Microorganisms in a Thoroughbred Mare

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**Background:** Small intestinal (SI) volvulus is defined as a rotation of greater than 180 degrees about its mesentery of a segment of jejunum or ileum. Horses of all ages have been affected. There is typically an acute onset of signs of mild to severe pain.

**Objectives:** The objective of this study was to evaluate the microbial pathogens of the duodenum, ileum, cecum, colon and rectum (feces) in associated with volvulus horse, and to determine whether rectal (fecal) samples are representative of proximal segments of the gastrointestinal tract.

**Materials and Methods:** A brown 26 years old mare, BCS (body condition score) 4 was found dead in stall in the morning. It was moved to a suitable area to conduct a post-mortem exam. The mare was examined in hanging position and then left lateral-recumbent. Advanced abdominal tympany was present. Clinical signs, laboratory data, surgical or necropsy findings, clinic-histopathological findings and outcome for horse with SI volvulus was obtained from medical records, and identified by manual review. Horsefeces and colon were collected in autopsy. Fecal material was scooped from the center of a freshly defecated bolus into sterile sample cups, which were placed into plastic anaerobe jars with PackAnaero sachets (Mitsubishi Gas Co. via Remel, Lenexa, KS) and transported to the laboratory. Alternatively, colon contents were collected from horse at the autopsy by direct incision into the colon immediately after the horse was autopsied. The samples were transported anaerobically to the laboratory.

**Results:** On opening the abdominal cavity; a large quantity of sanguineous, foul-smelling fluid with pus exited the perforated bowel wall (hemoperitoneum). Additionally, signs of an acute diffuse peritonitis were visible. The blood vessels of the stomach and intestines were distended. Small intestinal volvulus was observed in several segments (360 degree rotation involving the mesentery). This information may aid diagnosis and prognosis and guide to decision making for horses with this condition.

**Conclusions:** The present study results can be used to make a scientific assessment of prognosis in the pre-operative, operative, and post-operative management of horses with small intestinal volvulus. Bacterial infectivity results from a disturbance in the balance between bacterial virulence and host resistance. The "objective" of bacteria is to multiply rather than to cause disease; it is in the best interest of the bacteria to kill the host. Rectal samples were not entirely representative of intestinal compartments in the small or large intestine. This should be taken into account when designing studies using fecal sampling to assess other intestinal compartments, suggesting that parts of the intestinal microbiota were unique to each animal in this study.

**Keywords:** Intestinal Compartments; Fecal Bacteria; Small Intestinal

## 1. Background

Small intestinal (SI) volvulus occurs in 4.6% of horses with colic admitted to teaching hospitals in the USA (1). Early reported survival rates for small intestinal obstruction were low (2). In a recent retrospective study of 224 strangulating small intestine lesions, only 50% of horses recovered from surgery survived to discharge (3). The absolute twist of the intestine around the mesenteric attachment is named volvulus. This sort of disease is one of the remarkable causes of severe colic of equine abdomi-

nal cavity (1). SI volvulus is currently defined as a rotation > 180 of a segment of jejunum or ileum about its mesentery. The pathogenesis of primary volvulus is thought to involve hyperactive peristalsis, adjacent to an intestinal segment with temporary or permanent arrest of peristalsis, where the sudden arrest in forward peristalsis may cause the intestine to twist. SI volvulus may develop as a primary displacement or secondary to pre-existing lesions such as a lipoma (4), acquired inguinal hernia (5), mesodiverticular band (6), Meckel's diverticulum (7), mesenteric rents, vitelloumbilical bands, or adhesions

### Implication for health policy/practice/research/medical education:

To determine historical data, physical, signalment, clinical signs, clinical factors and results of laboratory analyses, that may affect morbidity and mortality in horses with SI volvulus unrelated to other causes.

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(8). The historical data, signalment, clinical signs, and outcome of horses with volvulus secondary to strangulating pedunculated lipomas have been described (5). The equine intestinal microbiota is complex and has an enormous impact on digestion, immune stimulation, pathogen protection and metabolism (1). Studies investigating the equine intestinal microbiota commonly use fecal material because of the ease of sampling (2). Significant differences in the microbial composition along the gastrointestinal tract have been reported in dogs and cattle (3, 4), but to date few studies have assessed whether microbial populations in feces are representative of proximal compartments of the GI tract in horses.

## 2. Objectives

To our knowledge, a large series of horses with primary SI volvulus has not been reported. The outcome and prognosis of surgical colic cases can be difficult to predict and is highly dependent on the nature and severity of the lesion (9). Therefore the purpose of our study was to investigate the historical data, pathological examination findings, results of laboratory analyses, and outcome of surgical and post-surgical intensive care of horses with a diagnosis of primary volvulus at surgery or necropsy.

## 3. Materials and Methods

### 3.1. Criteria for Diagnosis

June 2013, a brown 26 year-old mare, BCS (body condition score) 4 was found dead in the stall in the morning. It was moved to a suitable area to conduct a post-mortem examination (Figure 1A). The mare was examined in hanging position and then left lateral recumbency. Advanced abdominal tympany was present, medical records of a horse admitted to the Veterinary Teaching Hospital and large animal field services of Tehran University. Furthermore, case was identified from a clinico-histopathological database using the terms "volvulus" and "torsion." SI volvulus was defined as a rotation  $> 180^\circ$  of a segment of small intestine along the axis of the mesentery. Horse with a lesion recognized to predispose to volvulus (strangulating lipoma, mesenteric rent, mesodiverticular band, inguinal hernia, etc.) was excluded.

### 3.2. Case Presentation Findings

Clinical signs, laboratory data, surgical or necropsy findings, clinic-histopathological findings and outcome for horse with SI volvulus was obtained from medical records, identified by manual review.

### 3.3. Data Retrieved and Hospital Population

Data for horse was obtained from the medical record. The year of admission, age, breed, and sex were recorded. Collected historical information included any previous

episodes of abdominal pain and their nature (as reported by the owner), duration of pain signs before surgery, and drugs administered by the referring veterinarian.

### 3.3. Microbial Isolation and Identification of Horse Intestinal Bacteria and other Pathogens

Horse feces and colon were collected in autopsy. Fecal material was scooped from the center of a freshly defecated bolus into sterile sample cups, which were placed into plastic anaerobe jars with Pack Anaero sachets (Mitsubishi Gas Co. via Remel, Lenexa, KS) and transported to the laboratory. Alternatively, colon contents were collected from horse at the autopsy by direct incision into the colon immediately after the horse was autopsied. The samples were transported anaerobically to the laboratory, where they were immediately transferred to an anaerobic chamber with an atmosphere of 91 to 93% N<sub>2</sub>, 2 to 4% H<sub>2</sub>, and 5% CO<sub>2</sub> (Coy, Grass Lake, MI) and cultures were set up in broth and agar media. Glycerol was added to the remaining material to a final concentration of 10%, and the samples were frozen at  $-70^\circ\text{C}$  for later assays. Bacteria were isolated on *brucella* blood agar, *Bacteroides* bile esculin agar, laked blood kanamycin-vancomycin agar, egg yolk agar, *Bifidobacterium* selective agar, *Bacteroides vulgatus* selective agar, phenylethyl alcohol agar, and chocolate agar (all from Anaerobe Systems, Inc., San Jose, CA). All bacterial isolations were carried out in the absence of ceftiofur selective pressure. Cultures were incubated for 48 h at  $35^\circ\text{C}$  in the anaerobic chamber. Anaerobic (MTGE) enrichment broths were inoculated from anaerobic transport swabs collected at the time of sampling (Anaerobe Systems). These were cultured overnight anaerobically at  $35^\circ\text{C}$  and then subcultured to the same plate media described above for bacterial isolation. Additional media (Trypticase soy agar with 5% sheep blood, MacConkey agar, phenylethyl alcohol agar, and tetracycline agar from Remel) were inoculated, removed from the anaerobic chamber, and incubated in a 5% CO<sub>2</sub> aerobic incubator at  $37^\circ\text{C}$  overnight. The isolated bacteria were frozen in sterile SS stabilizer (8% nonfat dry skim milk, 5% sucrose, 0.5% thiourea) for subsequent evaluations. Finally, serology, virology and others were analyzed by laboratory experts that certain results have not been achieved from those tests.

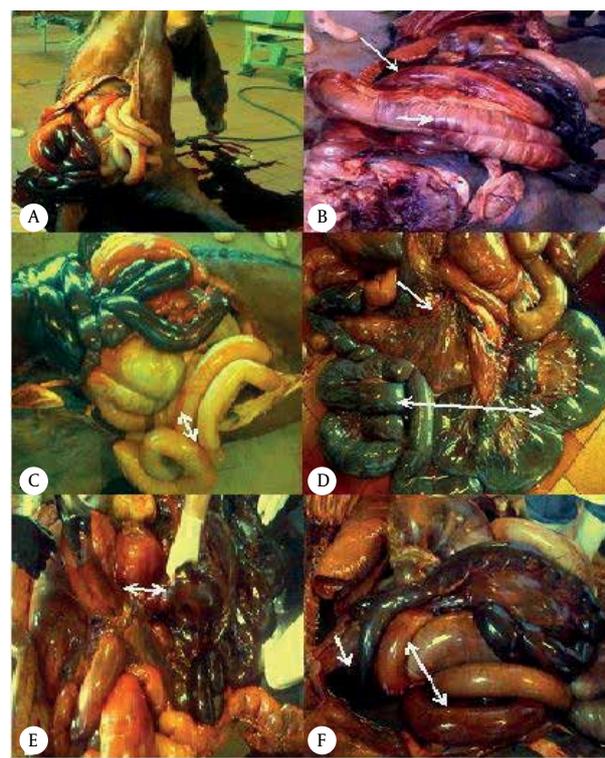
## 4. Results

In October 2012, A brown 26 year-old mare, BCS (body condition score) 4 was found dead in the stall in the morning. It was moved to a suitable area to conduct a post-mortem examination (Figure 1A). The mare was examined in hanging position and then left lateral recumbency. Advanced abdominal tympany was present. On opening the abdominal cavity; a large quantity of sanguineous, foul-smelling fluid with pus exited the perforated bowel wall (hemoperitoneum) (Figure 1F).

Additionally, signs of an acute diffuse peritonitis were visible. The blood vessels of the stomach and intestines were distended. Small intestinal volvulus was observed in several segments (360 degree rotation involving the mesentery). The affected loops discolored either dark red or black which was due to congestive hemorrhage and there was a line of demarcation between intact and affected portions (Figure 1 E and D). Also invasion of the putrefactive bacteria to the bowel wall was observed (an infarcted gangrenous intestinal wall is readily permeable to putrefactive bacteria) (Figure 1 D, E and F). The small intestine was distended with fluid and gas contained dark-colored ingesta mixed with blood. The mucosa was ulcerated and necrohemorrhagic enteritis was detected (Figure 1 B, and C). On handling these loops, the affected intestinal loops, easily identifiable by its red-brown discoloration and dark-colored fluid could be rubbed off the serosal layer. Multiple ecchymotic hemorrhages were diffusely present throughout the small intestine, mesentery, and large intestines (Figure 1 C). The stomach contained normal chymus and no abnormalities were seen though vascular dilatation and serosal hemorrhage. No parasites were observed in any portion of the gastrointestinal tract. The left dorsal colon was displaced with the pelvic flexure over the renosplenic ligament (known as entrapment of the colon by the nephrosplenic space). The pelvic flexure was hyperemic and distended with gas accumulation. The diaphragm was incised; a rush of air was heard to enter the thoracic cavity, a normal finding. As the diaphragm was incised deeper, a little serosanguinous pleural effusion exited the cavity. The left lung lobes and right caudal lung lobe were normal and no exudate was present but right cranial lobe was slightly hyperemic and hemorrhagic. The heart and pericardium had normal appearance. However, the necropsy findings indicated the death was due to complications arising from volvulus such as neurogenic and/or hypovolemic shocks, intestinal perforation and peritonitis. It seems likely this mare developed colic overnight and this contributed to her death. The mucosa was ulcerated and necrohemorrhagic enteritis was obvious. On handling these loops, the affected intestinal loops, easily identifiable by its red-brown discoloration and dark-colored fluid could be rubbed off the serosal layer. Multiple ecchymotic hemorrhages were diffusely present throughout the small intestine, mesentery, and large intestines. The horse death was due to volvulus, tissue ischemia, hypoxia, necrosis along with infarction and finally septicemia shock (probably due to necrosis or/and hypovolemic). The microbiology tests demonstrated the possible role of proteus mirabilis, especially clostridium perfringens, inducing toxemia or septicemia. Furthermore, the test result was negative for EVA virus. However, the necropsy findings indicate a diagnosis of death due to complications arising from volvulus such as neurogenic and/or hypovolemic shocks, intestinal perforation and peritonitis. It seems this mare

affected with colic overnight and this contributed to her death.

**Figure 1.** The Examined Mare



## 5. Discussion

There is little reported information on the clinical features of horses with primary small intestinal volvulus. We attempted to derive this information, based on a retrospective analysis of 1 horse admitted to one referral Veterinary Teaching Hospital (VTH) of the Tehran regional College of Veterinary Medicine is located on the Karaj teach campus in Iran. The outcome and prognosis for horses after surgery for colic can be difficult to predict and is highly dependent on the nature and severity of the lesion (10). In this study the compartment specific data was not analyzed separately for horse, and significant difference between the microbiota of horse was identified. Thus, the inclusion of horse could have influenced the compartment specific data and could explain the difference in results of our study (10). also found that the microbiota of the feces is representative of the distal colon; however, only certain bacterial species (*Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Streptococcus bovis*) were tested, therefore potentially biasing results. Schoster et al. (2) investigated the presence of *C. difficile* in various intestinal compartments of horses and found that rectal samples were only positive in 63% of animals from which *C. difficile* could be isolated from one or more

proximal compartments. The data from the present study lend further credence to concerns that the fecal microbiota is not representative of the entire gastrointestinal tract. It further supports the concerns raised about the accuracy of using fecal samples for investigation and interpretation of changes in the microbiota of proximal gastrointestinal compartments, which is commonly performed (5). To our knowledge, few studies report outcome in horses with primary SI volvulus (defined as volvulus unrelated to other apparent causes like incarceration, lipoma, mesenteric rent, etc.). Thus, our purpose was to investigate historical data, signalment, clinical signs, physical examination findings, results of laboratory analysis, surgical, necropsy and histopathological examinations of horse with a diagnosis of primary volvulus at surgery or necropsy, in relation to outcome (survival or non-survival). Our goal was to determine clinical and clinicopathologic factors associated with hospital mortality for large colon volvulus. Colic is one of the most difficult diseases to study with epidemiologic methods due to the large number of diseases cause colic (abdominal pain) as a clinical sign. Historically, there has been concern that analgesics administered before referral might mask signs of surgical colic on admission to referral hospitals (11). The increased frequency of severe pain on admission associated with previous but orphanol administration may reflect the fact that the most painful horses were administered but orphanol, and by admission, this was no longer effectively controlling their pain. Two factors should be considered when trying to prevent colic; farm factors and horse factors (12-14). Farm factors include management, use, feeding, and environment. The associated risks on farms with high rates of colic include poor parasite control, high concentrate levels in the diet, multiple sources of concentrates (including supplements which contain higher than suspected amounts of soluble carbohydrates), chronic deficiency in water, excessive use of NSAIDs, acute changes in hay or grain, and horses in training that are confined and fed large amounts of soluble carbohydrates and lesser amounts of roughage. Altering these risks with management has decreased the incidence of colic on farms with a higher than average annual colic rate. Farms with a high incidence of colic (> 10 colic cases per 100 horses per year) careful monitoring of the daily management and measurement of the energy, protein and fiber in the diet should be the first steps in assessing the farm for colic risk.

Incomplete data was a particular problem, and some assumptions were made. Where a specific historical factor, physical finding, or treatment was not recorded, it was assumed to be absent in that horse, provided there was other data for the same variable. In this horse, the variable may have been measured, but not recorded. Similarly, there was more detail in surgical reports compared with others. The case reported here was assumed to be the most common form of volvulus where the mesentery

twists the intestine into distinct spirals. Another weakness of retrospective studies is that the circumstances surrounding clinical decisions are not always evident from the record. Therefore, economic decisions regarding euthanasia, treatments administered, and the length of postoperative hospitalization are not always apparent and can influence the data. The horse in our study did not survive and was euthanatized. Although there was good clinical evidence in some cases that survival was unlikely (e.g. ruptured intestine), the historical data may be less accurate than the data directly recorded at the clinic, as it relies on information reported by third parties, and additionally in case of duration of pain and owner observation of the horse. The major weakness of our study was the lack of long-term outcome data. We believe that this data demonstrates that information on hospital outcome is still very relevant to clinicians and owners, making decisions at the time surgery is recommended.

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## Authors' Contribution

All authors have participated equally in this study.

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There is no conflict of interest.

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