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# COLIC SURGERY IN THE EQUINE PATIENT: NOVEL BIOMARKERS, POSTOPERATIVE MANAGEMENT, MICROBIOTAL INFLUENCE AND EPIDEMIOLOGICAL ANALYSIS ON THE ITALIAN TERRITORY

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# Chapter 1 – INTRODUCTION

Colic syndrome, defined as clinical signs of abdominal pain, is the most frequent emergency faced by equine veterinary surgeons and admitted to referral clinics [19], it also represents the most common cause of morbidity and mortality in managed equine populations [63]. Some studies have estimated that colic episodes per year can range from 3.5 to 10.6 every 100 equids [33,59,70,155,156], while the majority of these cases resolves spontaneusly or with medical therapy only, about 8% of them requires surgery or euthanasia [59,119].

First attempts of abdominal surgery in the horse go back to the late 1800s [95], but surgical treatment for colic has become commonly performed only from the 1970s [136]. Since then, our knowledge on epidemiology and pathophysiology of colic has remarkably increased, leading to advances in diagnostic tools, surgical technique and postoperative management, safer anaesthesia, and awareness of the importance of early referral; all these factors have caused morbidity and mortality rates to improve [48,100,147].

Nevertheless, furher studies need to be conducted to further investigate diagnostic and prognostic factors in order to better asses the clinical status of the patient, improve aftercare to increase survival rates and deepen our knowledge in the epidemiology of colic [48].

# Chapter 2 – ROLE OF BIOMARKERS IN COLIC PATIENTS

Traditionally, colic is classified as medical or surgical in nature [118]. Early recognition of which category a patient fits in is essential to rapidly intervene, and therefore improve the outcome [90]. Some abdominal affections of inflammatory origin, that have to be treated medically and in which surgery could only worsen the condition, may present with the same clinical signs as strangulations or displacements that require surgical intervention [46,65]. Therefore, as in human medicine, the study of novel biomarkers that may help differenciating between surgical and medical intestinal diseases has been and keeps on being a vital research area [22]. Unfortunately, among all investigated biomarkers, none seems to be completely accurate for disease diagnosis [97,116].

Moreover, surgical colics can be a burden both emotionally and economically to owners, making it important to give an accurate prognosis [45]. Some examples of biomarkers used as prognostic tools in colic patients include: blood lactate, plasma D-dimer, plasma muscle enzymes (aspartate aminotransferase and creatine kinase(CK)), serum amyloid A, serum haptoglobin, and bile acids [24,79,156,160,173].

Ideally, the most effective diagnostic marker should be both greatly sensitive and specific for intestinal ischemia, and also measurable in both blood and peritoneal fluid samples [85].

Abdominocentesis and peritoneal fluid analysis are commonly performed during examination of horses with colic syndrome or other abdominal diseases. Quantification of some biomarkers, and their correlation to their plasma

concentration, is a useful mean in differenciating between medical and surgical pathologies [94]. Among these molecules, the most important and more commonly evaluated in equine practice is lactate [138]. Nevertheless, other molecules such as D-dimers, transforming growth factor beta 1, CK, lactate dehydrogenase and bile acids have been studied as well [4,75,127,145].

The first aim of this PhD program was to search for new biological molecules to use as marker in order to more precisely, and possibly earlier, make a diagnosis and give the owner a prognosis.

In this process, the cooperation with the University of Pisa has been essential.

The main biomarkers our work groups focused on were procalcitonin, symmetric and asymmetric dimethylarginines.

## PREVALENCE AND PROGNOSTIC VALUE OF PROCALCITONIN EVALUATED DURING CELIOTOMY IN HORSES WITH COLIC

### Introduction

Procalcitonin (PCT) is the intracellular precursor of calcitonin, in healthy individuals it is produced by C-cells of the thyroid gland, then, in the intracellular environment, it is disrupted into N-terminal PCT, C-terminal katacalcin and active calcitonin. Therefore, no PCT is actually released into circulation in healthy humans and consequently it is no detectable. Furthermore, no enzymes that can breakdown PCT are present in plasma, hence, it remains in the circulatory system with a half-life of 25-30 hours [93]. Nevertheless, in septic patients, this molecule is produced and released in mutiple tissues, for example in lungs, intestine, liver and pituitary gland [77].

In human medicine, PCT evaluation has gained wide

application in the management of sepsis. In fact it has shown a high value as a prognostic tool for survival and as an aid to decide whether to stop antibiotic therapy [115,132].

In veterinary medicine, PCT has started to gain interest after it was demonstrated that it shows the same activity as in human patients [124,170,177].

In recent years, a new ELISA method for quantification of PCT in equine plasma has been developed [126]. Since then, several studies have been conducted investigating the concentration of PCT in septic and non septic horses and foals [16,17], horses with pneumopathies [11] and, more recently, in colic patients [109]. These studies have demonstrated that plasmatic concentration of PCT shows a significant increase in SIRS (sistemic inflammatory response syndrome) positive equines and in colic patients.

PCT has also been reported to be a functional biomarker, in human medicine, for the diagnosis of ischaemic intestinal damage [29].

The aim of the present work was to assess the possibility to measure the concentration of PCT in peritoneal fluid of horses undergoing exploratory celiotomy for colic syndrome, and to investigate the diagnostic and prognostic value of this protein.

**Materials and Methods**

The present study was performed on 13 horses referred for colic syndrome to the veterinary teaching hospital Giuseppe Gentile of the University of Bologna.

All owners signed an informed consent form to authorize the use of data collected from their horses for scientifical and teaching purposes.

*Case selection*

The patients enrolled in the present study were all adult horses referred for colic syndrome, which was confirmed at admission examination, and that required surgical treatment.

*Sampling, storing and analysis*

At admission, before any medication was administered, blood was collected from the jugular vein using a sterile syringe and 16G needle. Complete bloodwork and blood lactate measurement (mmol/L) (Lactate SCOUT+, SensLab, GmbH, Lepzig, Germany) were performed at admission time and a sample of whole blood was collected in a lithium heparin (LH-heparin) tube. Within 30 minutes from collection, this sample was centrifuged at 3000 rpm for 10 minutes, and the harvested plasma was frozen at -80°C and preserved this way untill analysis was performed. During surgery, as soon as the abdominal cavity was opened, a sample of peritoneal fluid was collected and placed in serum tubes, centrifuged and stocked in the same manner, lactate concentration was measured from this sample as well.

PCT concentration measurement was performed on all the samples in a single batch. Measurements were performed as previously reported [16,17] using a commercial kit, developed for equine species by Rieger et al (2014)[126] (Horse Procalcitonin ELISA kit, MyBiosource.com, Inc., San Diego, CA, USA).

The intra-assay coefficient of variation was determined from 10 replicates of equine plasma samples containing low and high PCT concentrations. These samples were obtained by the addition of standard PCT in equine blank samples. The inter-assay coefficient of variation was determined from values obtained by repeating the analysis of duplicate samples with low and high PCT concentrations in 5 different

assays. To establish the detection limit for equine plasma PCT, repeated PCT measurements were performed using equine samples with low PCT concentrations (<10.0 pg/ml). Samples were analyzed in 10 replicates in a single assay and in 5 different assays. The intra- and inter-assay coefficient of variation were <15% and the limit of detection of the method was 10 pg/mL.

Data regarding breed, sex, age, pathology and outcome of the horses included in the study were recorded (Table 1).

| Case n | Breed | Sex | Age (years) | Diagnosis | Outcome |
|---|---|---|---|---|---|
| 1 | Standardbred | F | 3.5 | Ileal impaction | Discharged |
| 2 | Crossbred | F | 32 | Transverse colon fecaloma | Discharged |
| 3 | Shetland pony | F | 4 | Transverse colon fecalith | Discharged |
| 4 | Italian saddlebred | F | 9 | Epiploic foramen entrapement | Euthanasia |
| 5 | Crossbred | G | 23 | Nephrosplenic entrapement | Discharged |
| 6 | Shetland pony | M | 8 | Sand impaction | Discharged |
| 7 | Standardbred | M | 4 | Inguinal hernia | Discharged |
| 8 | Quarter horse | F | 8 | Large colon volvolus | Discharged |
| 9 | Haflinger | F | 24 | Large colon right dorsal displacement | Discharged |
| 10 | Italian saddlebred | G | 15 | Epiploic foramen entrapement | Intra-operative euthanasia |
| 11 | Arabian | F | 18 | Small intestine volvolus | Discharged |
| 12 | Pure spanish horse | M | 10 | Inguinal hernia | Euthanasia |
| 13 | Italian saddlebred | G | 23 | Pedunculated lipoma | Intra-operative euthanasia |

Table 1: resuming data regarding breed, age, sex, diagnosis and outcome of the patients included in the study. F=female; G=gelding;

M=male.

*Statistical analysis*

No statistical analysis could be performed on the obtained data.

**Results**

Among the 13 horses included in the study 7/13 were females, 3/13 were stallions and 3/13 were geldings. The median age was 17.5 years old (mean 13.9, range 3-32). Horses were of different breed: italian Saddlebred (n=3), Standardbreds (n=2), Shetland pony (n=2), crossbred (n=2), Pure Spanish horse (n=1), Quarter horse (n=1), Haflinger (n=1) and Arabian (n=1).

Nine of 13 horses were discharged from hospital while 4/13 were humanly euthanized for poor prognosis.

Regarding peritoneal fluid PCT, the values measured on all the 13 samples were 0 pg/dl.

**Discussion**

Aim of the present study, which was the first poject of this PhD program, was to measure PCT concentrations in peritoneal fluid of equine patients undergoing colic surgery.

PCT values in peritoneal fluid of all 13 horses examined were under the detection limit of the test. The discouraging results obtained with the first 13 samples presented here led to abandoning this research. Moreover, during the same period of time a paper by Kilcoyne and colleagues (2020)[76], which analyzed the presence and the diagnostic value of PCT in the peritoneal fluid of horses with strangulating lesions, was published. Their results suggest that peritoneal PCT concentration can be a sensitive marker of ischemic lesions, whereas, as

previously showed by other, plasma PCT levels were not effective in differenciating between strangulating and non strangulating intestinal lesions [109,153].

The importance of peritoneal fluid analysis during examination for colic syndrome has been widely underlined in the past years. In particular, the analysis of the proteins that can be found and their diagnostic and/or prognostic value is gaining interest in research in order to find a biomarker as sensitive, but possibly more specific than lactate [76,138], which has become commonly evaluated in clinical practice due to the readiness of the commercial rapid tests that have been developed.

Peritoneal fluid is an ultrafiltrate of circulating plasma and therefore multiple circulating protein can be found in it. It has been showed that during strangulating colic, a greater amount of proteins associated with inflammation can be found in peritoneal fluid compared to plasma [9]. Therefore it is unlikely that PCT does not transfer from circulating plasma to peritoneal fluid, indeed Kilcoyne and colleagues (2020)[76] were able to measure it.

The differences in results do not depend on the type of test since the test utilized by Kilcoine and colleagues (2020)[76] was the same used in the present work.

The most plausible hypothesis is that there has been some issue in the test used to analyze the peritoneal fluid, either during transportation or production or storing, although we do not have any proof of that.

**Conclusions**

In conclusion, PCT has been previously proven by other authors to be a sensitive aid in diagnostic of strangulating intestinal lesions, and if more accurate and rapid tests to measure it will be developed it might enter the routine examination of colic patients.

## EVALUATION OF SYMMETRIC AND ASYMMETRIC DIMETHYLARGININES IN SIRS NEGATIVE AND SIRS POSITIVE HORSES WITH COLIC

### Introduction

Asymmetric dimethylarginine (ADMA) and Symmetric dimethylarginine (SDMA) are structural isomers of the same molecule. These N-methylated derivates of arginine are produced in all types of nucleated cells by methylation of arginine residues, then, during proteolysis, they are released into the circulation [12].

Circulating ADMA is trasported into major organs such as brain, kidney and liver for enzymatic degradation [152]. Otherwise, the majority of serum SDMA has a renal clearance, and only a small amount gets metabolized, making this molecule a good biomarker for kidney function, both in human and small animal patients [128,143].

Nevertheless, ADMA and SDMA have been investigated for their interference with nitric oxide synthase (NOS) and nitric oxid (NO) production, respectively. NO, which is produced by endothelial cells, is responsible for vasodilation on macrocirculation, and it improves blood flow through small capillaries by regulating vascular tone and erytrocites deformability [57]. Microcirculation function is compromised in septic patients, and SDMA is indirectly responsible for this mechanism by its down regulation on NO production. In fact, a significant increase in serum SDMA concentration is reported to be related to sepsis and mortality [103]. This correlation is probably due to the high sensitivity of renal function to septic assault, in fact if kidney elimination of the molecule is not working properly, plasma SDMA levels increase [174]. During COVID-19 pandemic, serum ADMA and

SDMA levels of COVID affected patients at hospital admissions have been proven to be positively related to death risk [56].

In recent years, an immunologic test for quantification of SDMA in equines has been validated and is available for clinical use [113]. Nevertheless, the clinical relevance of SDMA in assessing kidney diseases in horses is still debated [52].

Some inflammatory diseases and strangulating obstructions of the GI can lead to the clinical status of endotoxemia in equids. This condition has recently been given the term of systemic inflammatory response syndrome (SIRS) [101].

The aim of the present work is to evaluate ADMA and SDMA in colic SIRS positive and negative patients, and its possible use as an inflammation marker in equines.

**Materials and Methods**

The present study has been performed on a total of 54 horses hospitalized into two different italian veterinary teaching hospitals (VTHs): VTH Giuseppe Gentile of the University of Bologna, and VTH Mario Moderato of the University of Pisa on a one year period.

All owners signed an informed consent form to authorize the use of data collected from their horses for scientifical and teaching purposes.

Clinical Protocol has been approved by the Welfare committee of Pisa (protocol 2825/14).

*Population selection*

Horses enrolled in the present study were divided based on their SIRS score in three goups:

A. Control group: healthy female Thoroughbred and Standardbred horses that were hospitalized for reproduction.

B. Colic SIRS negative group: horses presenting for colic and evaluated SIRS negative.
C. Colic SIRS positive group: horses presenting for colic and evaluated SIRS positive.

Colic subjects were horses referred to the VTHs for colic syndrome related to the GI tract. SIRS status was evaluated based on clinical and hematological data collected during examination at admission [139]. In particular, body temperature, heart and respiratory rate, white blood cells (WBC) count, and band-neutrophils presence were considered. Normal reference values are reported in table 1 along with ranges of values used to determine SIRS positive patients.

| | Normal reference values | Pathologic values |
|---|---|---|
| BT (°C) | 37-38,5 | <37 o > 38,5 |
| HR (bpm) | <52 | >52 |
| RR (apm) | <20 | >20 |
| WBC (cell/ul) | 5000-12500 | <5000 o >12500 |
| Bands | Absent | >10% |

Table 1. SIRS *criteria* used to discriminate SIRS negative and SIRS positive equine patients in a 0-4 scale [139].

Each parameter is graded 0 if included in the physiological ranges, or 1 if pathological. SIRS negative patients are scored 0 or 1, whereas SIRS positive subjects are scored 2 or >2. Moreover, also Roy and colleagues (2017) [131] SIRS score has been applied to evaluate the patients, in which blood lactate value (physiological value <2.06 mmol/L) and mucous membrane evaluation are included as well (physiological: pink and moist; pathological: red, purple, cyanotic edges or white. Pale or icteric mucosae are not considered among abnormal parameters in Roy and colleagues SIRS criteria). Each parameters is graded 0 if in the physiological ranges, and 1 if pathological. This way, the

score scale il between 0 and 6.

*Sampling protocol, storing and analysis*

Blood samples were obtained from the jugular vein using a sterile 16G needle and a siringe in all subjects.

Samples from healthy horses belonging to the control group were collected once, whereas blood from colic horses was collected at admission time (T0), then 24 (T1), 48 (T2), 72 (T3) and 96 (T4) hours after admission.

Each sample was then divided as follows:

- One drop of whole blood was used to measure lactate value (Lactate SCOUT+, SensLab, GmbH, Lepzig, Germany).
- 1mL of whole blood was placed into an EDTA tube and analyzed for a complete blood cell count within 30 minutes from collection (ADVIA® 2120i Hematology System, Siemens Healthcare GmbH ©2023).
- 2,5 mL of whole blood were placed into LH-heparin tubes and then centrifuged at 3000 rpm for 5 minutes within 30 minutes from collection. Plasma was then place into an eppendorf and stored at -80°C.

All plasma samples were processed afterwards using the Ivanova and colleagues (2010) [64] method.

The analytical method developed involves a solid-phase extraction (SPE) technique using cation-exchange polymeric columns. The extracted samples were derivatized using o-phthaldehyde (OPA) as a reagent, with the addition of 3-mercaptoproprionic acid (MPA).

Separation of dimethylarginines was obtained through a reversed-phase high-performance liquid chromatography (RP-HPLC) method, optimized with isocratic elution with a C18 HAISIL HL column, and equipped with fluorescence detector.

The solid-phase extraction process is characterized by high recoveries (81.46±5.30% for SDMA and 81.81±2.65 % for

ADMA).
The described method enables a sensitive, reliable and reproducible analysis for the simultaneous quantification of ADMA and SDMA.

*Statistical analysis*
A commercial software has been utilized for all statistical analysis (GraphPad Prism 9, USA).
To verify statistical distribution of the data a Kolmogorov Smirnov test was applied. Since a non-gaussian distribution of the data was present, non-parametric tests were used.
Kruskal Wallis and Dunn's multiple comparisons tests were used to compare values of ADMA and SDMA between control cases and colic SIRS positive or colic SIRS negative patients at all timings.
Same tests have been applied to evaluate ADMA and SDMA values trends during time in colic groups.
A Mann Whitney test has been used to compare ADMA and SDMA values between SIRS negative ans SIRS potive patients at all timings.
Again, a Mann Whitney test has been applied to evaluate the difference in SIRS scores between SIRS negative and positive cases.
Cut off for significancy of the data was set at $p < 0,05$.

**Results**
Some of the colic horses were discharged or euthanized or died before the protocol time was completed, therefore it was not possible to collect all samples for every patient.
The control group included seven healty female Throughbred and Standardbreds aged between 2 and 13 years (median 11) hospitalized for reasons related to their reproduction activity.
A total of 47 colic horses were included in the study,

21/47 females, 4/47 stallions and 22/47 geldings, among them 26/47 (55,3%) SIRS negative and 21/47 (44,7%) resulted to be SIRS positive. SIRS negative horses were 21 Warmbloods, four Coldbloods and one draft horse aged between 4 and 30 years (median age 13). SIRS positive horses were 12 Warmbloods, three Coldbloods, two draft horses and four italian breeds, aged between 1 and 34 years (median age 11).

Among SIRS negative, 14/26 (53,8%) had a SIRS score 0-1/6 on a 0-6 scale and 12/26 (46,2%) scored 2-3/6. Among SIRS positive, 11/21 (52,4%) had a SIRS score 2-3/6, whereas 10/21 (47,6%) had a score of 4-5/6. No subject presented a 6/6 SIRS score.

Among colic horses 29/47 (61,7%) had a strangulating type of colic and 18/47 (38,3%) had a non strangulating colic. 13/29 (44,8%) horses presenting with a strangulating colic were SIRS positive, whereas 16/29 (55,2%) were SIRS negative. Among non strangulating colic horses 5/18 (27,8%) were SIRS positive and 13/18 (72,2%) were SIRS negative.

Regarding the outcome 31/47 (66%) horses were discharged, 15/47 (31,9%) were humanly euthanized for poor prognosis, and 1/47 (2,1%) spontaneously died. Among euthanized horses 7/15 (46,7%) were SIRS negative and 8/15 (53,3%) were SIRS positive. Among patients that were discharged 18/31 (58,1%) were SIRS negative and 13/31 (41,9%) SIRS positive. The horse that died spontaneously was SIRS negative.

In colic SIRS negative group SDMA concentration resulted statistically different from the control group at every measurement timing (graph 1) ($p<0,0001$), whereas ADMA concentration was statistically different at every timing except for T96 (graph 2) ($p=0,0593$).

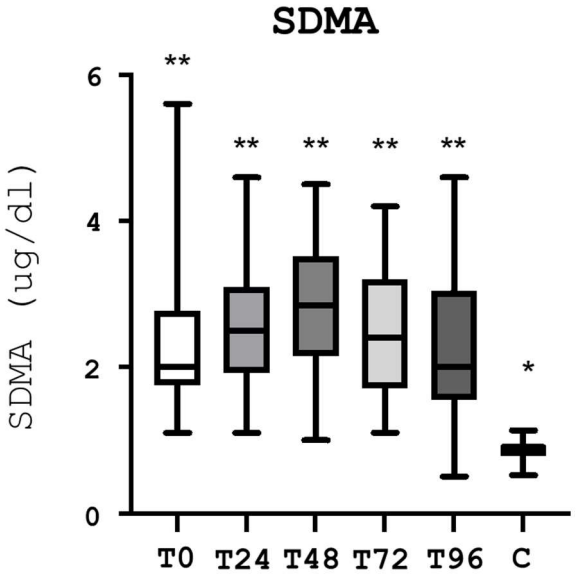
Regarding colic SIRS positive group SDMA concentration was statistically different from control group at every timing except for T72 (graph 3) ($p=0,0032$), whereas ADMA was

statistically different at every timing except for T24 and T96 (graph 4) (p=0,0209).

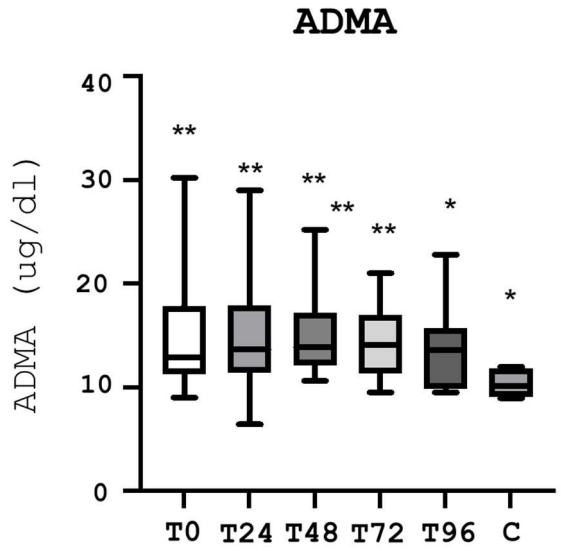
ADMA and SDMA concentration values did not change significantly during time neither in colic SIRS negative patients (p=0,9060 and p=0,2336 respectively), nor in colic SIRS positive group (ADMA p=0,6191 and SDMA p=0,7024).

No statistically significant differences have been found in ADMA and SDMA concentration values between SIRS negative and SIRS positive patients at every timing (Table 2).

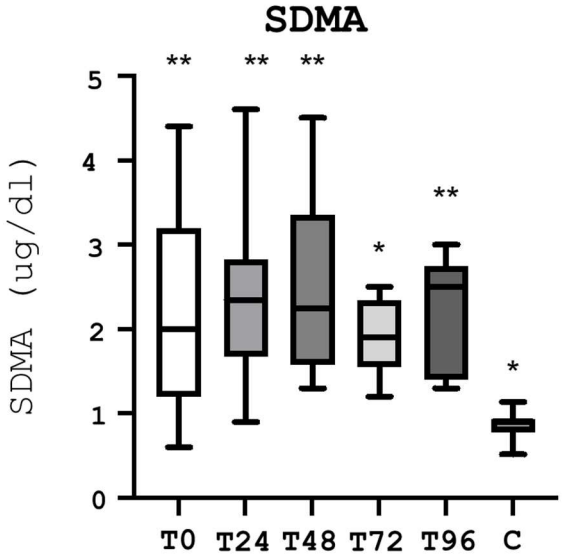
SIRS score was significantly different between SIRS negative and SIRS positive groups (p<0,0001).



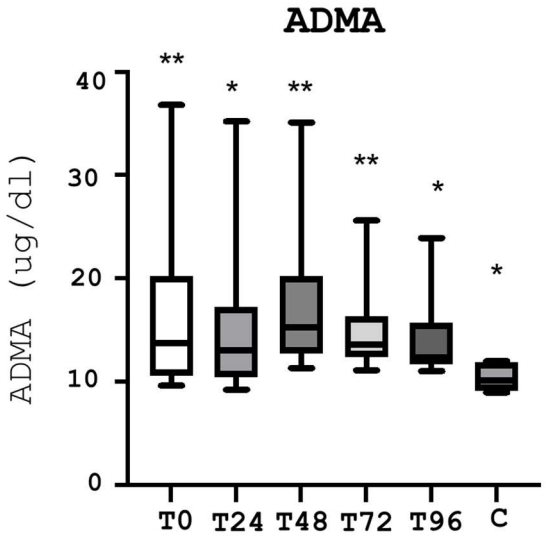

Graph 1. SDMA values at the different timings in colic SIRS negative horses vs control group (C). SDMA (ug/dl) has a significantly higher concentration in colic SIRS negative horses than in control group. The star (*) indicates p=<0,05.




Graph 2. ADMA values at the different timings in colic SIRS negative horses vs control group (C). ADMA (ug/dl) has a significantly higher concentration in colic SIRS negative horses than in control group, except for T96. The star (*) indicates p=<0,05.




Graph 3. SDMA values at the different timings in colic SIRS positive horses vs control group (C). SDMA (ug/dl) has a significantly higher concentration in colic SIRS positive horses than in control group, except for T72. The star (*) indicates p=<0,05.




Graph 4. ADMA values at the different timings in colic SIRS positive horses vs control group (C). ADMA (ug/dl) has a significantly higher concentration in colic SIRS positive horses than in control group, except for T24 and T96. The star (*) indicates p=<0,05.

| P value | T0 | T24 | T48 | T72 | T96 |
|---|---|---|---|---|---|
| SDMA | 0,6741 | 0,5388 | 0,3289 | 0,1418 | 0,6574 |
| ADMA | 0,5825 | 0,7234 | 0,4133 | 0,9185 | 0,7066 |

Table 2. p values obtained from comparison of SDMA and ADMA concentration values between colic SIRS positive and SIRS negative horses at every sampling time.

**Discussion**

This study, which results have been presented in a veterinary graduation thesis at the University of Pisa [158] is the first one considering ADMA and SDMA activity during SIRS status in horses presenting colic.

In human patients, ADMA and SDMA concentration significantly increase during septic SIRS status, and the values are positively correlated to the severity of the disease [174].

ADMA concentration in adult humans is reported to increase with age, in particular, in the geriatric patient, it can reach twice the value of a mid age person [151]. Also in

children, especially in neonates, ADMA levels are higher than in adults [169]. This difference in values has been suggested to be related to renal function, although never been proven [152]. In the equine population no correlation has been found between ADMA levels and age, although more studies on healthy subjects may be needed in the future to verify whether age might be a variable influencing ADMA measurements.

In human patients ADMA has been reported to increase during multiple pathological conditions, nevertheless among these, diseases affecting the GI tract are not much represented [152]. On the other hand, in the present study it was demonstrated how ADMA levels significantly increase during both strangulating and non stangulating intestinal lesions in equine patients.

Moreover, given the activity of ADMA as a NOS inhibitor, increasing in its plasma levels is associated with cardiovascular diseases both in human [142] and equine patients [42].

SIRS is a common complication of colic syndrome in horses. The excessive inflammatory response that is generated can lead to compromised cardiovascular function, multiple organ dysfunction syndrome, laminitis and ultimately death. Since SDMA is a good marker for renal function, which is deeply affected by SIRS, this molecule might also be a diagnostic marker for multiple organ dysfunction syndrome. Nevertheless in the present study there were no significant differences in SDMA concentrations between SIRS negative and SIRS positive patients. This finding suggests that ADMA and SDMA do not have a diagnostic or prognostic value in management of SIRS.

Similarly, in human medicine SDMA concentration has been reported to significantly increase in septic patients compared to healthy ones, nevertheless its values did not

differ between patients with sepsis and patients with septic shock [164].

In the present study SIRS subjects have been defined in accordance to Roy and colleagues SIRS criteria (2017), considering SIRS positive all horses that scored ≥2. Our results further confirm the sensitivity of the SIRS score, since it was significantly different between SIRS negative a SIRS positive groups ($p<0,0001$).

Another interesting finding was that ADMA and SDMA plasmatic levels did not significantly change through the different sampling timings. In human medicine concentrations of these molecules have been showed to remain high in response to chronic processes [80], nevertheless no studies have clarified their variation during healing [61]. Future studies with more sampling timings might be needed to further understand the variations in concentrations of these molecules during healing processes after colic syndrome.

*Limitations*

Limitations to the present work include the relatively low number of patients considered both in the control and in the colic groups, which might have negatively affected the statistical power of the analysis and the capability to identify significant differences between groups.

Moreover, the patients examined in the present study were referred to the VTHs after receiving different treatments at home and after being presenting colic syndrome for different time frames, adding variables which might have affected our results.

Also, since some patients have been discharged before the sampling protocol was completed, there is a lack of data regarding horses in which the pathology was rapidly resolved.

**Conclusions**

In conclusion it can be stated that ADMA and SDMA concentrations significantly increase in horses affected by colic syndrome, although these molecules are not diagnostic or prognostic markers of SIRS status.

Further studies might be needed to compare ADMA and SDMA concentrations in discharged and dead equine patients to evaluate possible prognostic cut-off values.

# Chapter 3 - A RETROSPECTIVE STUDY COMPARING TWO PROTOCOLS FOR POSTOPERATIVE TREATMENT AFTER COLIC SURGERY

Spadari, A., Giusto, G., Rinnovati, R., **Forni, G.**, & Gandini, M. (2021). A retrospective multicentric study comparing two protocols for postoperative treatment after colic surgery. In 13th International Equine Colic Research Symposium 2021 Abstracts. 9-10.

**Introduction**

Horses referred for colic that require a surgical approach are frequently haemodinamically unstable, being both dehydrated and hypovolemic. This volume loss may be due to water shift into third space. Therefore, fluid resuscitation is mandatory to restore intravascular volume in order to maintain tissue perfusion under general anaesthesia and post-operative period. Consequently, large amounts of crystalloid solutions, such as lactated ringers and 0,9% saline, are usually administered to colic patients [18,129].

In particular, post operative fluid therapy is a crucial element of management of both human and equid patients that underwent abdominal surgery for GI diseases, though it remains a controversial topic both in human and veterinary medicine and there are no specific guidelines available for fluid therapy in equine colic patients [7,105].

Several parameters have been considered to establish hydration status, among them, PCV and total solid (TS) can be promptly determined as they are easy to measure using cheap equipment [8].

Overhydration, meaning an amount of intravenous fluids exceeding the actual patient's needs, is known to be a

common practice in management of postoperative colic cases, however it has been demonstrated that in human patients it can be damaging to intestinal motility causing intramural oedema [20,137]. Moreover, it seems that overhydration might lower survival rate in equine patients by decreasing colloid osmotic pressure [123], also it has been observed that a reduced packed cell volume (PCV), due to excessive crstalloids administration, measured intraoperatively was predictive of failure to recover from anaesthesia and need for gastric decompression [43].
Aim of this study was to asses if a restricted post-operative fluid therapy protocol could improve prognosis of equine patients subjected to surgery for colic.

**Materials and Methods**

In the present "before-after" study, conducted in two Italian Veterinary Teaching Hospitals (VTHs), 205 horses that underwent colic surgery between 2015 and 2019 were enrolled. The study was conceived as a retrospective clinical study.

*Case selection*

Patients included in the present study were adults (age > 1 year old) equids referred to the VTHs of the Universities of Bologna and Turin (Italy), that recovered from exploratory celiotomy for colic syndrome.
Horses were divided in two groups depending on the post-operative fluid protocol they receveid:

- "Extended" protocol group (n=69): horses were administered a standard crystalloid fluid intravenous (IV) protocol for at least 24h, IV lidocaine, subcutaneous (SC) heparin, IV dimethylsulfoxide (DMSO), pre- and post-operative antibiotics, flunixin meglumine twice a day and gastroprotection.

- "Restricted" protocol group (N=136): horses were administered IV fluids only when deemed necessary based on the continuous monitoring of hydration status via PCV and TS measurements every 4 hours. Antibiotic, antinflammatory and gastroprotective therapies remained the same.

Both groups received a resuscitation fluid protocol, both before and during anaesthesia, of 20-80 ml/Kg of crystalloids, usually lactate ringer's unless differently needed to correct electrolytic disorders. Hypertonic saline was administered if needed based on the cardiovalscular status, PCV, TS and venous lactate values.

Both groups were allowed free water intake after recovery from surgery.

*Variables*

Variables evaluated for each horse included:

- Post-operative complications, meaning any deviation from normal post-operative course;
- Short-term survival, meaning survival untill discharge from the clinic
- Costs, considering only expenses substained by the hospital regarding the administered drugs in the post-operative period.

Data were collected from clinical reports and the hospital veterinary medical information system (FeniceVET®, ZakSoft srl, Bologna, Italy).

*Data analysis*

The commercial software MedCalc (MedCalc Statistical Software vers 14.8.1, Belgium) was used to perform the statistical analysis.

The most encountered post-operative complications were

post-operative reflux (POR) and incisional infection and therefore these two complications were taken into consideration for further statistical evaluation.
With the aim of comparing the costs between the two treatment groups, the horses were assigned to three different categories, depending on how much the drugs used for them in the post-operative period were. The first cost range was <500 €, second was >500 and >1000 € and the third was >1000 and <1500 €.
The short-term survival, the prevalence of complications and the costs category were evaluated and compared between the two groups using a Chi-squared test.
A p value <0.05 was considered statistically significant.

**Results**

Horses discharged in the "restricted" group were 115/136 (84,6%) whereas 54/69 (78,3%) were discharged in the "extended" group. Twenty horses in the "restricted" group developed post-operative reflux (POR) (14,7%) and 8 (15,9%) in the "extended" group. Other recorded complications were incisional infection, which were 18 (15,6%) in the "restricted" group and 10 (18,5%) in the "extended" group, and one case of laminitis in the "restricted" group.
Cases comprised in the lowest costs range (<500 €) were 97/205 (47.3%), and they all belonged to the "restricted" group; 90/205 (43.9%) cases were in the mid price range (500< € <1000), 51 of them belonged to the "extended" group and 39 to the "restricted" group; lastly, 18/205 horses were in the higher costs range (1000< € <1500) and they all belonged to the "extended" group.
All differences were not statistically significant, except for cost, that resulted lower for the "restricted" group.
Results and p-values are summarized in table 1.

| | Extended group | Restricted group | p-value |
|---|---|---|---|
| **Complications** | | | |
| POR | 15.9 % | 14.7 % | 0.6907 |
| Incisional infection | 18.5 % | 15.6 % | 0.9740 |
| **Survival** | 78.3 % | 84.6 % | 0.3546 |
| **Costs** | | | |
| <500 € | 0/205 | 97/205 | <0.0001 |
| 500< € < 1000 | 51/205 | 39/205 | |
| 1000< € < 1500 | 18/205 | 0/205 | |

Table 1: summary of the analized variables and the p-values obtained by the comparison between the two groups. As shown, only tha variable "costs" is significantly different.

**Discussion**

The present study is the first one to analyze, in a retrospective manner, the influence of a restricted fluid therapy protocol in the postoperative period in horses undergone colic surgery.

Our results showed that there were no significant differences in short term survival rates and incidence of postoperative complications between the "restricted" and the "extended" fluid therapy protocol groups. Traditionally, horses operated for colic were administered in the postoperative period a large amount of IV crystalloids, even for days, in order to keep the intestinal content hydrated. Nevertheless, fluids are drugs, and as such, they can be both life-saving aids and have severe adverse effects. Moreover, the use of IV route of administration, and the consequent bypass of the intestinal tract, where water uptake can be regulated, means that any mistake in fluid therapy calculation can lead to more severe effects, and since there is lack of precise guidelines, mistakes are easy to be made [34,105].

Until recently, the concept of overload has been widely ignored in adult equines, due to their large size and the consequent low risk perceived. However, given the evidence in human medicine that overhydration with crystalloids can be harmful [20,91], the concern regarding its implications on disease process and outcome has grown in equine practice as well. In fact, some studies have hypothesized that an excessive volume of cystalloids in horses might decrease PCV value to dangerously low levels, that were associated with reduced survival rate [123], failure to recover from anaesthesia and reflux [43].

In human medicine, some studies have shown how a restricted fluid therapy regimen is associated with a decreased risk of POR and better outcome [108,165]. On the other hand, in our study outcome and incidence of POR were not significantly improved in the restricted protocol group of horses, nevertheless, percentages of discharged horses were slightly higher, while percentages of POR were slightly lower. This might indicate that, even if little, some benefit has been achieved with the application of a restricted protocol. Moreover, by the application of this protocol, we might have reduced potential risk of adverse affects from administration of excessive amounts of crystalloids. In fact, in the multicentric study presented in the present thesis (Gandini et al 2023 unpublished data) it is shown how an amount of fluid greater than 44 ml/Kg in the postoperative period has a negative effect on survival rate. This difference in results might depend on the wider population considered in the multicentric study. Another previous study tried to correlate fluid administration and development of POR, although it did not report any difference between the group of cases, meaning horses that developed POR, and controls, patients that underwent colic surgery but did not develop POR [60].

Responsiveness to fluid administration is a key concept in human medicine [92], which requires the identification of measuring variables that can appropiately guide the so called goal directed fluid therapy (GDFT). Nevertheless, some of these measurements are much more challenging to perform in equine patients [141]. In the present study PCV and TS values were used to evaluate hydration status and decide wether to administer IV fluids or not. As previously reported, these parameters are useful aid in evaluating hydration status, especially when serial measurements are performed, besides being cheap and easy to estimate [8]. Nevertheless, both PCV and TS may be affected by metabolic conditions, some examples are: splenic contraction subsequent to distress, which can increace PCV levels; pre-existing anaemia, which may cover PCV increase, and pre-existing protein loss from cronic GI disease may mask an increase in TS. Other parameters that might have been used include body weight (BW), which is known to be useful in human medicine [73], although it may be difficult to evaluate changes in BW with the same precision in equine patients because of their large size and also not all horses are so compliant in mounting on the scale.

Results of the present study showed how costs tended to be significantly lower in the "restricted" group. According to previous authors. It is mandatory for equine surgeons to attempt to reduce the costs of colic surgery in order to allow more owners to afford life-saving emergency surgery [3,48]. One way to reach this goal is to apply a more selective approach in drugs usage [49]. Moreover, not keeping horses under unecessary fluid therapy for long time also allows equine hospitals to reduce costs not only in terms of drugs and materials used, but also in terms of in clinic personnel effort.

*Limitations*

Limitations to the present study include the numerical difference between the two groups, which mught have influenced the statistical analysis. Moreover, the clinical and pathological features of the horses belonging in the two groups were not considered in the present analysis.

**Conclusions**

In conclusion, the present study showed how a restricted postoperative fluid therapy can help reducing costs of colic surgery, avoiding higher postopertive complications and a decreased survival rate. Further, more standardized studies are needed to better assess the optimal approach in fluid therapy administratin in equine colic patients, and more reliable and precise aids to evaluate hydration status.

# Chapter 4 - EVALUATION OF HIND GUT MICROBIOTA IN HORSES UNDERGOING SURGERY FOR COLIC

**Introduction**

The term microbiota refers to the totality of microorganisms that inhabits a distinct space of the body, while the term microbiome indicates the corresponding genetic material [157].

Equids are non ruminant herbivors that have a large and complex gastrointestinal (GI) tract, exeptionally adapted for hind gut fermentation, specifically in cecum and large colon, where fibrolytic bacteria disrupt structural carbohydrates in order to produce energy for the body. Besides digestive function, hind gut microbiota takes part to immune system regulation and protection against pathogens [10,69].

Dysbiosis, meaning alterations to the composition of microbiota, has been related to numerous causes, among which: poor quality forage, diets high in starch, stress and age [81,167]. Recently, dysbiosis has also been associated with occurrence of colic, since modifications of relative abundance of some groups of bacteria, known as responsible for health maintenance in the GI tract, have been observed in affected horses [15,134,148). In fact, an increase in Proteobacteria phyla and a reduction in Firmicutes and Bacteroidetes, which represent the most abundant phyla in healthy equines, has been noticed in horses presenting for colic, although a cause-effect relationship has not been found yet [81].

Multiple studies have been conducted on association between changes in GI microbiota composition and manifestation of different disorders, such as colitis, laminitis and equine

grass sickness [30,82,99], since in human medicine this connection had already been proven for several diseases [71,175,176,178].

Most *in vivo* studies analyze microbiota from fecal samples, though the majority of bacteria resides in the cecum and large colon [68]. A recent paper, which collected fecal samples from the rectum and from colonic content intraoperatively, showed that fecal microbiota is not representative of quick changes of colonic bacterial community [133].

The aim of the present work was to analyze GI microbiota samples from colonic and cecal content during exploratory laparotomy in horses admitted for colic syndrome that required surgical treatment.

**Materials and Methods**

The present study was performed on 11 horses referred for colic syndrome to the veterinary teaching hospital Giuseppe Gentile of the University of Bologna.

All owners signed an informed consent form to authorize the use of data collected from their horses for scientifical and teaching purposes.

*Case selection*

The patients enrolled in the present study were all adult (age >1 year old) horses referred for colic syndrome due to GI affections, which was confirmed at admission examination, and that required surgical treatment.

Data regarding age, sex, breed and type of pathology are summarized in table 1.

| CASE | GENDER | AGE (years) | BREED | DISEASE | OUTCOME |
|---|---|---|---|---|---|
| Case 1 | F | 17 | Thoroughbred | SI strangulating (peduncolated lipoma) | Postoperative euthanasia |
| Case 2 | M | 16 | Italian saddlebred | LI strangulating (LC volvulus) | Intraoperative euthanasia |
| Case 3 | M | 4 | Frisian | SI strangulating | Intraoperative euthanasia |
| Case 4 | F | 11 | Italian saddlebred | SI nonstrangulating (ileal impaction) | Discharged |
| Case 5 | G | 19 | Irish hunter | SI strangulating (SI rupture) | Intraoperative euthanasia |
| Case 6 | F | 16 | Standardbred | LI strangulating (LC volvulus) | Postoperative death |
| Case 7 | M | 3 | Miniature horse | LI non strangulating (Trichobezoar in the small colon and LC impaction) | Discharged |
| Case 8 | G | 26 | Crossbred | LI nonstrangulating (Cecal impaction) | Postoperative euthanasia |
| Case 9 | M | 11 | Italian saddlebred | LI strangulating (LC volvulus) | Intraoperative euthanasia |
| Case 10 | G | 25 | Crossbred | LI non strangulating (nephrosplenic entrapment) | Discharged |
| Case 11 | F | 15 | Thoroughbred | LI strangulating (LC volvulus) | Intraoperative euthanasia |

Table 1: summary of horses included in the study, their gender, age, pathology (LI = large intestine, SI = small intestine, LC = large colon) and outcome.

*Sampling protocol and analysis*

During admission examination, at the same time as rectal palpation was performed, a sample of feces was collected from the rectum.

Two more samples were collected from horses during exploratory laparotomy: one from the cecum and one from pelvic flexure. These samples were collected while evacuating colotomy and tiflotomy were performed, all surgical actions were carried out for the patient's sake first and secondary for research purposes.

At the time of discharge or death one last fecal sample was collected from the rectum from each patient.

In order to have enough material to perform the analysis, at least two grams of feces had to be collected. Each sample was then placed in a sterile tube and was immediately frozen in liquid nitrogen and stored at -80°C untill examination was performed.

All test were performed in a single batch.

Illumina 16S rRNA protocol (V3-V4 regions) was used and the taxonomic quantification was performed by applying the VSEARCH-based classifier implemented in QIIME adopting the Greengenes 13_8 97% OUT dataset as reference.

*Statistical analysis*

Data of relative abundances of all phyla, classes, orders, families, genus and species for each horse were normalized upon the whole population.

For the present PhD thesis, it was decided to limit the statistical analysis to the families and phyla listed in the cecal and colonic contents.

Two pairs of groups were established to perform the statistical analysis: first pair was discharged vs non discharged, meaning horses that survived until return to their stable vs horses that underwent euthanasia or died

before discharge. The second pair of groups was based on the kind of pathology, distinguishing strangulating vs non strangulating disorders.
An unpaired student t-test was used to compare relative abundances of families and phyla between discharged and non discharged horses, and between horses with stranulating and non strangulating disorders.
A p value <0.05 was considered statistically significant.

**Results**

Regarding the analysis of phyla in the cecal content, relative abundance of *Proteobacteria* was significantly higher in the group of discharged horses compared to non discharge (p-value = 0.0147) (Figure 1), whereas relative abundance of *Bacteroidetes* was significantly increased in the "strangulating" group (p-value = 0.02184) (Figure 2).
Regarding the analysis of phyla in the pelvic flexure, the relative abundance of *Verrucomicrobia* was significantly higher in the "discharged" group (p-value = 0.0439) (Figure 3), whereas relative abundance of *Bacteroidetes* was significanlty increased in the "strangulating" group (p-value = 0.0283) (Figure 4).
The analysis of families in the cecal content showed that relative abundances of *Enterobacteriaceae* and *Enterococcaceae* were significanlty higher in the "discharged" group (p-values = 0.01470 and p-value = 0.00245 respectively) (Figure 1), in particular, *Enterococcaceae* were detectable only in horses that survived. In strangulating cases *Prevotellaceae* and *Paraprevotellaceae* families had significanlty higher relative abundances than in non strangulating ones (p-value = 0.0249 and p-value = 0.04642 respectively) (Figure 2), whereas *Mogibacteriaceae* abundance was increased in the "non strangulating" group (p-value = 0.04063).

The analysis of families in the pelvic flexure content showed no significant difference between horses that survived until discharge and horses that did not. Regarding the type of pathology, *Praprevotellaceae* and RF16 relative abundances were significantly increased in the "strangulating" group (p-value = 0.0186 and p-value = 0.0344 respectively), whereas *Christensenellaceae* had a higher relative abundance in the "non strangulating" group (p-value = 0.0472) (Figure 4).

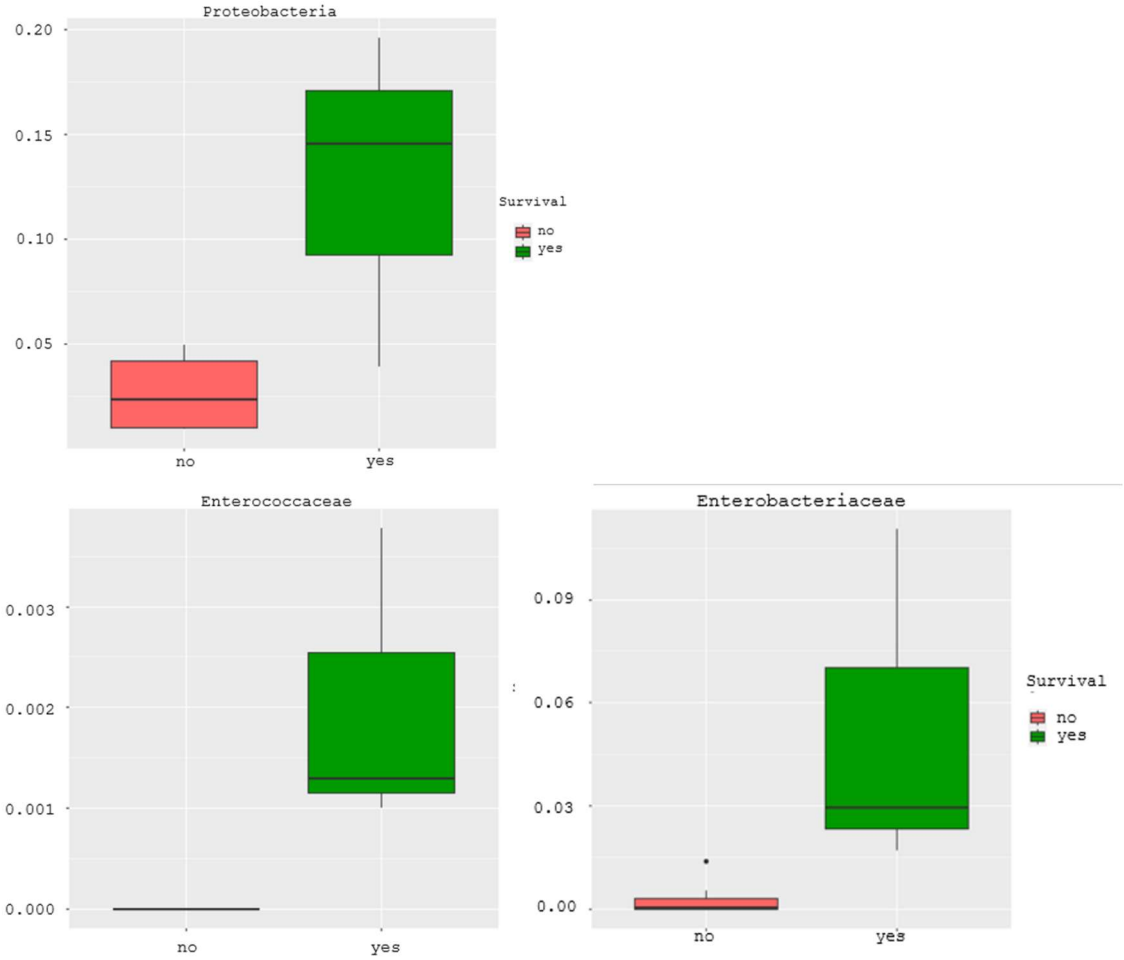



Fig. 1: box plot representing differences in phyla and families in the cecal content of horses that were discharged and horses that were not.

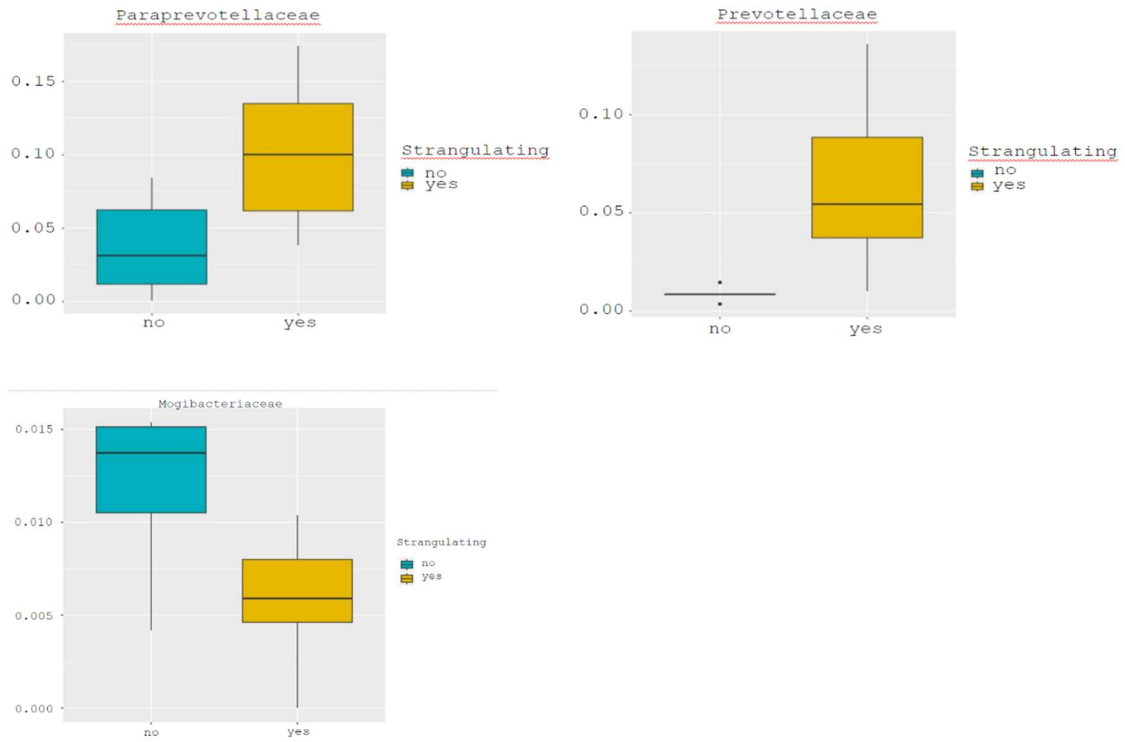



Fig. 2: box plot representing differences in phyla and families in the cecal content of horses with strangulating and non strangulating disorders.

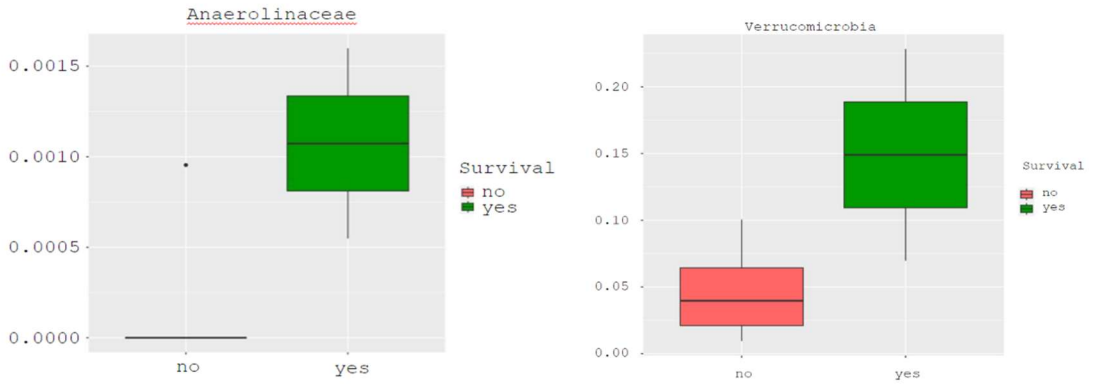



Fig. 3: box plot representing differences in phyla and families in the pelvic flexure content of horses that were discharged and horses that were not.

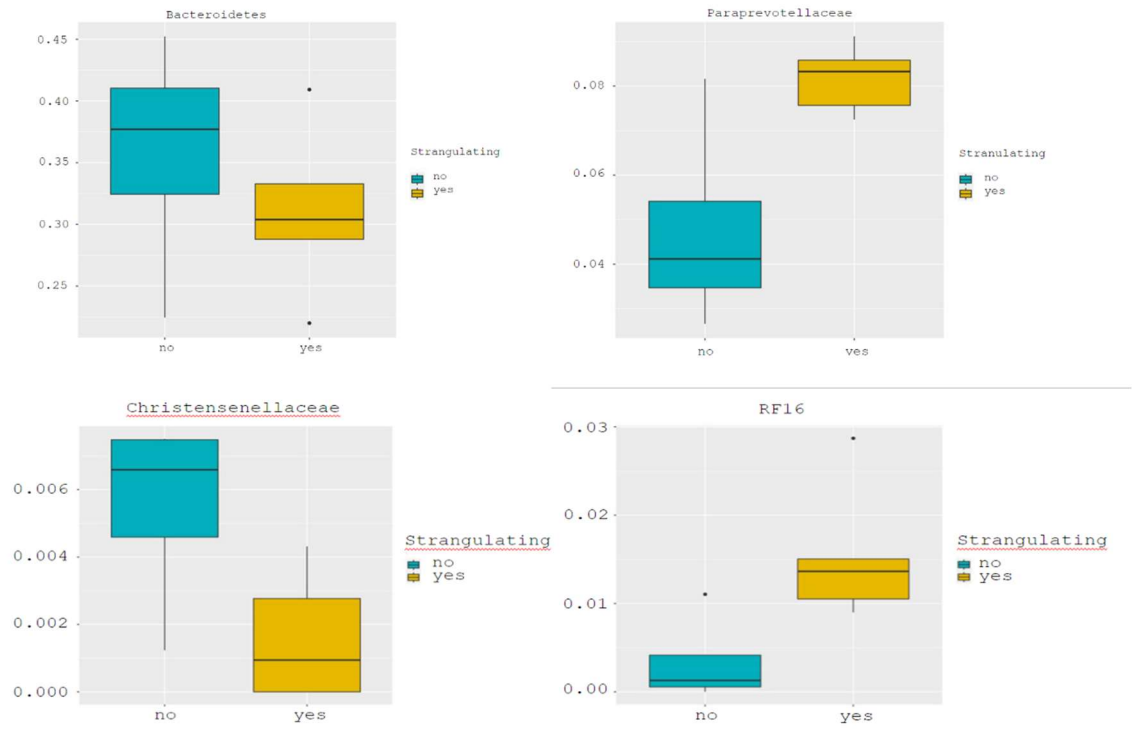



Fig. 4: box plot representing differences in phyla and families in the pelvic flexure content of horses with strangulating and non strangulating disorders.

**Discussion**

The results of the present study showed that some differences in cecal and pelvic flexure bacterial populations can be present between strangulating and non strangulating types of colic and between horses that survive till discharge from the clinic and horses that do not.

Colic syndrome is recognised as a major concern in the equne industry, nevertheless the aetiopathogenesis remains rather elusive [155]. Recently, many studies have been focusing on the role that intestinal microbiota may play in the course of this disorder [30,166,172], nevertheless a causal relationship between microbiota modifications and colic presentation has not yet been established [81]. As showed in a previous study, fecal microbiota does not always represent colonic one, especially during colic, meaning that changes in microbial population occur

suddenly, before alteration can be detected in the feces [133]. Therefore in the present study we decided to investigate microbiotal changes in the large bowel content during exploratory laparotomy in horses with strangulating and non strangulating disorders.

*Bacteroidetes* phylum was significanlty more present in strangulating colics both in the cecal and in the colonic content. This phylum has been repeatedly listed among the most represented in fecal microbiota of healthy adult horses: some studies reported *Bacteroidetes* to be the most abundant phylum [32,72,148], others ranked it as the second most abundant (38%) after *Firmicutes* (44%) [148], whereas other studies suggested a ratio of *Firmicutes*:*Bacteroidetes* of 4:1 [140]. These discrepancies in results may be explained by several factors such as geographic location, breed, exercise, diet and methodological technique of DNA extraction and sequencing platform [41,55]. Multiple authors that analized microbiota in horses with colic reported how in animals affected by large colon disordes *Bacteroidetes* abundancy overcomes *Firmicutes* abundancy. This change has been reported both in colonic content microbiota [133] and in fecal microbiota [30]. Nevertheless, in the present work, in contrast with literature, the tendency of *Firmicutes* abundancy was to be higher then *Bacteroidetes* abundancy in both colonic and cecal samples, only in two cases that presented strangulating disorders this ratio was reversed at the level of the cecum. In fact, the abundancy of Bacteroidetes was significantly higher in strangulating cases than in non strangulating, regardless of which intestinal tract the strangulation involved. Another study investigated possible association between strangulating disorders and changes in gut microbiota but found no correlation [149].

Our results showed that the relative abundance of

*Proteobacteria* was significantly higher in the cecal content of horses that were discharged compared to horses that died or were euthanized. This phylum has been reported to be one of the most represented in the ileum [37,84], therefore its proximity to the cecum might influence the abundance of *Proteobacteria* in the cecal content. This phylum has also been aknowledged to be more abundant in older horses [102], nevertheless, we did not find this connection with the horses that were discharged (aged three, eleven and 26). In human medicine, increasing in *Proteobacteria* abundancy in fecal microbiota is usually associated with GI disorders, included inflammatory bowel disease (IBD) [26,111], in fact, it is considered a marker of intestinal microbiota alteration [23,164]. Similarly, it has been repeatedly reported to increase in equine patients affected by colic [30,172]. Moreover, Stewart and colleagues (2019) [148] observed an association between *Firmicutes:Proteobacteria* ratio and the onset of colic: to a higher proportion corresponded a lower probability of developing colic. Another study showed a correlation between changes in fecal *Proteobacteria* abundancy and tract of intestine affected, in fact it was shown that it increases when lesions of the large intestine are present, and it decreases in horses with small intestinal lesions [149]. This finding does not correspond entirely to our results, since two horses that were discharged had disorders of the large intestine and one of the ileum. Interestingly, it has been shown by other authors how *Proteobacteria* abundancy increased overtime during hospitalization in healthy controls compared to colic horses [133].

Our results showed a significant increase in *Verrucomicrobia* phyla abundancy in the pelvic flexure content of survived horses. Pelvic flexure has been

previously reported to be a point of separation between distinct communities of bacteria, since large ventral colon and cecal populations are very similar to each other while large dorsal community is much similar to the one found in the feces [125]. *Verrucomicrobia* is among the most represented phyla in equine hindgut [31,41], and it has been previously reported to be more abundant in fecal samples of horses affected by colitis [6], equine metabolic syndrome (EMS) [40] and equine grass sickness (EGS) [82] compared to healthy controls. Similarly, in human medicine, variations in abundancy of this phylum have been correlated with metabolic disorders and obesity [25,51]. *Verrucomicrobia* is also known to be responsible for maintenance of intestinal mucine layer integrity and reduce bowel inflammation [44,83], this might partly explaine the correlation with short term survival in our patients. Dougal and colleagues (2012) [36] reported a higher abundancy of *Verrucomicrobia* compared to *Bacteroidetes* in the colonic content of healthy equines, which differs from our results, therefore it can be speculated that also during different types of colic this phylum decreases in abundancy in favour of *Bacteroidetes*.

In the present study, the family of *Paraprevotellaceae* was found to be significantly more abundant in strangulating types of colic, both in the cecal and in the pelvic flexure contents. Previous authors have identified this family, especially in the cecum of healthy subjects [68,126], although it has been aknowledged to have a potential impact on digestive physiology of the cecum and therefore deserves further investigation, up to now little is understood about its functional role in horses [125]. This family has been observed to increase in abundancy in the cecal content of horses fed a high starch diet [171], whereas its prevalence was reduced in the feces of pasture fed horses [47].

*Prevotellaceae* family was significantly more abundant in cecal content of horses with strangulating colics in the present work. It has been associated with many functions in the digestion process and it has been mainly identified with a great abundancy in the cecal environment [37,130,168]. In previous studies it has been showed how this family's relative abundancy is reduced in horses affected by colitis and more generally by colic [6,148], which is in contrast with our results.

Regarding RF16 family, our results showed a significant increase in pelvic flexure content of horses with strangulating disorders. Not much is present in literature about this family, nevertheless our results are in discordance with what previously described by Ericsson and colleagues (2016) [41], who reported a higher abundancy of this family in the cecal content of Quarter horses and Morgans.

In the present study, *Mogibacteriaceae* and *Christensenellaceae* abundancies were significanlty decreased in strangulating cases, in the cecal and in the pelvic flexure contents respectively. The first family has been reported to be more abundant in feces of healthy Spanish and Quarter horses [58], moreover it is aknowledged to have an immunomodulatory function in ileum and cecal content [83]. Probably relating to this function, other authors underlined the decrease in abundancy of *Mogibacteriaceae* in feces of horses with colitis derived from antimicrobial associated diarreah [5]. The family of *Christensenellaceae*, has been reported to be more abundant in feces of horses managed at pasture [13], fact that does not correspond to our population of horses with non strangulating disorders, therefore their management has not been a bias in our analysis. Other authors found an increase in *Christensenellaceae* in horses referred for

colic [148].

In the cecal content of the horses that survived we found an increased abundancy in *Enterobacteriaceae* and *Enterococcaceae* families. The first one is usually more abundant in the upper GI of healthy equines [41] and is aknowledged to be an indicator of dysbiosis both in human and equine patients [148], specifically, in people undergoing colorectal surgery, the subsiquent changes in intestinal microbiota are characterized by a significant increase in *Enterobacteriaceae* [110]. In equines both Enterobacteriaceae and Enterococcaceae abundancies have been reported to increase in patients with antimicrobial associated diarrhea [5]. Interestingly, in accordance to our findings, Ayoub and colleagues (2022) [6] found a higher abundancy of Enterobacteriaceae in the feces of horses that survived colitis and associated laminitis.

The family of *Anaerolinaceae* had an increased abundancy in pelvic flexure content of horses that were discharged in our study. A little is present in literature about this family, nevertheless it has been reported to be highly represented in right ventral colon, right dorsal colon and feces of healthy horses [125]. This might suggest that the patients that survived in our work did not have a highly compromised microbiota of the colonic content.

*Limitations*

Limitations to the present study include the limited number of cases and mostly the disparity between discharged and non discharged animals, which does not represent the trend of our hospital, but only the sampling period. Another limitation consists in the absence of healthy control cases.

**Conclusions**

In conclusion, our results suggest that some differences in hind gut microbiotal composition are present between horses that survive and horses that do not survive until discharge after colic surgery, and also between strangulating and non strangulating disorders. Some of these alterations are congruent with literature whereas some are reported in the present work for the first time.

Further studies are needed to better establish the influence of microbiota on equine colics onset, course and recovery.

# Chapter 5 - EPIDEMIOLOGIC STUDY IN THE ITALIAN TERRITORY INVESTIGATING SHORT-TERM SURVIVAL AND POSTOPERATIVE COMPLICATIONS RATES IN HORSE UNDERGOING COLIC SURGERY

Spadari, A., Gialletti, R., Gandini, M., Valle, E., Cerullo, A., Cavallini, D., Bertoletti, A., Rinnovati, R., **Forni**, **G.**, Scilimati, N. and Giusto, G., 2023. Short-Term Survival and Postoperative Complications Rates in Horses Undergoing Colic Surgery: A Multicentre Study. *Animals*, *13*(6), p.1107.

**Introduction**

Although much progress has been achieved through the years, abdominal surgery in equines, in particular for colic syndrome, still has a high death rate compared to other procedures [121,122]. Moreover, few improvements seem to have been made in decreasing occurrence of post operative complications, which can severly impact both the patient welfare and costs of treatment [48]. Therefore, in order to let owners and veterinarians make the most appropiate decisions about treatments of an equid, it is mandatory to understand the factors that affect prognosis [86].

Postoperative complications and survival rates have been widely investigated by several authors, which reported that type and severity of the disease, and treatment applied were the main factors affecting short term survival [27,39,50,86,88,117,166,150]. Moreover, factors such as experience of the surgical crew and type of postoperative care, which are reported to influence the outcome in human patients undergoing major surgeries [1,2], are likely to affect the outcome of equines undergoing colic surgery as well [89].

Short term outcome, meaning the post operative course till discharge, has been investigated in previous studies that date back to early 2000s [104], therefore, given the changes that have been carried through the years, an assessment of the current situation needs to be performed.
Several factors influence the occurrence of GI disease in equine patients and their survival after colic surgery. Each equid population has its own intrinsic and extrinsic variables in terms of stabulation, nutrition and even weather conditions, that might play a role in the onset of colic [62]. Thus, studies investigating predictive prognosis indices, incidence of post operative complications and survival rates, may not be comparable if conducted in different geographical areas.
So far, no multicentric studies have been performed investigating the italian population of equids undergoing colic surgery. In fact, the italian studies regarding outcomes and short-term complications of colic patients date back to the early 90s, and were conducted on small populations of horses [106].
The aim of the present study is to report the short-term postoperative complications and survival rates and to report pre-, intra and post-operative factors that could affect the short-term outcome of horses that underwent colic surgery in three Italian major referral centers.

**Materials and Methods**

In the present retrospective cohort study, conducted in three different surgical centres in Italy between 2018 and 2021, a total of 451 horses which had undergone colic surgery were enrolled.
No approval from a Welfare committee was necessary.

*Case records and variables*

Medical records of horses undergoing colic surgery in the Veterinary Teaching Hospitals (VTHs) of the Universities of Bologna, Perugia and Turin (Italy), between 2018 and 2021 were analyzed in a retrospective manner. Horse signalment, clinical and surgical data, short-term postoperative clinical features, treatment, complications and outcome were retrieved.

*Definitions*

Short-term postoperative complications were defined as any deviation from the normal postoperative course [28,35] before discharge. Short-term survival was defined as survival until discharge from the hospitals [104].

The outcome was categorised as positive or negative if horses survived or not at discharge.

*Statistical analysis*

Statistical analysis was performed using a statistical program (JMP Pro 16). Descriptive statistics were generated (mean ± S.D., median and range) for continuous data (count and percentage) and for categorical data of total horses that underwent colic surgery. Normality was assessed by Shapiro-Wilk test for continuous variables. The differences between survivors and non-survivors on horses hospitalized after surgery were assessed using a Mann-Whitney test for continuous variables and a chi-squared test for categorical variables. Any association between the surgeon's experience and outcome has been evaluated through Spearman's correlation. Significance was set at $P<0.05$. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for categorical data.

Areas under the receiver operating characteristic (ROC) curve were used in parameters resulted significant at the

univariate analysis to evaluate discrimination and provide specificity and sensitivity prediction values. Classes were then created according to ROC discriminant values to integrate in previous model as categorical variables.

**Results**

*Case details*

A total of 451 horses were included in the study, of which 76/451 (16.8%) were referred to VTH of Bologna, 162/451 (35.9%) to VTH of Perugia and 213/451 (47.2%) to VTH of Turin. Two hundred and one horses were females (44.5%), 183 were geldings (40.5%) and 67 were males (14.8%). Median age was 12 years (range=0.2-32), median weight was 491 Kg (range=30-740) and median body condition score (BCS) was 6 (range=2-9). Most of the horses were warmblood (53.88%), followed by hot blood (16.41%), pony (9.53%), American saddle breeds (9.09%), Standardbred (6.88%), crossbred (2.21%), cold blood (1.78%) and mule (0.22%).

*Clinical features at arrival*

Not all data were available for every admitted case and the number of animals for which that data was recorded, is reported in brackets (n =). Mean and median packed cell volume (PCV) and total plasma protein (TPP) at arrival were 41.2 +/- 6.7 and 40% (range: 25-72; n = 435) and 6.9 +/- 0.7 and 6.9 g/dL (range: 3-10.9; n = 418), respectively. Mean and median blood lactate at arrival was 3.8 +/- 2 and 3.1 mmol/L (range: 0.7-16.9; n= 296). In horses presenting with reflux, mean and median amount of reflux was 6.2+/- 4.9 and 4 liters (range: 0.5-36; n=98). Most of the animals were last seen healthy 24 hours before being admitted to hospital (22.6%; n=401). Data regarding when the animal was last seen healthy before being referred to hospital are shown in table 1.

| Hours | No. | % |
|---|---|---|
| >24 | 91 | 22.69 |
| 4-8 | 87 | 19.29 |
| 8-12 | 78 | 17.29 |
| 12-16 | 56 | 12.41 |
| <4 | 36 | 8.97 |
| 16-20 | 28 | 6.2 |
| 20-24 | 25 | 5.54 |

Table 1: Data on when the equids (n=401) were seen healthy last time (h) before being referred.

*Surgical features*

A total of 386 horses (85.5%) recovered from anaesthesia and 65 (14.4%) horses died during surgery or in recovery box. Fifty-five out of 65 horses (84.6%) underwent intraoperative euthanasia, of which 22/55 (40%) for intestinal rupture or owner's decision and remaining 33/55 (60%) for surgeon's decision. Of these 33 judged not operable, 14 were referred to the VTH of Bologna, 12 to the VTH of Perugia and 7 to the VTH of Turin. Twenty-one out of 33 horses intraoperatively euthanized had strangulating lesions of small intestine, 4/33 had non strangulating lesions of large intestine, 3/33 had strangulating lesions of large intestine, 3/33 had non strangulating lesions of small intestine, and in the remaining 2/33 cases there was involvement of other viscera. In 7/33 horses, reasons for intraoperative euthanasia were reported (2 horses were euthanised because of endotoxemia and 5 for involvement of too much intestine). Six horses out of 65 (9.2%) did not wake up from the anaesthesia and 4/65 horses (6.1%) died spontaneously during surgery.

In the 451 horses that underwent surgery, the large intestine was involved in 56.3% of cases, the small

intestine in 42.1% of cases and in the remaining cases (1.5%) other viscera were involved. The large colon was the most involved intestinal tract (46.1%), followed by jejunum (20.1%) and ileum (9.7%). Detailed data of intestinal tract involved were reported in table 2.

Right dorsal displacement (15.74%) was the most encountered pathology, followed by large colon volvulus (14.19%), nephrosplenic entrapment (9.09%) and epiploic foramen entrapment (6.87%). List and % of diseases were summarised in table 3.

Strangulating lesions were recorded in 39.9% of cases: specifically in 70/254 (27.5%) cases with large intestine diseases and 110/191 (55.8%) cases with small intestine diseases. An anastomosis was performed in 25.4% of cases, of which most common types were jejuno-jejunostomy (35.2%), followed by jejunal-caecal bypass (20.9%) and jejuno-caecostomy (18%). List and % of types of anastomosis performed was reported in table 4. Mean and median duration of surgery, in horses recovered from anaesthesia, were 1.73 +/- 0.7 and 1.58 hours (range: 0-5.17; n=384). Details relating surgeons' experience are reported in table 5. Mean and median amount of pre and intraoperative fluid therapy were 15 +/- 16.1 and 10 L (range: 0.3-118; n = 316).

| Intestine | No. | % |
|---|---|---|
| Large colon | 208 | 46.11 |
| Jejunum | 91 | 20.17 |
| Ileum | 44 | 9.75 |
| Jejunum - Ileum | 40 | 8.86 |
| Small colon | 24 | 5.32 |
| Caecum | 14 | 3.1 |

| | | |
|---|---|---|
| Duodenum - Jejunum | 11 | 2.43 |
| Pelvic flexure | 7 | 1.55 |
| Mesentery | 4 | 0.88 |
| Stomach - Spleen | 3 | 0.66 |
| Ileum - Caecum | 2 | 0.44 |
| Duodenum | 2 | 0.44 |
| Liver | 1 | 0.22 |

Table 2: List and % of viscera involved in 451 equids undergone colic surgery.

| Disease | No. | % |
|---|---|---|
| Right dorsal displacement (RDD) | 71 | 15.74 |
| Large colon volvulus (LCV) | 64 | 14.19 |
| Nephrosplenic entrapment | 41 | 9.09 |
| Large colon impaction | 31 | 6.87 |
| Epiploic foramen entrapment (EFE) | 30 | 6.65 |
| Pedunculated lipoma | 26 | 5.76 |
| Ileal impaction | 22 | 4.87 |
| Inguinal hernia | 21 | 4.65 |
| Small colon focal impaction | 21 | 4.65 |
| Small intestinal volvulus | 18 | 3.99 |
| Small intestine obstruction | 14 | 3.1 |
| Adhesions | 13 | 2.88 |
| Caecal impaction | 9 | 1.99 |

| | | |
|---|---|---|
| DPJ | 8 | 1.77 |
| Jejunal impaction | 7 | 1.55 |
| Ileal hypertrophy | 6 | 1.33 |
| Mesenteric rent | 5 | 1.1 |
| Jejuno-jejunal intussusception | 5 | 1.1 |
| Caecal intussusception | 3 | 0.66 |
| Extramural mass | 3 | 0.66 |
| Gastrosplenic entrapment | 3 | 0.66 |
| Omental rent | 3 | 0.66 |
| Extraperitoneal haematoma | 2 | 0.44 |
| Abscess | 2 | 0.44 |
| Diaphragmatic hernia | 2 | 0.44 |
| Mesodiverticular band | 2 | 0.44 |
| Nephrosplenic entrapment of small intestine | 2 | 0.44 |
| POI | 2 | 0.44 |
| Caecal rupture near J-Ce anastomosis | 1 | 0.22 |
| Abdominal hernia | 1 | 0.22 |
| Focal eosinophilic enteritis | 1 | 0.22 |
| Hepatic abscess | 1 | 0.22 |
| Ileo-caecal intussusception | 1 | 0.22 |
| Jejuno-ileo-caecal intussusception | 1 | 0.22 |
| Caecal hypertrophy | 1 | 0.22 |

| | | |
|---|---|---|
| Duodenal entrapment | 1 | 0.22 |
| Gastric impaction | 1 | 0.22 |
| IV grade rectal prolaps | 1 | 0.22 |
| Lymphoma | 1 | 0.22 |
| Mesenteric abscess | 1 | 0.22 |
| Mesenteric ischemia | 1 | 0.22 |
| Small intestine neoplasia | 1 | 0.22 |
| Typhlitis | 1 | 0.22 |

Table 3: List and % of type of diseases in 451 equids undergone colic surgery.

| Anastomosis | No. | % |
|---|---|---|
| Jejuno-jejunostomy (JJ) | 40 | 37 |
| Jejuno-caecal bypass (JCE BYPASS) | 22 | 20.4 |
| Jejuno-caecal (JCE) | 21 | 19.4 |
| Jejuno-ileal (JI) | 8 | 7.4 |
| Hibrid jejuno-ileo caecal (HJICE) | 7 | 6.3 |
| Colo-colic (COCO) | 6 | 5.6 |
| End-to-end of small colon (SCO-SCO) | 4 | 3.7 |
| Jejuno-ileo-colic (JICO) | 2 | 1.9 |

Table 4: List and % of types of anastomoses reported in 108 equids undergone colic surgery.

| Surgeon | On total horses (%) | No. of surgeries | Intraoperative Euthanasia | Relaparotomy (%) | Postop euthanasia | Discharged/ hospitalized | Discharged/ total |
|---|---|---|---|---|---|---|---|
| 1 | 9.5 | 13.1 | 16.3 | 2.8 | 25.0 | 72.2 | 60.5 |
| 3 | 5.3 | 9.6 | 29.2 | 0.0 | 29.4 | 70.6 | 50.0 |
| 2 | 3.5 | 4.8 | 12.5 | 14.3 | 21.4 | 64.3 | 56.3 |
| 4 | 33.3 | **39.6** | 13.2 | 6.1 | 19.1 | **74.8** | **64.9** |
| 5 | 5.7 | **18** | 0.0 | 0.0 | 11.5 | **88.5** | **88.5** |
| 6 | 42.6 | **69** | 13.0 | 4.8 | 12.5 | **82.7** | **72.0** |
| Spearman correlation | | | | | | r= 0.83 | r= 0.77 |

Table 5: Details on surgeon's experience and effect on short term outcome.

*Postoperative clinical features*

Mean and median PCV and TS were 34.5 +/- 6.9 and 34% (range: 22-64; n = 206) and 65.3 +/- 0.8 and 5.4 g/dL (range: 3.2-8.1; n = 205), respectively. Mean and median Delta PCV were -6.1 +/- 8.8 and -6% (range: -45-36; n = 207). Mean and median Delta TS were -1.5 +/- 1.1 and -1.4 g/dL (range: -7.8-1.7; n = 202).

*Short-term postoperative complications*

Eighteen out of 386 (4.6%) horses underwent relaparotomy in the postoperative period for recurrence of clinical signs of colic. Five out of 18 horses (27.7%) had intestinal adhesions, 3/18 (16.6%) intestinal impaction, 2/18 (11.1%) per each large colon volvolus (LCV), peritonitis or POI, and 1/18 (5.5%) per each had acute abdominal herniation, right dorsal displacement (RDD), anastomotic obstruction or intussusception. Nine out of 18 horses (50%) were discharged alive, 5/18 (27.8%) underwent postoperative euthanasia (2 for abdominal pain persistence, 1 for adhesions, 1 for peritonitis and 1 for acute abdominal herniation) and 4/18 (22.2%) underwent intraoperative euthanasia.

Pyrexia was the most common postoperative complication and was recorded in 27% of 386 horses recovered from anaesthesia, with single or multiple episodes (50%), respectively. Surgical site infections were recorded in 25% of horses. Postoperative colic was recorded in 23% of cases, clinical signs of piroplasmosis were recorded in 21.8% of horses, and postoperative reflux in 20% of cases. Diarrhea was recorded in 10% and thrombophlebitis was recorded in 7.7% of cases. Complete list and % of postoperative complications were reported in table 6.

| Complication | No. | % |
|---|---|---|
| Pyrexia | 107 | 22.4 |
| Surgical site infection | 100 | 25.1 |
| Clinical signs of piroplasmosis | 91 | 22.9 |
| Postoperative colic | 72 | 18.1 |
| Postoperative reflux | 62 | 15.6 |

| | | |
|---|---|---|
| Diarrhoea | 40 | 10 |
| Thrombophlebitis | 29 | 7.3 |
| Hernia | 25 | 5.7 |
| Other complications | 22 | 5.5 |
| SIRS | 17 | 4.2 |
| Laminitis | 11 | 2.7 |
| Hyperlipemia | 8 | 2 |
| Wound dehiscence | 7 | 1.7 |
| Myopathy | 7 | 1.7 |
| Piroplasmosis | 4 | 1 |
| Hemoperitoneum | 1 | 0.2 |

Table 6: List and % of postoperative complications reported in 386 horses recovered after colic surgery.

*Short-term survival and predictive indicators*

Overall short-term survival rate was 68.5%, while it was 80% for the 386 horses hospitalized after surgery. Prognosis predictive indices were evaluated only on hospitalized horses, excluding animals undergone intraoperative euthanasia. The use of parameters of subjects judged inoperable, in the opinion of individual surgeons, or underwent euthanasia for economic reason to identify risk factors for prognosis of horses operable and operated could represent a confounding factor. The characteristics of these subjects were in fact considered separately from those who survived surgery. The criteria of judgement for defining an inoperable subject are subjective and therefore can vary greatly between centres and surgeons.

There was no significant association between sex, weight,

breed and survival, while age influenced the outcome. Horses with age >14 years were 2.3-times more likely to have a negative outcome than horses between 10 and 14 years (p=0.0276). Horses with BCS between 4 and 6 and >6 had a 2.83- and 3-times higher probability of positive outcome than horses with BCS <4 (p=0.0136; p= 0.0044). The cut-off to define the risk BCS value is 7 (spec. 65%; sens. 51%). PCV, TS and blood lactate values, and amount of reflux appear to be good predictors of outcome. Horses with PCV >50% (p<0.0003) and TS value <5.7 (p=0.0034) and >7.4 (p=0.0207) at arrival had a higher probability of poor prognosis than horses with different values. Blood lactate <1.2 mmol/L had a higher probability of positive outcome than horses with blood lactate >6.6 mmol/L (p = 0.05). The presence of reflux did not increase the probability of a negative outcome, but an amount of reflux <0.018 L/Kg BW was associated to a higher probability of positive outcome (p=0.0304). Referral time did not affect the outcome. Horses with pathology of other viscera than intestine (e.g. spleen, liver) had a 7.3-times higher probability of negative outcomes than horses with intestinal disease (p=0.0316). However, the outcome was not influenced by the type of pathology. Horses with non strangulating lesions had a 2.18-times higher probability of positive outcome than horses with strangulating lesions. In horses that did not undergo anastomosis, the probability of a positive outcome was 2.3-times higher than in horses which underwent intestinal anastomosis (p<0.0029). Outcome was not affected by the type of anastomosis performed. Surgery lasting more than 2 hours and 24 minutes was more likely to result in negative outcome (p< 0.0001). Surgeon experience in terms of numbers of horses operated per year affected the outcome. A mean number of horses operated per year >18 was correlated to a better short-term outcome (r=0.83).

Furthermore, an amount of crystalloids > 44 ml/Kg was associated to a higher probability of poor prognosis (p=0.0208). PCV value after surgery also influenced the outcome: a PCV between 26 and 43% was associated with a higher probability of positive outcome (p=0.0105). Furthermore, the outcome was better if the variation of PCV (delta PCV) was between -8 and 0 than if it was less than -8 (p=0.0156). The TS value after surgery and delta TS also affected the outcome as a TS value after surgery below 4.5 g/dl was associated with a worse outcome (p=0.0229) and a loss <2.2 g/dl was associated with a higher probability of negative outcome (p=0.0089).

**Discussion**

The present work, which is also under review for publication, represents the first multicentric study across the italian territory investigating short-term postoperative complications, short-term survival rate and possible risk factors affecting the short-term outcome of equines that underwent colic surgery.

Short-term survival rate in the present work was 80%, which is slightly lower than the one reported in some previous studies [39,50,87], but higher than what was described by others [27,117,159,162].

Multiple predictive indices associated with short-term survival were shown in the present work, some of which are similar to those presented in previous studies examining different populations. In particular, PCV, blood lactate value at arrival, BCS, the type of intestinal lesion, presence of anastomosis, duration of surgery and surgeon experience appeared to be prognosis predictive indices both in the present studies and in previous ones [21,48,66,78,86,104,120,121,136,161,162]. Nevertheless, in this study, also the amount of cristalloids administered

resulted to affect the postoperative course, which was not investigated before.

Age of horses underging colic sugery has been previously reported to be associated with short-term outcome [114], as it appears in the present work, although with different age ranges. Nevertheless, there is some literature in disagreement about the actual influence of age [86].

No significant association was found between breed, weight or sex and survival rate in the present study, nor in previous ones from other authors [86].

The results about the influence of BCS, PCV, TS and blood lactate at arrival on the outcome were in line with those reported in other studies [66,78], although with higher cut-off values, which may be related to the features of the italian horse ppopulation.

Regarding type of lesion, execution of an anastomosis, surgeon and duration of the surgery, their effect on short-term outcome was also comforming with what has been previously reported in literature [86,104,120,122,162]. In fact, non strangulating lesions, which affected more frequently the large intestine [48], were more likely to have a favourable short-term outcome (> 88%) [27,48,87,98,107,144]. On the other hand, strangulating lesions of large bowels tend to have a lower effect on short-term survival rate [87], since they are more frequently associated to pre- or intraoperative death or euthanasia [27,98] due to the severly compromised clinical and haematological status they often cause.

Regarding small intestine, previous studies have shown a lower short-term survival rate in horses with strangulating small intestinal lesions that required resection and anastomosis, compared to those with simple obstructions [86,114]. This data is in line with what is reported in the present study, in which patients that did not receive an

anastomosis had a 2.3-times greater probability to survive till discharge than horses on which an anastomosis was performed. Nevertheless this finding is in contrast to what is reported by other authors [38,50,117]. Nevertheless, it must be considered that leaving a strangulated tract of small intestine in place means exposing the patient to the risk of developing endotoxaemia or reperfusion injury and therefore an increased probability of postoperative ileus, laminitiis, adhesions and death [86,87,104,121]. The decision making of whether to perform an enterectomy or not represents a key point in abdominal surgery. The absence of an anastomosis might positively affect long-term survival [123], although it could lead to the development of postoperative complications that may require a relaparotomy and thus decrease the possibility of a favourable outcome [86,112]. Not all cases presents with obvious signs of necrosis that suggest an enterectomy is mandatory and no objective parameters have been identified, therefore the choice whether to perform the anastomosis or not resides only in the surgeon's opinion. Thus, the outcome depends also on the experience and judgement of the surgeon.

Another interesting finding of the present study is that the type of anastomosis performed seems to not affect the outcome, a result different from what previously reported. In fact, it has been reported that survival rate of horses that underwent an end-to-end anastomosis was higher than the ones that requred a jejunocecal anastomosis [120]. However, the type of anastomosis performed strongly depends on the pathology that has to be treated, thus the outcome might not be related to the surgical procedure itself but also to the type of intestinal lesion. Strangulating lesions of the small intestine tend to cause a distension of all the bowel proximal to it, therefore if a distal tract is interested the whole small intestine might be

affected by the distension, thus a worse prognosis and a higher rate of postoperative complications are expected than in cases where the strangulation is more proximal and therefore it affects a shorter tract.

However, in order to have better informations in this regard, prospective studies comparing different types of anastomosis for the same pathology are needed.

As previously reported, also in the present study an increase in duration of surgery negatively affected the short-term survival rate [121]. In fact, a longer surgery may play a role in the development of neurological signs during both recovery phase [67,162] and early postoperative period [96], moreover it could increase the probability of developing surgical site infections [74]. Duration of surgery, in turn, is determined by several variables such as surgeon experience, type of pathology or necessity to perform an anastomosis. All these factors directly influence the outcome as well, therefore it is difficult to discriminate whether the duration variable itself has a high influence on the outcome or it just has a marginal effect [87].

Short-term survival rate in the present study was also affected by the surgeon's experience, intended as number of patients operated per year, as previously reported by other authors as well [21]. Nevertheless, the number of patients operated per year might as well depend on the policy of each hospital, which might be biased towards surgery, leading to operating also a portion of horses that would have recovered with medical treatment, and therefore also a higher survival rate [14].

Regarding the use of intravenous cristalloids infusion, results from this study showed it had a positive influence on the outcome. Nevertheless, it was shown that when the amounts of administered fluid was above 44 ml/Kg it

negatively affected the short-term survival. In fact, although the great importance of cristalloids administration to counteract hypovolemia, hypotension, hypoperfusion and ensure normal cardiac output, which is mandatory in horses presenting with colic synrome, an excessive use can lead to hypervolemia, haemodilution, coagulopathy, pulmonary oedema, and organ dysfunction [7]. Moreover, it has been described how a "goal-directed" fluid administration in horses that underwent laparotomy for strangulating lesions of the small intestine was able to reduce postoperative complications while being less expensive and reducing hospitalization time [54].

*Limitations*

Only univariable analysis of data was conducted in the present study, consequently possible confounding effects between variables may not have been considered. Furthermore, not all differences among centers were taken into account but overall results were considered. It is important to underline that the weather, the prevalence of breeds and the activity of horses may differ between north and central regions of Italy. Moreover, only effects on short-term survival were considered. Further studies could be carried out to evaluate the possible differences between the three Italian populations considered and long-term survival. Furthermore, the evaluation of the effect of predictive indices on long-term survival could lead to different results than those obtained in this study. Further prospective studies should be conducted to confirm the effect of predictive indices considered in this study on short-term survival.

**Conclusions**

This is the first multicentric study in Italy evaluating

short-term outcomes of horses that underwent colic surgery. This study gave indications on factors that affect the short-term survival. Previous studies have been carried out investigating the same topic, but on different equine populations and in different geographical areas. However, results of the present work suggest that predictive indices previously reported on different populations are applicable also on Italian equine population.

# Chapter 6 – GENERAL CONCLUSIONS

The present PhD thesis represents the continuation of a work of research on colic syndrome in the equid patient, especially surgical colic, that has been initiated and carried on in the University of Bologna for a long time.

Human medicine is in continuous evolution to always increase quality and efficiency of treatments, and so is veterinary medicine.

Better education of referring veterinarians [48], novel diagnostic aids that offer an early and more precise diagnosis and prognosis [85], new surgical techniques [53] and more specific training of surgeons, and goal-directed postoperative treatments [54] are key subjects to offer a better quality of treatment to equid patients affected by colic syndrome and improve survival rates. Therefore, further researches are needed in these fields.

This work also underlines the importance of conducting multicentric studies, especially in the Italian territory, in which the number of horses is not as high as in other countries, in order to increase the number of cases and improve results reliability of the scientific studies.

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