ASSOCIATION OF RISK FACTORS WITH COLIC IN HORSES WITH SPECIAL EMPHASIS ON NUTRITION

INAUGURAL-DISSERTATION

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LIST OF ABBREVIATIONS

°C  Celcius
°F  Fahrenheit
µkat/L  U/l x 0,0167
ALP  Alkaline phosphatase activity
AST  Aspartate transaminase
C. difficile  Clostridium difficile
C. perfringens  Clostridium perfringens
CFU  Colony forming units
Fig.  Figure
fl  Femtoliter
g  Gram
GGT  γ-Glutamyltransferase
GLDH  Glutamate dehydrogenase
GMD  GDP-β-mannose-4,6-dehydratase
H  Hour
Kg  Kilogram
L  Liter
MCHC  Mean cell hemoglobin concentration
MCH  Mean cell hemoglobin
MCV  Mean cell volume
mg  Milligram
PCV  Packed cell volume
pH  Hydrogen ion concentration
Tab.  Table
TIS  Animal hospital information system
TP  Total plasma protein
U/L  Units per liter
VFA  Volatile fatty acids
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1. INTRODUCTION

The term colic refers to abdominal pain. The manifestations of pain generally are pawing, rolling, lying down, kicking at the abdomen, looking at the flanks, sweating or attempting to urinate (ROSE and HODGSON, 1993). Some physiological or anatomic factors such as no vomiting ability, unfixed position of the left colon, long mesentery of the small intestine, cecum being a blind sac, upward movement of ingestion and narrowing of the lumen at the pelvic flexure predispose horses to colic (ROSE and HODGSON, 1993). Colic may occur due to different causes. In horses, most of these problems involve the gastrointestinal tract, but diseases of other structures within or associated with the abdomen such as the kidneys, liver, uterus, peritoneum or spleen may also result in signs of colic (TRAUB-DARGATZ et al., 2001). Colic in the content of this thesis is defined as pain originating in the enteric-system, its associated ligaments, the mesentery and the peritoneum. The causes of pain are many, and as such are not a matter of investigation in this study. Especially those forms of colic that need reference to an equine hospital have a high risk of death. The mortality rate for colic cases was 0.45 deaths per 100 horse years in the UK study (HILLYER et al., 2001), 0.5 deaths per 100 horse years in a Michigan study (KANEENE et al., 1997) and 0.7 deaths per 100 horse years in a Virginia and Maryland study (TINKER et al., 1997b).

The role of many risk factors of colic is still not completely clear or not yet identified. These may be the results of interaction and confounding. Identification of risk factors is important because prevention of disease by controlling modifiable, exposure risk factors can be possible.

Horse feeding in principle is not complicated in spite of the fact that feeding is one of the most common discussed topics concerning colic. Feeding and watering management such as type of feed and amount of feed intake, hygienic quality of feed, a change in diet and access to water were identified as putative risk factors for colic. These are all controllable risk factors. For example, hay of poorer quality is often less digestible, thereby predisposing colonic impaction (COHEN and GIBBS, 1999). Change of diet composition (COHEN et al., 1995; COHEN and GIBBS, 1999; COHEN and PELOSO, 1996; HUDSON et al., 2001; REEVES et al., 1996; TINKER et al., 1997a) and inadequate water supplies (REEVES et al., 1996) are associated with the increased risk of colic. However, the role of watering practices has not yet clearly been determined (HUDSON et al., 2001).

Activity of the horse, housing and pasture practices may also be risk factors for colic, and these are also controllable. Activity level depends on the duty of the horse. Intense activity (TINKER et al., 1997a), activities associated with stress (KANEENE et al., 1997) and any change in activity (COHEN and GIBBS, 1999) increased the risk of colic. Year-long pasture indicated lower colic risks than horses living indoors (COHEN and GIBBS, 1999). Moreover, anthelmintic administration is also an alterable risk factor. Parasites like small or large strongyles and tapeworms can cause colic. COHEN and GIBBS (1999) found the risk of colic increased when horses did not receive any regular deworming treatment; in contrast, COHEN et al. (1995) identified no association between colic and the type of anthelmintic administered or the parasite control program.

Individual factors are non-alterable factors, although these factors are putative risk factors of colic. Age, sex and breed were found to be risk factors with contrary conclusions in several studies. For example, TINKER et al. (1997a) found that the horses between 2 and 10 years old were at increased risk for colic, while COHEN and GIBBS (1999) found that horses
older than 10 years of age had an increased incidence of colic. However, another study conducted by HUDSON et al. (2001) found that age was not significantly associated with colic. EDWARDS and PROUDMAN (1994) revealed that geldings were more prone to colic than females and stallions, although the exact reason for this is unknown. The opinion of ABUTARBUSH et al. (2005) was that mares and stallions are better cared for, because these are valuable breeding animals. Considering breed, REEVES et al. (1996) found Arabian horses were more than twice as likely to become colic cases, whereas ABUTARBUSH et al. (2005) concluded that no breed was predisposed to colic.

Weather-temperature may have an effect on occurring of colic cases, but the causality is not clear. MBAROMETRIC (2002) proposed both that colic incidence could increase during warmer months of the year due to dehydration from sweating, and contrary-wise, colic incidence could increase during the colder months of the year due to decreased water intake.

The first step for diagnosis of colic is having good anamnesis. At the time a case is admitted to hospital, owners have to answer questions or even fill out questionnaires. History is valuable for helping to find the cause of the disease. Many questions should be asked either to the owner or caretakers. Some must be about the individual horse (such as age, breed, sex and weight) and the rest about management of the horse (such as feeding and activity) (MBAROMETRIC, 2002). The second step is clinical examination, including evaluation of effects of previous medication, severity of pain, heart rate (beats/min), rectal temperature, respiratory rate (breaths/min), auscultation of intestine, rectal palpation, capillary refill time (seconds), mucous membrane color, packed cell volume (%) and total plasma protein (gm/dl) (MBAROMETRIC, 2002), and in special cases an abdominal paracentesis must be performed.

Several studies (BRAUN et al., 2002; ORSINI et al., 1988; PARRY et al., 1983a; PARRY et al., 1983b; VAN DER LINDEN et al., 2003; WIRTH, 1986) were to determine the effects of blood parameters on prognosis of colic. Bile acids concentration was found to be a more sensitive indicator of disorders related to colic than other serum parameters (SEIFERT, 1983). Alkaline phosphatase activity (ALP) is valuable for aiding in the decision for surgery and prognosis (SAULEZ et al., 2004).

Amines are produced by bacteria in the cecum and large intestine of horses (BAILEY et al., 2003b). Many amines (tryptamine, tyramine, putrescine, cadaverine, histamine, spermidine and spermine) may have the potential to cause peripheral vasoconstriction if released into the circulation from the gastrointestinal tract (BAILEY et al., 2003a).

Several studies reported that Clostridium perfringens (GRIFITHS et al., 1997; HERHOLZ et al., 1999; JOBST et al., 2002; PERK et al., 1993) and Clostridium difficile (JOBST et al., 2002; PERK et al., 1993) and Salmonella spp. (ERNST et al., 2004; JOBST et al., 2002; KIM et al., 2001; PALMER et al., 1985) were isolated from feces of horses with gastrointestinal diseases. However, in a few other studies in which there was no detection of C. difficile in any of the feces samples from horses with colic (BAVERUD et al., 2003). Neither was C. perfringens and Salmonella isolated from diarrheic foals (ARROYO et al., 2004), nor was Salmonella isolated in gastric reflux or feces of horses with anterior enteritis (GRIFITHS et al., 1997). Apparently regional differences can result in great differences of microbial isolations.
The aim of this work was:

- To identify the impact of feeding and watering practices, hygienic quality and quantity of the feed, individual factors, weather-related factors and management factors that may influence the development of colic in an equine hospital population in Vienna.

- To reveal possible associations, especially the activity of serum alkaline phosphatase and serum bile acids with colic in horses. The rationale for this was that these parameters could be helpful in decision for surgery and for giving an outcome of prognosis.

- To determine the presence of specific biogenic amines in blood and feces from horses with colic.

- To isolate and investigate the association of prevalence of *Clostridium perfringens*, *Clostridium difficile* and *Salmonella spp.* in horses and colic. In addition, the effect of colic on fecal pH was investigated.

Consequently, these aims have the single important point of providing equine clinicians with a better understanding of details of important risk factors of colic occurrence, which are useful for diagnosis and prognosis of colic.
2. REVIEW OF LITERATURE

2.1. Clinical history of colic, treatment procedure of colic and mortality rate

Any history of colic forms found an increased risk for further colic episodes (COHEN et al., 1995; COHEN and GIBBS, 1999; REEVES et al., 1996; TINKER et al., 1997a). There is the risk to develop colic multiplied by 3 to 4.6 (COHEN and GIBBS, 1999; NATHANIEL, 2005). There is no definite explanation for that, but it may be attributable to environmental, managerial or physiological factors (COHEN et al., 1995). Horses that have history of colic and were previously treated by surgery are at significantly increased risk of further colic episodes (COHEN et al., 1995; COHEN and PELOSO, 1996; COHEN and GIBBS, 1999).

TINKER et al. (1997b) reported that 13 % of 1427 horses enrolled in Virginia and Maryland in the USA that had one or more colic episodes during the previous year. HILLYER et al. (2001) reported that a total of 509 episodes of colic occurred on thoroughbred training premises in the British Isles in 1997. Of these horses 84.6 % had only one episode, 14.9 % had 2-5 episodes and 0.5 % had more than 5 episodes. DABAREINER and WHITE (1995) reported that 32 % of the horses in 147 horses, which were suffering from large colon impaction had at least one more colic episode after the first colic and that was possibly due to permanent intestinal damage or converting colon dysfunction to original impaction. In contrast, MORRIS et al. (1989) found no correlation between history of previous colic and any form of causes.

Historical information relating to frequency of recurrent periods of colic and their duration appeared to be helpful in terms of prognosis. In a study by HILLYER and MBAROMETRIC (1997), horses with frequent (> 3 episodes /year) recurrences of transit colic had a relatively high mortality rate (53 %). Transit colic in this study was defined as colic that either improved within 24 hours without any treatment or did not recur within 24 hours following medical treatment. In contrast, horses with infrequent recurrences of transit colic mostly had non-specific or spasmodic colic with a very low mortality rate (4 %). Horses with recurrent prolonged colic defined as colic signs present for more than 24 hours regardless of medical treatment, and it had an intermediate mortality rate (31 %).

Surgically treated colic cases remained longer in the hospital than medical cases (ABUTARBUSH et al., 2005; FREDERICO et al., 2006; RHOADS et al., 1997). A significant difference was identified for duration of hospitalization between the medically treated and surgically treated cases of small colon impaction (FREDERICO et al., 2006; RHOADS et al., 1997) and large colon impaction (FREDERICO et al., 2006). RHOADS et al. (1997) reported mean durations of hospitalization for horses with small colon impaction treated medically and surgically, and those were 7.2 days and 10.7 days, respectively. FREDERICO et al. (2006) also reported mean durations of hospitalization for horses with small colon impaction treated medically and surgically, and those were 7.6 days and 12.8 days, respectively. Additionally, the same authors reported mean durations of hospitalization for horses with large colon impaction treated medically and surgically, and those were 4.8 days and 9 days, respectively.

Regardless of whether medical or surgical treatment is required, the prognosis appears to be good for horses with small colon impaction (FREDERICO et al., 2006; RHOADS et al., 1997) with 91 % of horses treated medically and 95 % of horses treated surgically surviving to discharge (FREDERICO et al., 2006). In one study, the case fatality risk for surgical treatment was 31 %, while the case fatality risk for non-surgical colic was 10 % (KANEENE...
et al. 1997). Significantly more animals with surgical colic were euthanized than were animals with medical colic, which can be explained by the fact that horses with surgical conditions are more likely to carry a poorer prognosis that those with a medical problem (ABUTARBUSH et al. 2005). Most horses with large colon impaction can be treated medically, and those horses have a lower fatality rate than horses treated surgically due to the possibility of inter operative bowel rupture. Therefore, the risk of bowel rupture should be considered when using surgery as a treatment for that kind of colic (DABAREINSER and WHITE, 1995). Finally, cases of epiploic foramen entrapment have a lower long-term probability of survival than all other types of surgical colic (ARCHER et al. 2004).

2.2. Feeding Practices

2.2.1. Feed Hygienic Quality and Quantity

The most common defects in food are included high contents of molds, yeasts and bacteria (MEYER et al. 1986). These contaminants indicate poor hygienic quality of the feedstuff. These conditions have a negative effect on nutritive value and palatability and furthermore cause digestive disorders (KAMPHUES, 1996). Frequent other problems identified by MEYER et al. (1986) are unsuitable physical condition of feed such as hay and straw chopped too short (causing colic), crumbling pellets and dusty hay (respiratory disorders), and sticky mixed feed (colic and stomach rupture). Increased yeast concentrations were particularly associated with colic (MEYER et al., 1986). KAMPHUES (1996) found that high levels of mites and molds in the fore, and in lower frequency higher contamination by yeasts and bacteria. The same author concluded that, besides the negative effects of these loads on the nutritive value and the palatability, the main risk lies in causing digestive disorders. Silages, molassed oats and sometimes concentrates were loaded with yeast in a lower frequency but higher contamination than hay and straw. Cereals were more contaminated with mites and molds. In addition, oats were especially highly contaminated with bacteria. After the intake of oats and mixed feeds of poor hygienic quality, a history of digestive disturbances was reported for most cases. Horses suffering from chronic digestive disorders unrelated to feed of poor quality often respond to treatment with enzyme preparations such as mixtures of proteases, amylases and lipases (MEYER et al., 1986).

SLIWINSKY et al. (2005) conducted a study on 159 feedstuffs from 40 horse farms or stables and found that only a few hay samples were contaminated, and some grass silage showed high levels of yeasts. Two thirds of oat samples had high bacterial contamination and 20 % were contaminated with molds. On the other hand barley and maize had lower levels of contamination. 30 % of all concentrate feed, both home mixes and branded products, had very high levels of bacterial contamination. COENEN and KIENZLE (1992) conducted a study in which 442 feed samples were analyzed for hygienic quality by sensory evaluation, amounts of lipopolysaccharids, and counts of bacteria and molds. Two thirds of 152 oat samples were damaged, 15 % of mixed feeds were rejected and 39 % of hay samples were unsatisfactory.

The Oryzaephilus surinamensis, the saw toothed grain beetle is commonly found in the stored grain. The adults cannot fly and must be introduced from contaminated grain. Adults live an average of 6 to 10 months, females lay between 43 and 285 eggs during their lifetime, and these eggs hatch in 3 to 5 days when environmental conditions are optimal (26 to 29 °C). Results of the contamination of the grain by the beetle are reduced grain weight and quality (CALVIN, 2001).
Colchiculum autumnale is commonly called meadow saffron, autumn crocus or naked ladies. All parts of the plant, but especially the flowers and seeds, are poisonous, and it contains two structurally similar alkaloids, which are colchicines and colchicine. Both alkaloids are able to withstand drying and storage without losing their toxic properties. In most animals, Colchiculum autumnale poisoning causes abdominal pain due to effects on the gastrointestinal system (COOPER and JOHNSON, 1990). Colchicines poisoning was diagnosed in Germany in horses that developed colic following feeding of newly delivered batch of hay, which was heavily contaminated by Colchiculum autumnale (about 1.48 % of total mass). Pathological findings indicated colchicines intoxication (KAMPHUES and MEYER, 1990).

A change in the quality or the quantity of food and in the time of feeding increased the risk of colic (COHEN et al., 1995; COHEN and GIBBS, 1999; PROUDMAN, 1991; REEVES et al., 1996). Therefore, changing to a poorer quality type of hay during a two-week period predisposes a horse to colic. Because this hay is less digestible, and possibly it causes alterations in the colonic pH, the VFA production, the colonic micro flora, and causes disorders of intestinal function, it predisposes horses to colonic impaction according to COHEN and GIBBS (1999).

2.2.2. Type and Amount of Dietary Components

Several studies showed contrary results regarding the effects of nutrition on occurrence of colic. The major component of the horses’ diet is forage, which provides energy, protein, minerals, vitamins, and other essential nutrients in amounts capable of supporting a horse at maintenance or in light work. When energy needs are high (e.g., growth, gestation, lactation or heavy work), grain supplementation becomes important (LEWIS, 1995b). COHEN et al. (1995) could not show that the types of concentrate (oat and sweet feed) and hays fed to horses with colic and without colic differed. Furthermore, COHEN and GIBBS (1999) also did not find an association between feeding a particular type of concentrate and colic.

Other authors suggest that grain diets are associated with increased risk of colic, because they decrease the water content in the colon due to less fiber, which binds to water and also increases gas production, thereby creating the environment needed for tympani and displacement (NATHANIEL, 2005). For each 1 kg increase in the amount of whole grain-corn consumed, the risk of colic increased over three-fold; however, a 1 kg increase in the amount of non-roughage feed consumed (when all non-roughage concentrate feeds were combined) was associated with a 12 % reduction in colic (REEVES et al., 1996). Feeding more processed feeds such as pellets or sweet feeds increased the risk of colic (TINKER et al., 1997a). Pelleted grain was associated with large colon displacement or volvulus, and pelleted roughage was associated with large colon impaction (MORRIS et al., 1989). Feeding horses grain after being brought from pasture and then keeping them in stalls increased the risk of colic, especially colon tympani and displacement, but feeding horses grain and then turning them out decreased the risk of colic (NATHANIEL, 2005).

It is normal custom that the amount of concentrate feed given is proportional with the amount of exercise or training undertaken (ARCHER and PROUDMAN, 2006; TINKER et al., 1997b). Under grain overload conditions, significant amounts of starch can enter the hindgut, and it can negatively affect fermentation. The negative effects include increased lactate production, decreased hindgut pH, shifting proportions of VFA and occurrence of health problems (HUSSEIN and VOGEDES, 2003) such as colitis and endotoxemia, possibly
resulting in signs of colic (HUDSON et al., 2001). Compared with horses that were fed < 2.7 kg of oats per day, horses consuming > 2.7 kg of oats per day were 2.2 times more likely to develop colic; however, this result did not mean feeding oats exclusively at amounts > 2.7 kg of oats/day was associated with colic, because oats were often fed in combination with other concentrates (HUDSON et al., 2001). Furthermore, no significant differences were detected between duodenitis-proximal jejunitis and other forms of colic in the amounts fed daily to each horse of pelleted concentrate, oats or other concentrates; however, there was a significant difference between duodenitis-proximal jejunitis and other forms of colic in the amount of total concentrate fed (Median 4.1 and 2.7, respectively; COHEN et al., 2006). However, MORRIS et al. (1989) found no association between the amount of grain or hay fed and colic.

High carbohydrate loads can undergo rapid fermentation in the intestine and possibly lead to colic (REEVES et al., 1996). Overload starch consumption causes alteration in colonic micro-flora and colonic damage. When a horse consumption of carbohydrate overload the resulting acidification shifts bacterial population toward lactic-acid producers instead of volatile fatty acid producers. The acid and hyperosmolality damaged colonic mucosal barrier, allowing absorption of endotoxins and other large molecules. Mucosal mast cells respond to acid by releasing histamine, which causes increased capillary permeability and sub-mucosal edema. Increases in tissue pressures and in mucosal permeability may result in net fluid secretion and protein loss into lumen as shown in figure 1. (CLARKE et al., 1990).

Effect of the feeding frequency on the occurrence of colic is not well known. REEVES et al. (1996) and HUDSON et al. (2001) did not find significant association between feeding frequency of grains or concentrates and colic prevalence. TINKER et al., (1997a) reported
that the risk of colic increased 6-fold for horses that received high amounts of concentrate compared to those horses that received no concentrate at all. However, feeding high amounts of concentrate divided in 3 portions or more a day did not reduce the risk associated with high levels of concentrate intake. Additionally feeding more than twice was increased the risk of colic in this study. NATHANIEL (2005) proposes that the increased risk of colic may not be due to the feeding frequency but an increased daily intake of grain.

The risk of gastric ulcers can be decreased by modification of the diet management by providing continuous feeding of good quality grass or alfalfa hay, minimizing sweet feed and substitution of grains, in order to decreases fermentation resulting in volatile fatty acids (Tab 1., BUCHANAN and ANDREWS, 2003).

Table 1 Combination of preventative and therapeutic treatment for gastric ulcers (adapted from BUCHANAN and ANDREWS, 2003)

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Hay</th>
<th>Feeding</th>
<th>Husbandry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race training / intensive training</td>
<td>Alfa alfa hay</td>
<td>Free choice feeding</td>
<td>Stall confinement</td>
</tr>
<tr>
<td>Moderate training / show/performance horses</td>
<td>Alfa alfa hay</td>
<td>Free choice feeding when in stall</td>
<td>Stall confinement with limited pasture turnout</td>
</tr>
<tr>
<td>Pleasure horse</td>
<td>Alfa alfa hay / grass hay</td>
<td>On pasture</td>
<td>Maintained on pasture</td>
</tr>
</tbody>
</table>

2.2.3. Change in Diet

Diet and changes in diet (TINKER et al., 1997a), such as recent change in diet (previous two weeks) and poor-quality roughage (MEHDI and MOHAMMAD, 2006), are important risk factors for colic. Horses that recent changed in diet (two weeks prior to examination) were 2 to 5 times more likely to develop colic than horses that did not change their diet (COHEN et al., 1995; COHEN and GIBBS, 1999). One change in concentrate feeding during the year multiplied the risk factor for colic by 3.6, while more than one change in concentrate feeding during the year multiplied it by 2.2 relative to no changes; more than one change in hay feeding during the year multiplied it by 2.1 relative to no changes (TINKER et al., 1997a).

Recent change in type of grain and amount of grain or concentrate fed conceivably increased the risk of colic (HUDSON et al., 2001, TINKER et al., 1997a). Moreover, recent change in hay increased the risk of colic by 9.8 (COHEN and GIBBS, 1999). HUDSON et al. (2001) found that a recent change in the type of hay, such as from alfalfa to coastal hay was not significantly associated with colic, but horses that had a recent change in amount of hay fed (either an increase or decrease) were at a greater risk of colic, and a recent change in batch of hay was the variable significantly associated with colic in this study.

2.2.4. Access to Water

Access to water is important for decreasing the risk of colic (COHEN et al., 1995). Horses that had access to outdoor enclosures without a continuous supply of water were more
than twice as likely to get colic (OR = 2.2, 95 % CI = 1.2 - 4.3) than horses that had an adequate supply of water on outside enclosures (REEVES et al., 1996). With respect to the water source, horses that drank water from a bucket, stream, or pond were more likely to have gastric rupture than horses that had access to water from an automatic waterer. This association may have occurred because horses that drink water from buckets may have extended periods without water unless the buckets are refilled frequently. Also, ponds or streams may dry up, freeze over, or become brackish or contaminated and deprive the horses of fresh water source (KIPER et al., 1990). However, HUDSON et al. (2001) found that the type of watering practice (none were without access to water > 4h/day) was not significantly associated with colic. KANEENE et al. (1997) conducted a study to determine any association between risk reduction of colic and the use of water heaters in cold weather to prevent water freezing, but there was none.

2.3. Management Factors

2.3.1. Housing and Pasture Practices

Year-long pasture presented lower risk of colic than indoor living conditions (COHEN and GIBBS, 1999). REEVES et al. (1996) found that horses access to one pasture had lower risks than access to either two or three different pastures during the 4-week period prior to presentation of colic (OR = 0.8 and 0.4, respectively). In addition, horses that had access to four or more pastures had an even higher colic risk (OR = 2.4). The movement of a horse away from its natural grazing environment appears to be strongly associated with an increased risk of simple colonic obstruction and distension (HILLYER et al., 2002). However, type of pasture (MORRIS et al.1989; TRAUB-DARGATZ et al., 2001) and pasture quality and percentage of pasture with edible vegetation or stocking density (TRAUB-DARGATZ et al., 2001) were not significantly associated with colic. Both increasing the hours stabled and decreasing the hours at pasture were highly correlated and associated with an increased risk of simple colonic obstruction and distension in horses (HILLYER et al., 2002). Additionally, horses that spent 100 % of their time in the stable were reported to be at increased risk of colic when compared to horses that spent no time in a stable (HUDSON et al., 2001).

Change in stables may predispose horses to colic (COHEN and GIBBS, 1999; TINKER et al., 1997a). A recent change (two weeks prior to occurrence of colic) in stabling was associated with increased risk of colic (COHEN and GIBBS, 1999). This was the most commonly reported risk factor for spasmodic colic according to PROUDMAN (1991). However, COHEN et al. (1995) could not find any association between recent change in stabling and development of colic.

2.3.2. Duty of the Horse

Increased duration of acid exposure is directly related to the daily duration of exercise. Gastric pH decreased rapidly at the beginning of walking and continued to decrease during trotting and galloping exercise. Increased intra-abdominal pressure during intense exercise causes gastric compression pushing acidic contents into the proximal region of the stomach. Thereby, squamous lesions in the stomach tend to develop when horses are in intensive training programs (LORENZO-FIGUERAS and MERRITT, 2002). This may have an effect on development of gastrointestinal upset, since horses that exercised at least once per week were 1.6 times more likely to develop colic than horses that were not exercised (COHEN and GIBBS, 1999).
The activity level is related to the duty of the horse. Horses used for events, racing or in active training were at increased risk of colic relative to horses used for other less strenuous activities. It can be deduced from this that activity level is an important risk factor for colic (COHEN, 1997). Intense activity (TINKER et al., 1997b) and activities associated with stress such as foaling (KANEENE et al., 1997) presented increased risk for colic. The mature horses used for lessons or those not uses at all (pets, retired, on pasture with no stated purpose) had the lowest colic incidence rates when compared to horses used for events or in training (TINKER et al., 1997b). Trotters were more likely to have gastric ulcers than pacers, and racing horses were more likely to have gastric ulcers than horses at rest (DIONNE et al., 2003). A change in activity level such as exercise restriction increased the risk of developing large colon impaction (DABAREINER and WHITE, 1995). However, two studies (COHEN et al., 1995; REEVES, 1996) found that activity of the horse was not significantly associated with colic.

2.3.3. Administration of Anthelmintic Drugs

Parasitic infections still cause significant losses to the equine industry, either directly as a result of colic or indirectly as a result of reductions in condition and performance (BARRETT et al., 2004). In foals, recent deworming may cause intestinal obstruction and that may result from rapid death of intraluminal ascarid (REID et al., 1995). The risk of colic was increased in the seven-day period following anthelmintic administration (COHEN and GIBBS, 1999).

Increased the deworming frequency (KANEENE et al., 1997) and using a regular anthelmintic program (COHEN and GIBBS, 1999) had a positive effect on colic risk. Furthermore, anthelmintic treatment during the two-week period prior to examination decreased the risk of colic in one study (HUDSON et al., 2001).

Tapeworms are located in places like the mass around the ileoceccal valve, and that may prevent the transit from ileum to the cecum (PROUDMAN et al., 1998). BARRETT et al. (2004) showed that horses were 5 times less likely to suffer from tapeworm-associated colic twelve weeks after an anthelmintic treatment. Horses were half likely to suffer from mixed infection-associated (roundworms and tapeworms) colic than they had been before treatment. Despite effective anthelmintics are commonly used, colic due to parasites still occurs. The resistance of certain Strongyle species (mainly Cyathostomum) to the anthelmintics used, and the outbreak of 'new' parasites like tapeworms (Anoplocephala perfoliata), which occupy the ecosystem, might explain the continuing problem (LOVE, 1997). Anaplocephala perfoliata infection is a risk factor for both ileal impaction colic and spasmodic colic, and there was a linear relationship between infection intensity and the risk of spasmodic colic (PROUDMAN et al., 1998). In the USA (LOVE, 1992), a recent development in equine control is a formulation of pyrantel pamoate given in feed on a daily basis. This application form causes the death of ingested and infective Strongyle larvae prior to penetration of intestinal mucosa. Perhaps the single most important point regarding Strongyle control is that there is no ideal prophylaxis program applicable to every system of horse management. Ideally, the program should be custom-designed with consideration of the various epidemiological features that might affect the transmission of parasites from one host to another.

Clinical cyathostomiasis is usually related to the sudden release of large numbers of the larval stages of small Strongyle from the mucosa of the large intestine. It causes typical
clinical signs including colic, ventral abdominal edema and diarrhea (LYONS et al., 2000). UHLINGER (1990) conducted a study in 4 herds (29 to 40 animals per herd) over a 5-year period. Despite the horses were subsequently controlled by anthelmintic schedules, they were suffered from colic. Thus, the data suggests that cyathostomes were responsible for the colic seen in these animals. This may be a result of resistance of small Strongyle to most of the currently used anti-parasitic drugs (LYONS et al., 2000).

Not all authors agree on the role of the frequency of anthelmintics administration, the recent administration of anthelmintics and the type of anthelmintic used. These factors were not significantly associated with colic in two studies (COHEN et al., 1995; MORRIS et al., 1989).

2.4. Individual Factors

2.4.1. Gender

Geldings appeared to be less affected than stallions by colic in one study (KANEENE et al., 1997). Contrary-wise, geldings were more susceptible for colic than stallions in two other studies (ABUTARBUSH et al., 2005; COHEN and PELOSO, 1996). This may be explained by many mares and stallions being valuable breeding animals, and therefore are better cared for than geldings (ABUTARBUSH et al., 2005). Chronic, intermittent colic was more likely to occur in geldings this could be due to complications of castrations, which may contain peritonitis or infections of the spermatic cord with streptococcal or staphylococcal bacteria (COHEN and PELOSO, 1996). In addition, geldings have a significantly greater risk of pedunculated lipoma obstruction than stallions or mares (EDWARDS and PROUDMAN, 1994). On the other hand, NATHANIEL (2005) did not find that sex was significantly associated with the simple forms of colic. Neither gastric rupture (KIPER et al., 1990) or small colon impaction nor large colon impaction (FREDERICO et al., 2006) appeared to behave a gender preference. Several studies (REEVES et al., 1989; REEVES et al., 1996; TINKER et al., 1997a; TRAUB-DARGATZ et al., 2001) support the idea that there is no real association between gender and colic.

2.4.2. Breed

Thoroughbreds were found to be at an increased risk of colic compared with Arabians, quarter horses and horses of other breeds according to HUDSON et al. (2001). In contrast, Arabian horses were found to be at higher risk for colic than any other breed in other studies (COHEN et al., 1995; COHEN and PELOSO, 1996; TINKER et al., 1997a). The risk of colic for Arabians was even found to be more than two fold (COHEN and GIBBS, 1999). Also, another study (REEVES et al., 1996) also revealed that Arabian horses were more than twice as likely to be colic cases, whereas Standard bred horses were nearly half as likely to be colic cases compared with the thoroughbred group. Another study (MEHDI and MOHAMMAD, 2006) revealed that crossbred horses had the highest susceptibility to colic in a population of thoroughbred, Turkmen and Arabian breeds. However, KANEENE et al. (1997) and ABUTARBUSH et al. (2005) could not show any breed predisposition for colic.

In detail, the various causes of colic and breed predisposition were also examined in several studies. EDWARDS and PROUDMAN (1994) found that ponies had a significantly higher risk of suffering from pedunculated lipoma obstruction, whereas thoroughbreds and thoroughbred crossbreds had a significantly lower risk of disease. In addition, COHEN (1997) found that the impaction of the small colon appeared to be more frequent for ponies.
MORRIS et al. (1989) revealed that quarter horses comprised 48.1% of the large colon displacement or volvulus, and there was a high incidence of Arabians in groups with ileal impaction (29.4%) and transit small intestinal distension (27.8%). FREDERICO et al. (2006) could not find that breed was a significant risk factor for small colon impaction or large colon impaction in the studied population. In the population of KIPER et al. (1990) revealed that gastric rupture was not significantly associated with breed.

2.4.3. Age

To date, several studies have examined the effects of age on occurrence of colic, but the results are confounding. In one study (REEVES et al., 1996) with 402 colic horses, mean age was 8.5 years. Another study (RHOADS et al., 1997) with 84 small colon impaction cases, mean age was 5.8 years (range 7 months to 26 years).

Horses aged 2 to 10 years had a higher risk of occurrence of colic (TINKER et al., 1997b), and being in this age interval multiplied the risk for colic by 2.8 compared to horses < 2 years old in one study (TINKER et al., 1997a). Increased age increased the risk for colic (KANEENE et al., 1997). Horses in between 5 and 10 year-old were more likely to suffer from spasmodic colic (PROUDMAN, 1991). Horses > 8 years old were more likely to have chronic, intermittent colic (≥ 2 episodes during the preceding 12 months). This age relationship could be explained with physiologic and anatomic abnormalities caused by age (COHEN and PELOSO, 1996). Horses older than 10 years had increased incidence of colic (COHEN and GIBBS, 1999). In contrast, age was not significantly associated with occurrence of colic in two other studies (COHEN et al., 1995; HUDSON et al., 2001).

2.5. Weather-related factors

There is a firm believe by many veterinarians that weather changes have influence on occurrence of colic, but most of the previous studies have not been able to find scientific evidence for an increased colic risk with weather changes (NATHANIEL, 2005). A recent study in Texas found that weather change during the three-day period prior to examination multiplied the risk of colic by 3.2 (COHEN and GIBBS, 1999). In contrast, PROUDMAN (1991) found no relation between weather-related factors like monthly temperature, change in monthly temperature, monthly rainfall and rainfall weighted for temperature and the incidence of colic over a two year period study in the UK. Increase in incidence of colic was not directly related to weather, but rather to management changes (e.g., no turn out exercise) caused by the weather (NATHANIEL, 2005).

However, seasonal effects may play a role since HILLYER et al. (2001) and PROUDMAN (1991) found a trend towards an increased colic incidence during spring and autumn. HILLYER et al. (2001) reported that the highest numbers of cases were during April, September and November in the UK, which was more or less confirmed by ARCHER et al. (2006) who reported that the highest numbers of cases were in the months of April / May and again in October / November / December in the UK. This may be explained by changing grass quality or changing management practices at these times of the year rather than changing weather conditions. TRAUB-DARGATZ et al. (2001) reported that the prevalence of colic cases was higher in spring than in summer or autumn. The attempt by PROUDMAN (1991) to blame monthly rainfall as factor for effecting grass growth and as such a factor for increased colic incidence failed.
2.6. Bacteriology of feces

2.6.1. Clostridia species (*Clostridium perfringens* and *Clostridium difficile*)

The Clostridium species are large (0.3-1.3 x 3-10 μm), Gram-positive, anaerobic, endospore-producing rods, and under the microscope, they appear as long drumsticks. Optimum growth of the pathogenic Clostridia occurs at 37 °C. Many of the pathogenic clostridia are normal inhabitants of the intestinal tract of animals and often cause endogenous infections. Other clostridia are commonly present in soil and cause exogenous infectious from wound contamination or by ingestion (QUINN et al., 1994).

*Clostridium perfringens* is the only species that produces a capsule in animal tissues (QUINN et al., 2004). The feces of healthy horses contained few *C. perfringens*. Mostly only low numbers are cultured from feces of healthy horses ranging from 10 CFU/g to 1000 CFU/g (DIVERS 2002; KEMILAIEN et al., 2005). However, the numbers of *C. perfringens* in the feces may increase in horses with diarrhea (DIVERS, 2002). *Clostridium perfringens* was detected in feces of horses with gastrointestinal disease in several studies (GRIFFITHS et al., 1997; HERHOLZ et al., 1999; JOBST et al., 2002). GRIFFITHS et al. (1997) could detect *Clostridium perfringens* in 30.3 % of the fecal samples, which belonged to surgical colic horses. JOBST et al. (2002) isolated *Clostridium perfringens* in feces in 52.2 % of the horses, which were undergoing medical or surgical treatment for colic. However, *C. perfringens* was isolated in 48.8 % (n = 39) of the horses upon arrival in the clinic, in 37.5 % (n = 30) of the horses during clinical treatment and in 13.8 % (n = 11) of the horses when they were discharged from the clinic.

GREISS et al. (1996) examined the bacterial composition of the caecal contents. Colony counts for Clostridium reached more than log 6/g intestinal contents in 50 % (5/10) horses with colic, while only *C. perfringens* was cultured in low numbers, less than log 1/g, from one of the healthy horses (n = 6). Among the Clostridium species identified were the potentially pathogenic species *C. perfringens* and *C. difficile*. Clostridial infection is also associated with non-gastrointestinal problems. Considering 37 cases suffering from clostridial myonecrosis, in 29 cases of *C. perfringens* infections were found, and 25 of those were pure *C. perfringens* infections (PEEK et al., 2003).

*C. perfringens* enterotoxin can rarely be found in the feces of healthy horses, but may be found in normal feces of horses with colic (DIVERS, 2002). Colonisation by *C. perfringens* and subsequent toxin product can occur within a short time course as is shown by a case reported by HOWARD-MARTIN et al. (1986). The case, a 1-day old foal with a history of colic died within 2 hours of admission at the colic. Histopathologically, the superficial intestinal mucosa was already completely necrotic, and numerous large, gram-positive rods covered villi which appeared to be *Clostridium perfringens* at isolation. DONALDSON and PALMER (1999) reported that the prevalence of *Clostridium perfringens* enterotoxin in feces of adult horses with diarrhea was 16 % and was detected in 10 % of horses with colic regardless of whether or not they had diarrhea. According to these authors its 3 possibilities may explain this finding:

- These horses may have had preexisting sub-clinical infections with enterotoxigenic *C. perfringens*, and a change in the intestinal environment attributable to colic then triggered proliferation of *C. perfringens* and subsequent production of *C. perfringens* enterotoxin.
Colic may have been attributable to ingestion of enterotoxigenic C. perfringens by a susceptible horse. Because fecal samples were obtained after hospitalization, nosocomial acquisition of enterotoxigenic C. perfringens was possible.

Clostridium difficile is another significant nosocomial pathogen for equine (SLOVIS, 2003). Pathogenic strains of C. difficile produce either toxin A, B or both in the intestinal tract. Toxin A is an enterotoxin, which causes both hypersecretion and cytotoxicity. Toxin B causes severe intestinal inflammation and necrosis. C. difficile is seldom found in normal equine feces (DIVERS, 2002), and was isolated from 1 in 3 normal healthy foals age < 14 days (BAVERUD et al., 2003). A great source of hospital and occasionally farm environmental contamination may be antibiotic-treated foals, which may shed the toxigenic C. difficile in normal feces (DIVERS, 2002).

BAVERUD et al. (2003) could not isolate C. difficile from any of the feces samples of horses with colic, whereas JOBST et al. (2002) detected C. difficile in 21.6 % of the feces samples of horses with colic. Remarkably, only one case was positive upon arrival in the clinic while in the remaining horses C. difficile was isolated during hospitalization. Support of this finding was by a case report of PERK et al., (1993). A case of a 2 ½ year old with mild abdominal pain for 2 days was colonized by C. difficile at the 5th day of its hospitalization. This may be explained by lack of sensitivity of assays to detect C. difficile or may indicate that C. difficile was a secondary problem. WEESE et al. (2006) detected no difference considering the incidence of diarrhea or colic between Clostridium difficile associated diarrhea, which had diarrhea as the presenting complaint while colic was the initial complaint, and non-Clostridium difficile associated diarrhea, which had diarrhea as the main presenting complaint while colic was the initial complaint. Moreover, JOBST et al. (2002) reported that the presence of C. difficile was higher in horses that underwent surgery (66.7 %) than in those treated medically. It was concluded that the colonisation and multiplication of C. difficile is supported by colic surgery including a perioperative antibiotic treatment and withholding of nutrition. ARROYO et al. (2004) conducted an experimental study with nine 1-day-old pony foals that were inoculated intragastrically with spores or vegetative cells of C. difficile. Within 24-72 hours after challenge with spores of vegetative cells of C. difficile in 8 out of 9 foals showed clinical signs ranging from mild abdominal discomfort and pasty feces to colic and watery diarrhea. From this it was concluded that both spores and vegetative cells of C. difficile are capable of rapidly colonizing the gastrointestinal tract, producing toxins, and inducing clinical signs such as abdominal discomfort and pasty feces to colic. Clostridium difficile spores survive in inoculated equine feces for at least 4 years, both indoors and outdoors (BAVERUD et al., 2003).

2.6.2. Salmonella spp.

Salmonella spp. is a genus of rod-shaped, facultative, aerobic, gram negative bacteria and belongs to the Enterobacteriaceae family. Salmonella usually gains access to the gastrointestinal tract via the fecal-oral route. The most common source of exposure to foals is via another horse (SLOVIS, 2003). Young animals are more susceptible to Salmonella infections due to less well established micro flora within the gastrointestinal tract (ERNST et al., 2004; SLOVIS, 2003). Therefore, foals with gastrointestinal tract disease are more likely to shed Salmonella organisms in their feces than are adult horses (ERNST et al., 2004).

In a study (GRIFFITHS et al., 1997), with five anterior enteritis cases were assayed for Salmonella spp. in gastric reflux, small intestinal contents and feces, but none were
isolated in any cases. On the other hand, HIRD et al. (1986) found that horses admitted because of colic were 4.2 times more likely to carry *Salmonella* as those admitted for other reasons. JOBST et al. (2002) detected *Salmonella* in only 1.9 % of feces samples in 153 colic horses. Serotypes of these were as follows: *Salmonella typhimurium*, *Salmonella typhimurium var.*, *Salmonella enteritidis*. Furthermore, horses with colic may be shed *Salmonella* at the time of hospitalization (PALMER et al., 1985). The highest percentage of positive samples was detected during the period from the fourth to the seventh day of hospitalization (DUNOWSKA et al., 2004). ERNST et al. (2004) reported that fecal *Salmonella* shedding was detected at least once in 13 % of horses (n =465) hospitalized because of gastrointestinal tract disease. KIM et al. (2001) detected *Salmonella* organisms in feces at least once in 9 % horses (23/246) hospitalized because of colic at the Colorado State University Veterinary Teaching Hospital. This was at > 72 hours after initial hospitalization. In 56 % of the cases *Salmonella* detection was even possible in less than 24 hours after hospitalization, while 22 % of the cases were found to be shed to *Salmonella* after a period of 24 to 72 hours of hospitalization. A similar study (PALMER et al., 1985) at the University of Pennsylvania reported *Salmonella* organism in 13 % of 100 colicky horses. It was concluded that 8 horses were infected before admission. Whether the remaining 5 horses were infected before or after admission could not be determined. Equine patients were 3.5 times more likely to shed *Salmonella* organisms if travel time to the teaching hospital was > 1 hour (KIM et al., 2001). Stress associated with abdominal pain, transport to the hospital and hospitalization may all compromise the immunologic status of carrier horses (PALMER et al., 1985).

Moreover, patients were more likely to shed *Salmonella* organisms if diarrhea was evident ≤ 6 hours after hospitalization and if duration of hospitalization exceeded 8 days (KIM et al., 2001). DONALDSON and PALMER (1999) were unable to isolate *Salmonella spp.* from fecal samples (n = 30) collected from any horse that developed diarrhea as a complication of colic.

Adult horses with gastrointestinal tract disease that underwent abdominal surgery were more likely to shed *Salmonella* organisms than were adult horses that did not undergo abdominal surgery (ERNST et al., 2004; RHOADS et al. 1999). Twenty per cent of the horses that underwent surgery for colic (PARRAGA et al., 1997) and 22 % of the horses that underwent surgery for impaction of small colon (RUGGLES and ROSS, 1991) yielded positive fecal cultures for *Salmonella*. This makes clear that horses with gastrointestinal tract disease that undergo abdominal surgery suffer substantial amounts of stress (ERNST et al., 2004) and must be regarded as potential shedders.

### 2.7. Fecal pH

The normal fecal pH of adult horses is between 6.8 and 8.0 (MEYER and COENEN, 2002). For foals the normal fecal pH is 6.58 (NICOL et al., 2002). Fecal pH in adult horses of less than 6.8 indicates dysfermentation in the hindgut, and fecal pH in adult horses of less than 6.0 indicates a high level of carbohydrate fermentation in the cecum and/or colon (MEYER and COENEN, 2002). Metabolic acidosis occurs most frequently in association with severe obstructive gastrointestinal disease, and the underlying causes of acidosis in these situations are either increased base loss and / or reduced peripheral perfusion causing a switch to predominantly anaerobic metabolism in tissues with a consequent build up of lactate (TAYLOR, 2002).

WILLIAMSON et al. (2007) conducted a survey of feeding, management and fecal pH of 16 thoroughbred racehorses in the North Island of New Zealand and found that acidic fecal
pH was associated with diets of 4 kg of grain as the only form of concentrate together with offering ≤ 2.25 kg hay / day. Regardless of the source, grain supplementation (rolled barley, corn, naked oats, or oats) decreased fecal pH from 7.04 to an average of 6.74 (HUSSEIN et al., 2004). These authors suggest that on alfalfa diet could be supplement with grain at levels not exceeding 0.2 % of body weight (dry matter basis) without affecting nutrient digestion negatively or exposing the horse to health problems such as colic, laminitis, or post feeding acidemia.

RICHARDS et al. (2006) found a strong negative relationship between fecal pH and fecal propionate concentration. Fecal starch concentrations were also negatively related to fecal pH. This fact provides some further evidence that hindgut starch fermentation occurs and also suggests that fecal pH may be useful for monitoring starch digestion in horses fed high levels of grain concentrate. In addition, ZEYNER et al. (2004) found that the concentration of short-chain fatty acids increased and the buffering capacity decreased when the hay intake declined, although the amount of starch consumed by the horse remained consistent. Therefore, the same author suggests that the feeding sequence influences the synchronism of fermentation independent of the amount of starch presented for fermentation.

2.8. Biogenic amines in feces and blood

The two largest subgroups of small-molecule neurotransmitters consist of amino acids and biogenic amines (SJAASTAD et al., 2005). Amines are produced by the decarboxylation of amino acids by bacteria in the cecum and large intestine (BAILEY et al., 2003b). Dopamine, epinephrine and norepinephrine belong to the catecholamine, and are derived from the amino acid tyrosine, whereas serotonin (5-hyroxytryptamine) and histamine are synthesized from tryptophan and histidine, respectively (SJAASTAD et al., 2005). Monoamines such as serotonin, tryptamine and tyramine which are found in the cecum of the horse can potentially induce hemodynamic disturbances in the digit, resulting in laminar ischemia and therefore triggering laminitis (ELLIOTT and BAILEY, 2006). Polyamines such as putrescine, spermidine and spermine are small aliphatic amines, and the concentration of polyamines plays a fundamental role in proliferating, growing and regenerating tissues (PEULEN et al., 2002).

In one study by BAILEY et al., (2003a), fifteen amines (methylamine, ethylamine, propylamine, isooamylamine and isobutylamine, tryptamine, tyramine, kynuramine, phenylethylamine, putrescine, cadaverine, histamine, diaminoheptane, spermidine and spermine) were identified in equine caecal and colonic contents, all at concentrations greater than 1μM. Any significant difference in the concentration of these amines when comparing colonic contents from normal horses with those from colic cases was not seen. The plasma concentration of these amines was below the limits of detection. Consequently, these data show that many amines are present in the equine hindgut, some of which may have the potential to cause peripheral vasoconstrictive if released into the circulation from the gastrointestinal tract.

CRAWFORD et al. (2007) conducted a study with 5 normal and 6 laminitis-prone ponies and fed a inulin to mimic a change from a basal hay diet to lush spring-summer pasture. Methylamine, ethylamine, propylamine, isooamylamine, isobutylamine, tryptamine, tyramine, phenylethylamine, putrescine, cadaverine, histamine, diaminoheptane, spermidine, and spermine were measured in fecal samples and isoamylamine, phenylethylamine, tryptamine, and tyramine were measured in plasma samples from all of the ponies. An increase was observed in the fecal concentrations of a number of amines, including
tryptamine and tyramine. However, no differences in any of the measured compounds were observed between the group of normal ponies and those predisposed to laminitis, indicating that differences in the intestinal micro flora or mucosal barrier do not account for this predisposition. BAILEY et al. (2003b) found that the addition of fermentable carbohydrate was followed by an increase in the numbers of *Streptococcus spp* and *Lactobacillus spp*, and these bacteria are capable of producing vasoactive amines, which play a role in the pathogenesis of acute laminitis.

HODSON et al. (1989) reported that plasma histamine levels of 11 control horses were not significantly different from 6 control horses with colic and from 8 horses with grass sickness. Levels were high in horses with early (0.557 ± 0.088) or per acute (0.853) grass sickness. These results indicate that high plasma histamine levels found in grass sickness may be caused by the spill over from much higher levels present in the gastrointestinal tract.

Dopamine and serotonin are neurotransmitters; dopamine plays an important part in the area of the brain that controls movement, and serotonin is found in those parts of the brain that are concerned with conscious activity (PILLINER and DAVIES, 2004). Serotonin is contained in both enterochromaffin cells of the gut wall and in platelets (BAXER et al., 1989). In foals as in adults, a relatively large number of serotonin immunoreactive cells were detected in all proportions of the gastrointestinal tract (FINK et al., 2006). Serotonin plays an important role in gastrointestinal smooth muscle contraction (DHASMANA et al., 1993; FINK et al., 2006) or relaxation (DHASMANA et al., 1993) and is also an important constrictor mediator controlling digital blood flow (ELLIOTT and BAILEY, 2006). Moreover, the presence of muscular serotonin receptors induced contraction in equine jejunal longitudinal muscle (DELESALLE et al., 2006).

Tyramine is the most potent amine, causing significant serotonin release (at a concentration of 0.8μM) (ELLIOTT et al., 2003). In addition, the same authors also found that the aromatic amines (i.e., tyramine, tryptophan, and phenylethylamine) caused displacement of serotonin from equine platelets at lower threshold concentrations than did the aliphatic monoamines (i.e., isoamylamine and isobutylamine). Furthermore, tryptamine is the most potent cecum-derived amine, causing vasoconstriction in vitro and in vivo through direct activation of serotonin receptors and displacing serotonin from platelets (ELLIOTT and BAILEY, 2006). An experimental study (BAILEY et al., 2004) was conducted by i.v. tryptamine infusion to six Thoroughbred horses found that tryptamine caused significant decreases in digital arterial and venous blood flow and 65% of increases in plasma 5-HT (serotonin) concentration. The same authors suggest that amines produced by bacteria in the equine hindgut, if released into the circulation following carbohydrate overload, may contribute to digital vasoconstriction, thereby expressing their selective effect on the digital vasculature over the systemic circulation. This effect may be directly or indirectly via displacement of serotonin from platelets.

2.9. Blood analysis

2.9.1. Serum bile acids

The primary bile acids are synthesized from cholesterol in hepatocytes by its hydroxylation mediated by 7α-hydroxylase (MEYER and HARVEY, 1998). Cholic acid is the major primary bile acid in the dog and cow, whereas chenodeoxycholic acid is the major acid in the horse and human (MEYER and HARVEY, 1998; WASHIZU et al., 1991). In normal horses, 100% of the bile acids are released into the duodenum, but 5 to 10% of bile
acids are lost in the feces during the entero-hepatic cycle (MEYER and HARVEY, 1998). Bile acids facilitate fat absorption and are normally absorbed via sodium-dependent active transport pumps, which are thought to be localized in the ileum, and sodium-independent organic anion-transporting polypeptides, the localization of which are unknown (MEIER and STIEGEL, 2002). Age and sex had no effect on plasma bile acid concentrations in horses (WEST, 1989).

Serum bile acid concentrations in horses has a high specificity for diagnosing the presence of acute and chronic liver disease, but little or no specificity in identification of the type of dysfunction (ENGEKING, 1989). WEST (1989) found that there was no increase in total plasma bile acid concentrations outside the normal range in horses with gastrointestinal disease, except for two horses (n = 26), but it was increased in all forms of equine hepatic disease (hepatic necrosis, lipidosis, neoplasia, cirrhosis, n = 38).

Serum bile acids levels may be related to diet fed to horses. BERTONE et al. (1992) conducted a study on considering serum total bile acids in adult healthy horses (n = 4) in relation to caeliotomy and jejunoocecostomy. It appeared that 27 weeks after ileal resection and jejunoocecostomy, cholesterol was significantly decreased, but serum bile acids remained normal. This may be due to diet because horses were fed grass hay, which contains no fat or stimulus for bile acid production and secretion.

2.9.2. Alkaline phosphatase activity (ALP)

In horses as in other animals, alkaline phosphatase activity originates in cells from several organs such as the liver, heart, muscle, kidney, intestine and pancreas (MEYER and HARVEY, 1998). Equine alkaline phosphatase is a very heterogeneous protein, and normal horse serum does not contain significant renal or intestinal derived alkaline phosphatase (ELLISON and JACOBS, 1990). A large proportion of equine serum alkaline phosphatase is of osseous origin (TRUEMAN et al., 1983). FROSCHER and NAGODE, 1979 studied that samples of tissues were obtained from clinically normal horses (n = 10) immediately after death at an abattoir and were examined for alkaline phosphatase activity. As shown in figure 2, granulocytes contained large amount of ALP, but all portions of the large intestine were low in alkaline phosphatase activity (cecum, large colon, small colon).

Figure 2. The alkaline phosphatase activity per gram of material of the sources tested (n = 10): JE = jejunum, CE = cecum, LC = large colon, SC = small colon, LV = liver, KD = kidney, GR = granulocytes (adapted from FROSCHER and NAGODE, 1979).
The stage of pregnancy is an influential factor on the level of ALP activity in serum. The mean total ALP activity of early pregnant mares (2-4 months pregnant; 190 ± 54 IU/L) were significantly higher than those of late pregnant mares (9-11 months pregnant). The decrease in serum ALP activity during pregnancy forms strong evidence that the placental isoenzyme is not present in the circulation of mares in the advanced stages of pregnancy (ELLISON and JACOBS, 1990). In newborn and very young horses, two different isoenzyme fractions normally appeared in the serum: 1) liver and 2) bone ALP (THOREN-TOLLING, 1988). Pony foal serum had a high total ALP activity that continually decreased over the 21 days. Bone ALP activity constituted 80% of the total activity for the first 5 days, whereas liver ALP activity dropped only slightly during the 21 days. Intestine ALP activity was not found during this period (HANK et al., 1993). During the first year of life, the bone isoenzyme fraction slowly disappears, and in normal cases, only liver ALP could be detected after the first year of life (THOREN-TOLLING, 1988). Therefore, ALP activity decreased with age, especially during the first year of life (table 2, LEPAGE et al., 1990).

Table 2 Alkaline phosphatase levels in serum of healthy female Standardbred horses (adapted from LEPAGE et al., 1990)

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>n</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>8</td>
<td>856 ± 228a</td>
</tr>
<tr>
<td>1.5 - 2.5</td>
<td>9</td>
<td>339 ± 54a</td>
</tr>
<tr>
<td>3.5 - 20</td>
<td>33</td>
<td>351 ± 71a</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± SD for all the groups

The diagnostic and prognostic relationships of alkaline phosphatase activity in serum or peritoneal fluid were examined in several studies (BRAUN et al., 2002; DAVIES et al., 1984; HOFFMANN et al. 1983; MILNE et al. 1990; SAULEZ et al. 2004).

SAULEZ et al. (2004) conducted a study with 126 horses with colic and found that the serum ALP<sub>i</sub> and ALP<sub>j</sub> activity in horses with colic cannot be used to predict the probability of surviving. In contrast, measurement of peritoneal fluid ALP<sub>i</sub> and ALP<sub>j</sub> activity aided in the identification of more severe lesions and horses needing surgery. MILNE et al. (1990) also showed that surgical cases were unique in having blood-stained peritoneal fluid with a high alkaline phosphatase activity. BRAUN et al. (2002) conducted a study considering the usefulness of measuring ALP activity in the diagnosis of small intestinal injury. Analysis of peritoneal fluid from 50 horses with acute abdominal disease revealed that just 20 had increased ALP activities (> 200 IU/L), nevertheless, ALP activity in serum in horses with colic remained within the normal range.

ALP activity in serum or peritoneal fluid could also be used in the differential diagnosis between small intestinal strangulating obstruction and proximal enteritis, or grass sickness and surgical colic in horses. Horses with proximal enteritis had significantly higher serum GGT, AST and ALP activities than horses with small intestinal strangulating obstruction (DAVIS et al., 2003).

WIRTH, (1986) found that serum ALP activity had no prognostic value in a case study with 825 horses with colic where in 181 were killed or died.
3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Animals, definition of colic and non-colic horses

A total of 2743 patients were selected between August 1, 2006 and August 1, 2007 from the Equine Clinic (EC) located at the University of Veterinary Medicine Vienna. Of the 333 patients with colic, several patients were observed to have had multiple episodes of colic, bringing the total cases observed to 366. A colic horse was defined as a horse suffering from an acute gastrointestinal disorder, and a non-colic horse was defined as a horse examined because of any illnesses other than colic or for routine veterinary procedures (control) by veterinarians at the Equine Clinic.

3.1.2. Sampling and data collection

3.1.2.1. Feed samples

Colic horses: Horse owners were informed concerning free feed evaluation personally or by phone. Thereby, a total of 155 samples of feedstuffs, which consisted of 45 hay samples, 35 straw samples, 30 cereal samples, 21 oat samples, 17 pellet samples, 6 bran samples and 1 mash sample, were collected from 51 colic horses during the study period.

Non-colic horses: A total of 66 feed samples, which consisted of 24 hay samples, 14 straw samples, 12 cereal samples, 9 oat samples, 6 pellet samples and 1 mash sample, were collected from 26 non-colic horses during the study period.

3.1.2.2. Feces and blood samples

Colic horses: The feces samples that were taken from the rectum at the date of colic horses’ admission to the Equine Clinic are called “first feces samples (FFS)”. Feces samples that were taken three days after the first feces samples from the stable (if the feces samples were fresh) are called “second feces samples (SFS)”. Nevertheless, a second feces sample was only obtained when the hospitalization lasted longer than three days. Both first feces samples and second feces samples were packed in plastic cups so that excess barometric was eliminated (HERHOLZ, 1998). Consequently, a total of 177 FFS and 70 SFS were collected during the study period, and these were kept frozen at – 20° C degrees.

The blood samples that were taken from the external jugular vein into the tubes (VACUETTE, Z Serum Sep. Clot Activator 9 ml) at the day of colic horses’ admission are called “first blood samples (FBS)”. The blood samples that were taken three days after the first blood samples from the catheter are called “second blood samples (SBS)”. If a horse had no catheter, the second blood sample was not taken. The samples were approximately 9 ml, and they were immediately centrifuged with 4.5 krpm for 10 minutes. Then the blood serum was separated, and each blood serum was divided into two. One of them was examined for activity of serum alkaline phosphatase and serum bile acids, and the other one was stored at – 20° C in the freezer until the biogenic amines evaluation. Eventually, a total of 187 FBS and 26 SBS were collected from the colic cases during the study period.
Non-colic horses: A total of 34 fresh feces samples, which constituted 14 samples from the Research and Teaching Hospital (Rehrgras), 10 samples from Orthopedic Clinics and 10 samples from the Surgery department of the Equine Clinic, were collected from non-colic horses during August 2007.

Blood samples were not collected from non-colic horses. The values of the central laboratory at the University of Veterinary Medicine Vienna were used as reference range.

3.1.2.3. Data collection

3.1.2.3.1. Individual factors and feeding- and management-related factors

Individual factors and feeding- and management-related factors in this study were obtained from either the hospital medical records (TIS = animal hospital information system) or from the questionnaires (appendix, p 82-84).

The following data were obtained from medical records for colic horses and non-colic horses in this study:
- date of examination,
- reason for examination (colic case or non-colic case),
- type of treatment of the case of colic (surgical or medical),
- the ultimate outcome of the case of colic (recovery or death),
- gender, breed, age (day-month-year),
- duty of the horse,
- previous history of colic,
- anthelmintic administration,
- recent anthelmintic administration (during the two-week period prior to examination),
- change in diet (in type or amount) during the two-week period prior to examination,
- change in water consumption (increased or decreased).

Specific data on feeding, watering, housing and pasture practices were not available from the medical records. Therefore, a questionnaire was developed to obtain information on the following risk factors:
- amount of hay fed daily,
- amount of concentrate fed in a meal,
- amount of oats fed daily,
- amount of squeezed oat fed daily,
- amount of corn fed daily,
- amount of cereal fed daily,
- amount of pellet fed daily,
- total amount of concentrate fed daily,
- number of meals of concentrate daily,
- source of water (installed bucket or automatic waterer),
- foreign material intakes (soil, sand, wood, or shavings litter),
- pasture duration (by the hour, full time, or also at night),
- type of stable (stable with paddock, stable without paddock or stabled and some hours at pasture),
- change in stable conditions.

Due to missing data, not all variables could be observed completely for all horses.
3.1.2.3.2. Weather-related factors

Meteorological information for the Vienna area was obtained from www.orf.at in the form of daily temperatures (°C) and barometric pressures (hPa) between August 1, 2006 and August 1, 2007.

3.2. Methods

3.2.1. Feed Quality of Sensory Evaluation

The quantity of feed and hygienic quality of feed were examined by feed sensory evaluation (hand examination, color and odor examination, whether including foreign material), as previously described (KAMPHUES et al., 2004) and shown in table 3a-b, 4a-b, 5a-b, 6a-b, 7a-b. For hygienic evaluation the samples were also examined under microscope, and samples that indicated sign of mold or yeast were confirmed by microbiological investigation (See 3.2.2.). Then the scores were totaled. The final score appears on the chart, identifying quantity of nutrients in the food and identifying hygienic status of the sample, as shown in table 8, 9, 10. Reports of each examined feed sample were sent to the owners, along with a recommendation about the feed sample by post within three weeks (appendix, p. 85).

Table 3a Hay quantity of nutrients in food (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantity of nutrients in food (Energy, protein content, acceptability)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>soft, lots of leaves (not many bloom)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>not many leaves</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>poor leaves (many bloom)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>hard like straw</td>
<td>0</td>
</tr>
<tr>
<td>Odor</td>
<td>nice aroma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>less hay odor</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>no hay odor</td>
<td>0</td>
</tr>
<tr>
<td>Color</td>
<td>dark green</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>slightly faded</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>strongly faded</td>
<td>1</td>
</tr>
<tr>
<td>Foreign material</td>
<td>macroscopic free</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>small amount of sand -/ small amount of soil</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>large amount of soil (root)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3b Hay hygienic quality (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hygienic Evaluation (Risk of deterministic health effect)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>Dry</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>slightly clammy</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>clammy-moist</td>
<td>-5</td>
</tr>
<tr>
<td>Odor</td>
<td>strange odor</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>moldy odor</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>moldy-rotten odor</td>
<td>-10</td>
</tr>
<tr>
<td>Color</td>
<td>dirty gray</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>locally gray-white</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>diffuse discolored</td>
<td>-5</td>
</tr>
<tr>
<td>Foreign material</td>
<td>free</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>some</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>a lot</td>
<td>-10</td>
</tr>
<tr>
<td>Poisonous plant</td>
<td>(-5) to (-10)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4a Straw quantity of nutrients in food (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantity of nutrients in food (Energy, protein content, acceptability)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>typical straw</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>bulky (less-no leaves )</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>woody</td>
<td>0</td>
</tr>
<tr>
<td>Odor</td>
<td>typical straw odor</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>not aromatic</td>
<td>0</td>
</tr>
<tr>
<td>Color</td>
<td>intensive-light, cute-blond</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>faded</td>
<td>1</td>
</tr>
<tr>
<td>Foreign material</td>
<td>free</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>small amount of sand</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>large amount of sand-soil</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4b Straw hygienic quality (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hygienic Evaluation (Risk of deterministic health effect)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>dry-brittle</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>slightly clammy</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>clammy-moist elastic</td>
<td>-5</td>
</tr>
<tr>
<td>Odor</td>
<td>free of foreign odor</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mold-moisture odor</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>fusty odor</td>
<td>-10</td>
</tr>
<tr>
<td>Color</td>
<td>light</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>dirty gray-brown black</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>locally gray-white/black-red discolor</td>
<td>-10</td>
</tr>
<tr>
<td>Foreign material (mold, pest, mites, weed)</td>
<td>free</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>some</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>large amount</td>
<td>-10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5a Oat quantity of nutrients in food (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantity of nutrients in food (Energy, protein content, acceptability)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the weight of 1L cereal?</td>
<td>heavy</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>light</td>
<td>0</td>
</tr>
<tr>
<td>Odor</td>
<td>typical, intensive acidic</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ammonia alcoholic</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>roast</td>
<td>0</td>
</tr>
<tr>
<td>Taste</td>
<td>typical or not</td>
<td>2 / 0</td>
</tr>
<tr>
<td>Macroscopic View</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contamination</td>
<td>Clean, intensive clean</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Dust, dirt, foreign (chaff, awn, husk)</td>
<td>1</td>
</tr>
<tr>
<td>Botanic</td>
<td>Part of foreign cereal or foreign plant or not</td>
<td>5 \ 1</td>
</tr>
<tr>
<td>Color</td>
<td>Brown-black color free or not (due to overheating)</td>
<td>10 \ 1</td>
</tr>
<tr>
<td>Size / Shape</td>
<td>Very thick</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Very thin</td>
<td>1</td>
</tr>
<tr>
<td>Ingredients</td>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>germ</td>
<td>1</td>
</tr>
<tr>
<td>Lateral cut</td>
<td>Clear-white endosperm</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Discolored-consistency</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

24
Table 5b Oat hygienic quality (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hygienic Evaluation (Risk of deterministic health effect)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>Dry</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Clammy</td>
<td>-3</td>
</tr>
<tr>
<td></td>
<td>Moist</td>
<td>-5</td>
</tr>
<tr>
<td>Odor</td>
<td>Moldy, rotten, sweet, yeast, alcoholic, roast, chemical free or not</td>
<td>0 / -5</td>
</tr>
<tr>
<td>Taste</td>
<td>Not nice-bitter free or not</td>
<td>0 / -1</td>
</tr>
<tr>
<td>A) Macroscopic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contamination</td>
<td>Sand, humus, dirt, feces of mice, germ free or not</td>
<td>0 / -5</td>
</tr>
<tr>
<td>Botanic</td>
<td>Ergotamine</td>
<td>-5</td>
</tr>
<tr>
<td>Color</td>
<td>Intensive clear, typical color / dirty grey, brown-black, red violet, green (immature), red (Fusarien toxin)</td>
<td>0 / -5</td>
</tr>
<tr>
<td>Size / Shape</td>
<td>Smooth or Atrophy, untypical shape</td>
<td>0 / -5</td>
</tr>
<tr>
<td>Ingredients</td>
<td>Break corn, fissure on the surface, hole or other destruction due to pests, germ free or not</td>
<td>0 / -5</td>
</tr>
<tr>
<td>Lateral cut</td>
<td>yellow-grey until brown or black free or not</td>
<td>0 / -5</td>
</tr>
<tr>
<td>B) Microscopic</td>
<td>Dirt on the surface, grey-white-black dot on the surface (sign of mold) free or not</td>
<td>0 / -5</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6a Cereal quantity of nutrients in food (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantity of nutrient in food (Energy, protein content, acceptability)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>hard</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>a little bit soft</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>soft</td>
<td>0</td>
</tr>
<tr>
<td>Odor</td>
<td>nice aroma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>less cereal odor</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>no cereal odor</td>
<td>0</td>
</tr>
<tr>
<td>Color</td>
<td>typical cereal color</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>atypical cereal color</td>
<td>1</td>
</tr>
<tr>
<td>Foreign Material</td>
<td>macroscopic free</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>small amount of foreign material</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>large amount of foreign material</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6b Cereal hygienic quality (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hygienic Evaluation (Risk of deterministic health effect)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>Dry</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>slightly clammy</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>clammy-moist elastic</td>
<td>-5</td>
</tr>
<tr>
<td>Odor</td>
<td>no strange odor</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>moldy odor</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>moldy-rotten odor</td>
<td>-10</td>
</tr>
<tr>
<td>Color</td>
<td>Brown</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>diffuse discolored</td>
<td>-5</td>
</tr>
<tr>
<td>Foreign Material (mold-pest, mites)</td>
<td>Free</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Some</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>large amount of</td>
<td>-10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7a Pellet quantity of nutrients in food (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quantity of Nutrient in Food (Energy, protein content, acceptability)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>Hard</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>a little bit soft</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Soft</td>
<td>0</td>
</tr>
<tr>
<td>Odor</td>
<td>nice aroma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>less pellet odor</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>not pellet odor</td>
<td>0</td>
</tr>
<tr>
<td>Color</td>
<td>typical pellet color</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>atypical pellet color</td>
<td>1</td>
</tr>
<tr>
<td>Foreign material</td>
<td>macroscopic free</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>small amount of foreign material</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>large amount of foreign material</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7b Pellet hygienic quality (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hygienic Evaluation (Risk of deterministic health effect)</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation by hand</td>
<td>dry</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>slightly clammy</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>clammy-moist elastic</td>
<td>-5</td>
</tr>
<tr>
<td>Odor</td>
<td>no strange odor</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>moldy odor</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>moldy-rotten odor</td>
<td>-10</td>
</tr>
<tr>
<td>Color</td>
<td>brown</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>diffuse discolored</td>
<td>-5</td>
</tr>
<tr>
<td>Foreign Material (mold-pest, mites)</td>
<td>free</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>some</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>large amount of</td>
<td>-10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8 Score of hay, pellet and cereal quantity of nutrients and hygienic status (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Quantity of nutrients in food</th>
<th>Score</th>
<th>Hygienic Status</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>20 to 10</td>
<td>High quality</td>
<td>0 to -5</td>
</tr>
<tr>
<td>Poor</td>
<td>9 to 0</td>
<td>Low quality</td>
<td>-6 to -30</td>
</tr>
</tbody>
</table>

Table 9 Score of oats quantity of nutrients and hygienic status (adapted from KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Quantity of nutrients in food</th>
<th>Score</th>
<th>Hygienic Status</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>60 to 30</td>
<td>High quality</td>
<td>0 to -14</td>
</tr>
<tr>
<td>Poor</td>
<td>29 to 0</td>
<td>Low quality</td>
<td>-15 to 46</td>
</tr>
</tbody>
</table>

Table 10 Score of straw quantity of nutrients and hygienic status (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Quantity of nutrients in food</th>
<th>Score</th>
<th>Hygienic Status</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>20 to 8</td>
<td>High quality</td>
<td>0 to -5</td>
</tr>
<tr>
<td>Poor</td>
<td>7 to 4</td>
<td>Low quality</td>
<td>-6 to -30</td>
</tr>
</tbody>
</table>
Table 11 Benchmarks of the feedstuffs (KAMPHUES et al., 2004)

<table>
<thead>
<tr>
<th>Feed Types</th>
<th>Total bacteria (CFU / g)</th>
<th>Yeast (CFU / g)</th>
<th>Mold (CFU / g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>&gt; 10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Silage</td>
<td>&gt; 10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Straw</td>
<td>&gt; 10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Oats</td>
<td>15*10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>50*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;4&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Cereal</td>
<td>-</td>
<td>&gt; 10&lt;sup&gt;4&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Bran</td>
<td>8*10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>80*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
<td>70*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
<tr>
<td>Mash</td>
<td>5*10&lt;sup&gt;6&lt;/sup&gt; critical</td>
<td>50*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
<td>40*10&lt;sup&gt;3&lt;/sup&gt; critical</td>
</tr>
</tbody>
</table>

3.2.2. Microbiological investigation

3.2.2.1. Fungal investigation

The procedure given by the International Dairy Federation (IDF) 100B:1991 was followed for determining yeast and mold counts, as described below. Rose-Bengal Chloramphenicol Agar Base (Merck, Germany; VASDINYEI, et al., 2003) was used. An initial 10<sup>1</sup>, 10<sup>2</sup>, 10<sup>3</sup> dilution of feed sample material was performed. The prepared medium was stored at 2-8 °C in the dark to prevent toxic photo-oxidation of the dye. 1 g of presumptive feed samples was aseptically added to 9 ml Ringer solution in a sterile tube, and mixed gently with a lab shaker (GFL3015) for one hour to dissolve the supplement completely. Before beginning the examination, the medium was boiled until completely melted, and then cooled 45 ± 1 °C for 15 minutes in the water bath. 1 ml of the initial suspension was then transferred to empty Petri dishes. 12-15 ml of the culture medium was poured into the Petri dishes, and then carefully mixed. It is repeated for each dilution. Plates were incubated at 25 °C for five days. After the incubation period, the yeast and mold were identified by morphological appearance and microscopic examination (appendix 87, picture 1).

Method of calculation: Retain dishes containing more than 10 and fewer than 300 colonies are enumerated.

Method 1. Calculation of colonies

\[
\sum c \times \frac{(n_1 + 0.1 \cdot n_2)}{d} = N
\]

(\textit{International IDF Standard 100B:1991})

Where:
\(\sum c\); is the sum of colonies counted, all dishes retained
\(n_1\); is the number of the dishes retained in the first dilution
\(n_2\); is the number of the dishes retained in the first dilution
\(d\); is the dilution factor corresponding to the first dilution
The result is the estimated number of microorganisms per gram, and that is evaluated according to benchmark of each feed sample (table 11). A total of 15 hay samples, 20 straw samples, 10 oat samples, 8 cereal samples, 2 bran samples, 2 mash samples from colic horses and 5 straw samples, 3 oat samples from non-colic horses were presumptive with yeast or mold signs. Therefore, these samples were investigated with this method for enumeration of yeast and molds.

3.2.2.2. Total bacterial content

The International Dairy Federation procedure (IDF 100B:1991) was followed for total bacterial count. 1 g of feed samples were aseptically added to 9 ml of Ringer solution in a sterile tube and mixed gently with a lab shaker (GFL 3015) for one hour to dissolve the supplement completely. After the feed sample was prepared, 1 ml of the initial suspension was transferred to an empty Petri dishes and 12-15 ml Plate Count Agar (Casein-Peptone-Dextrose Yeast Agar, Merck, Germany) was added as a medium. The $10^3$, $10^4$, $10^5$ dilutions of the feed samples were used. Plates were incubated at 30 °C for 72 hours under aerobic conditions. After the incubation period, each colony, including the pinpoints (appendix 87, picture 2), were counted and calculated with the method above (Method 1.). A total of 9 hay samples, 20 straw samples, 9 oat samples, 5 cereal samples, 2 bran samples, 2 mash samples from colic horses and 5 straw samples, 3 oat samples from non-colic horses were investigated according to this procedure for total bacterial content, and were evaluated with respect to the benchmark (table 11).

3.2.3. Specific bacteria investigation in feces:

The agars and additional necessary products used in specific bacteria detection in feces are listed in table 12 and 13, respectively.

Table 12 Agars used in bacteria detection

<table>
<thead>
<tr>
<th>Agar</th>
<th>Company / Country</th>
<th>Growth bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Enrichment Broth</td>
<td>Merck/Germany</td>
<td>Salmonella Enrichment</td>
</tr>
<tr>
<td>Brilliant-Green-Phenol-Red Lactose Sucrose Agar</td>
<td>Merck/Germany</td>
<td>Salmonella</td>
</tr>
<tr>
<td>Tryptose Sulfite Cycloserine Agar (TSC)</td>
<td>Fluka/Switzerland</td>
<td>C. perfringens</td>
</tr>
<tr>
<td>Clostridium difficile agar</td>
<td>Oxoid/England</td>
<td>C. difficile</td>
</tr>
<tr>
<td>Gelose Trycase Soja (TSA-D)</td>
<td>Bio Merieux-sa/France</td>
<td>Pure colony</td>
</tr>
</tbody>
</table>

Table 13 Additional necessary products used in specific bacteria investigation in feces

<table>
<thead>
<tr>
<th>Other Products</th>
<th>Company / Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defibrinated horse blood</td>
<td>Oxoid / England</td>
</tr>
<tr>
<td>Stomacher bag</td>
<td>Seward / England</td>
</tr>
<tr>
<td>Peptone water</td>
<td>Merck / Germany</td>
</tr>
<tr>
<td>Genbox anaer</td>
<td>Bio Merieux sa / France</td>
</tr>
<tr>
<td>Anaer indicator</td>
<td>Bio Merieux sa / France</td>
</tr>
<tr>
<td>API 20A</td>
<td>Bio Merieux sa / France</td>
</tr>
<tr>
<td>API 50CH</td>
<td>Bio Merieux sa / France</td>
</tr>
<tr>
<td>API 20E</td>
<td>Bio Merieux sa / France</td>
</tr>
</tbody>
</table>
3.2.3.1. Clostridium perfringens culture

The ISO / DIS 7937:1997 and the Government of Canada Health Products and Food Branch Ottawa (2001) procedures were followed for identification of *Clostridium perfringens*. Accordingly, the steps were:

- **Preparation:** 5 g of feces material was added to 45 ml Peptone water to a sterile tube and mixed gently for one hour with a lab shaker (GFL 3015) to dissolve the supplement completely.

- **Agar and dilution:** The Tryptose Sulfite Cycloserine Agar (STAEMPFLI, H. R. et al., 1992) was used with the $10^1$, $10^2$, $10^3$ dilution of sample material. 0.1 ml of the initial suspension was transferred to the prepared medium petri dishes, and spread by a glass loop. The plates were always duplicated.

- **Incubation:** Plates were incubated at 44 °C for 18-24 hours under anaerobic conditions that were supplied by the product of Genbox anaer and indicated by Anaer indicator.

- **Enumeration:** Selected plates containing black color (STAEMPFLI, H. R. et al., 1992), which contained 20-200 colonies in a diameter 1-2.5 mm, not including pinpoint black colonies, were counted (Government of Canada Health Products and Food branch Ottawa, 2001).

- **Pure colony:** The Gelose Trycase Soja (TSA-D) was used with 5 % defibrinated horse blood. The prepared TSA-D was stored in the refrigerator no longer than seven days. Selected presumptive colonies from the appropriate plates were subcultures on prepared TSA-D medium Petri dishes. The plates were incubated at 37 °C for 24 hours under anaerobic conditions.

- **Confirmatory test:** Consequently, presumptive colonies exhibited biochemical confirmation (Government of Canada Health Products and Food Branch Ottawa, 2001) with API 20A, which gives rapid identification (appendix 88, Picture 3). The presumptive pure colony (always a young culture) was obtained on TSA-D blood agar to API 20A Medium, and then an API 20A strip was prepared, which was then incubated at 36 °C for 24 hours in an anaerobic Genbox.

- **Reading API 20A:** After incubation, the strip was read according to the API 20A reading table. Thereby, bacteria species were identified with respect to the apiweb results.

**Examined samples:** The 177 FFS, 70 SFS and 34 feces samples from non-colic horses were examined for prevalence of *C. perfringens* by this method.

The method was confirmed by using *C. perfringens* mikropak.

3.2.3.2. Clostridium difficile culture

The ISO / DIS 7937:1997 procedures were followed for identification of *Clostridium difficile*. The steps were:

- **Preparation:** The preparation step followed was identical with *C. perfringens* test method.
• **Agar and dilution:** The *Clostridium difficile* Agar Base with *C. difficile* supplement and defibrinated horse blood were the necessary supplements for agar. The prepared medium was stored at 2-8 °C, no longer than 5-7 days. 0.1 ml of the initial suspension was transferred to the prepared medium petri dishes and spread by a glass loop. The $10^1$, $10^2$, $10^3$ dilution of sample material on duplicate plates was performed.

• **Incubation:** Plates were incubated at 35 °C for 18-24 hours under anaerobic conditions. Anaerobic conditions were supplied in an identical procedure with *C. perfringens*.

• **Enumeration:** The white colonies were presumptive for *C. difficile* (appendix 88, Picture 5), and those were subcultures on prepared medium TSA-D Petri dishes.

• **Pure colony and confirmatory test:** Pure colony and confirmatory test steps followed were identical to *C. perfringens* test method.

**Examined samples:** The 177 FFS, 35 SFS and 34 feces samples from non-colic horses were analyzed for prevalence of *C. difficile* by this method.

The method was confirmed by using mikropak *C. difficile*.

3.2.3.3. **Salmonella spp. culture**

The ISO2002 / FDIS 6579 Standards were followed for *Salmonella spp.* investigation in feces. According to this procedure:

• **Pre-enrichment:** The 10 g feces sample and 90 ml peptone water was incubated at 37 °C ± 1 °C for 18 ± 2 hours in the stomacher bag.

• **Selective Enrichment:** The 0.1 ml of culture was added to 10 ml Salmonella Enrichment Broth and that was incubated at 41.5 °C ± 1 °C for 24 ± 3 hours. The $10^1$, $10^2$, $10^3$ dilution was used, and duplicate selective enrichment was done.

• **Plating-Out:** From the cultures obtained in Selective Enrichment, the selective solid media, which is Brilliant-Green-Phenol-Red Lactose Sucrose Agar (BPLS) (JOBST, 2001), was initially prepared for three dilutions for duplicate plates and incubated at 35 °C for 24 hours.

• **Enumeration:** The presumptive *Salmonella spp.* colonies are pink surrounded by a red zone. *Salmonella spp.* colonies are red because the bacterium does not ferment lactose or sucrose; however, *Escherichia coli* are yellow because of acid produced, which decreases the pH during the lactose and / or sucrose fermentation (appendix 89, picture 6 and picture 7; JERGENSEN et al., 2000).

• **Confirmation:** Colonies of presumptive Salmonella were subcultured on the TSA-D plates at 37 °C for 24 hours, and their identity was confirmed by biochemical test API CHB/E (included API 50CH and API 20E).

**Examined samples:** The initial 93 FFS and 34 feces samples from non-colic horses were analyzed for prevalence of *Salmonella spp.* by this method.
3.2.4. Measuring fecal pH

5 g of feces material was added to 45 ml distilled water (RICHARDS et al., 2006) in a sterile tube and mixed gently for 1 hour with lab shaker (GFL3015) to dissolve the supplement completely. The pH of each homogenized sample was measured with a glass electrode (Beckman Φ 40 pH meter). The pH meter was calibrated by typical calibration procedure using standardized buffer solutions, pH = 4 and pH = 7, immediately before the measurement. 177 FFS, 70 SFS and 34 control feces samples’ pH were measured by this procedure.

3.2.5. Determination of biogenic amines in feces and blood

Biogenic amines in both plasma and feces were measured by rapid high performance liquid chromatography (RP-HPLC), as previously described (BOCKHARDT et al., 1996). Products that were used in determination of biogenic amines are listed in table 15.

Standard solutions: All amine standards (serotonin, dopamine, tryptamine, tyramine, spermidine-trihydrochloride, spermine-tetrahydrochloride, histamine, cadaverine and putrescine) were purchased as hydro-chloride salts of the highest purity available. Stock solutions of the biogenic amines 10 μmol / ml or 2.5 μmol / ml were made by dissolving in 0.1 N HCL containing 0.2 % TDPA (3.3'-thiodiopropionic acid) as an antioxidant. 1.7 diaminoheptane and 1.6 diaminohexane were used as internal standards at the same concentration. They were kept refrigerated at -20 °C. Composite amine standards were prepared from stock solutions to yield an overall concentration of 250 nmol / ml per component for determination of biogenic amines in plasma and feces.

Amines extraction for feces samples: Between 1.0 and 5.0 g of thoroughly homogenized solid feces samples were dispersed with 10 ml of 0.1 mol / l hydrochloric acid solution containing 0.2 % TDPA (3.3'-thiodipropionic acid) and the internal standard in a centrifuge vial using an Ultra-Turrax homogenizer for 2 minutes. It was centrifuged at 10 000 rpm for 10 minutes and the supernatant was filtered through a 0.45-μm RC-membrane.

Derivatization procedure: Reaction buffer medium (0.2 mmol / 1 NaHCO₃) consisted of 840 mg NaHCO₃ in 40 ml HPLC-water, adjusted to pH 9.0 with diluted NaOH and made up to 50 ml with HPLC-water. Dilution buffer was prepared from a mixture of 50 ml acetonitrile, 25 ml ethanol and 25 ml of mobile phase A. Dabsyl chloride reagent (4-Dimethylaminoazobenzene-4'-sulfonyl chloride; 12.4 mmol / l) was prepared by dissolving 40 mg dabsyl chloride in 10 ml acetone following ultrasonic treatment for 15 min and filtering through an 0.45-μm RC-membrane into brown glass vial and finally stored at -20 °C. Manual derivatization: 200 μl dabsyl chloride reagents were pipetted into a vial. 10 μl biogenic amine standards or 10 μl sample extract (feces or plasma extract) and 190 μl reaction buffer were subsequently loaded into the vial. It was incubated in a water bath at 70 °C for 15 minutes. While heating, the vial was mixed during the incubation period. After cooling at 12 °C in the sample tray for 5 min, 400 μl of the dilution buffer was added. The assay was centrifuged again at maximum 10 000 rpm for 5 min before being injected.

High-performance liquid chromatography: The gradient-HPLC equipment (Waters, Eschborn, Germany) consisted of a Water 717 plus auto sampler and a Water PDA-996 photodiode array detector interfaced to computer-software (Chromleun dionex, Germany), which was used for system controlling peak integration. Dabsyl derivatives of biogenic amines were separated on a 150 x 3.0 I.D. mm column filled with 3 μm ACE 3 C18 (ACT-Advanced Chromatography
Technologies, Scotland), including a guard cartridge of 4.0 x 3.0 mm C18 (ODS-Octadecyl, Phenomenex, Germany). The column was temperature-controlled at 40° C. 10 μl of the derivatized samples were injected. Mobile phase A consisted of HPLC-water. Mobile phase B consisted of acetonitrile. Dabsylated amines were eluted at a flow rate of 0.55 ml / min using the gradient profile listed in table 14. The detection was at λ = 436 nm and the data acquisition rate was 5 Hz. A linear relation between the area of the peak and amine concentration was observed between 0.5 and 500 pmol for all amines under investigation.

Table 14 Scheme of linear elution gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solvent A (%)</th>
<th>Solvent B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>4.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>6.0</td>
<td>30.0</td>
<td>70.0</td>
</tr>
<tr>
<td>24.0</td>
<td>18.0</td>
<td>82.0</td>
</tr>
<tr>
<td>28.0</td>
<td>3.0</td>
<td>97.0</td>
</tr>
<tr>
<td>32.9</td>
<td>3.0</td>
<td>97.0</td>
</tr>
<tr>
<td>33.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>40.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>

Table 15 Products used in determination of biogenic amines

<table>
<thead>
<tr>
<th>Product</th>
<th>Company</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>Aldrich</td>
<td>Germany</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Fluka</td>
<td>Germany</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Tyramine</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Spermidine-trihydrochloride</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Spermine-tetrahydrochloride</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Histamine</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Putrescine</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>1,7 diaminoheptane</td>
<td>Fluka</td>
<td>Germany</td>
</tr>
<tr>
<td>1,6 diaminohexane</td>
<td>Aldrich</td>
<td>Germany</td>
</tr>
<tr>
<td>3,3'-thiodiopropionic acid</td>
<td>Fluka</td>
<td>Germany</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>Fluka</td>
<td>Germany</td>
</tr>
<tr>
<td>RC-membrane (Minisart RC 15)</td>
<td>Satorius</td>
<td>Germany</td>
</tr>
<tr>
<td>Acetonitrile (HPLC gradient-grade)</td>
<td>Fisher Scientific</td>
<td>Germany</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Merck</td>
<td>Germany</td>
</tr>
<tr>
<td>Dabsyl chloride</td>
<td>Fluka</td>
<td>Germany</td>
</tr>
<tr>
<td>Acetone (LiChrosolv)</td>
<td>Merck</td>
<td>Germany</td>
</tr>
<tr>
<td>Vial</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
</tbody>
</table>
3.2.6. Evaluation activity of alkaline phosphatase and concentration of serum bile acids

Both serum alkaline phosphatase activity and serum bile acids levels were measured by Auto analyzer Hitachi 911 at the Central Laboratory, University of Veterinary Medicine Vienna. The serum activity of alkaline phosphatase was measured by the International Federation of Clinical Chemistry (IFCC) method at 37 °C and the supplement was p-nitrophenylphosphate. The serum bile acids were measured by enzymatic color test, and the supplement was 3-α-Hydroxysteroiddehydrogenase. The values of serum alkaline phosphatase activities and the serum bile acids were compared with the Central Laboratory reference value, which is < 250 U/L and < 20 μmol/L, respectively.

3.3. Statistical analysis

The SPSS 14.0 (Statistical Package for the Social Sciences) was used to perform statistical analysis. For all analyses, a value of $p < 0.05$ was considered significant.

The feed intake, number of meals of concentrate daily, the parameters pertaining to feed hygienic quality, the parameters pertaining to quantity of nutrients in feed, all of foreign material intakes, watering methods, all of the housing and pasture practices parameters, type of colic, type of treatment, the outcome of recovery and death of colic, all of the weather-related factors were analyzed by using descriptive statistics, Chi Square test and one way ANOVA.

Univariate analyses were performed to examine the association of age, gender, breed, duty of the horse, administration of anthelmintic, recent deworming, change in diet, and change in water consumption with colic, using conditional logistic regression analysis. Variables that were found to be associated with colic in univariate analysis were included in multivariate analysis for the final model. The final evaluation was done by logistic regression analysis with stepwise removing of variables depending on $p$-values. Associations derived from conditional logistic regression were characterized by odds ratio (OR) and their 95 % confidence intervals (CI). An odds ratio $> 1$ implies increased risk and an OR $< 1$ implies decreased risk.

The agreement between the owners assessment and valid assessment concerning mold in straw is measured by Cohen’s kappa coefficient. Interpretation of kappa is shown in table 16.

Table 16 Kappa interpretation (SACHS, 1992)

<table>
<thead>
<tr>
<th>Kappa</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;0.10$</td>
<td>Poor agreement</td>
</tr>
<tr>
<td>$0.10 - 0.40$</td>
<td>Slight agreement</td>
</tr>
<tr>
<td>$0.41 - 0.60$</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>$0.61 - 0.80$</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>$0.81 - 1.00$</td>
<td>Almost perfect agreement</td>
</tr>
</tbody>
</table>

34
The data were analyzed by the one-sample Kolmogorov-Smirnov test for normal distribution. Data of activity of alkaline phosphatase and serum bile acids were investigated by Mann-Whitney test when data were not normally distributed and by unpaired T-test when data was normally distributed between medically and surgically treated colic horses, and also between survived and death colic horses.

Furthermore, horses examined for serum alkaline phosphatase activity, were also split into a medically survived (MA) and died (MD) groups; and a surgically alive (SA) and dead (SD) group. Differences in serum alkaline phosphatase activity between MA and MD, between MA and SA groups were analyzed by Mann-Whitney test, between MD and SD, between SA and SD groups were analyzed by unpaired T-tests. In addition, differences between serum alkaline phosphatase activity for the same colic horses on the day of admission and three days after admission were analyzed by Wilcoxon test. Differences between the serum bile acids value for the same colic horses on the day of admission and three days after the admission were analyzed by pair T-test.

Logistic regression was used to analyze risk factors for Clostridium perfringens fecal shedding in colic horses, and it was confirmed by Chi-square test. The Mann-Whitney test was used to compare the value of fecal biogenic amines between colic and non-colic horses.

Mann-Whitney tests were used to analyze differences between fecal pH values for colic horses on the day of admission and non-colic horses. Unpaired-T-tests were used to analyze differences between fecal pH values for second feces samples from colic horses and non-colic horses. Differences in fecal pH for the same colic horses on the day of admission and three days after the admission were analyzed using paired T-test. In addition, the data considering fecal pH was divided into acidic or alkali, and Chi-square test was used for comparing proportions.
4. RESULTS

4.1. Frequency, treatment procedure of colic and mortality rates

A total of 2743 patients (horses) arrived at the Equine Clinic of the University of Veterinary Medicine Vienna between August 1, 2006 and August 1, 2007. Of the 333 patients referred with colic, some patients were sent in multiple occasions; 305 (91.6 %) had one episode, 25 (7.5 %) had two episodes, one (0.3 %) had three episodes and two (0.6 %) had four episodes of colic, which brings the total cases observed to 366.

A substantially higher percentage of equine colic cases, 80.6 % (295 / 366), received medical treatment than the surgery, 19.4 % (71 / 366). No significant difference was found between case fatality rate of the medically and surgically treated horses (table 17, p = 0.059).

Table 17 Case fatality rate for surgical and non-surgical colic horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Case fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medically treated</td>
<td>13.5 % (40 / 295)</td>
</tr>
<tr>
<td>Surgically treated</td>
<td>22.5 % (16 / 71)</td>
</tr>
</tbody>
</table>

The effect of age was investigated on probability of surgery, but the mean age of horses with colic that received surgery (11.02 ± 6.48) was not significantly different from the horses that received medical treatment (11.85 ± 6.18; p = 0.317). Duration of hospitalization was also examined, and it appeared that surgical cases remained in the hospital significantly longer (16.36 ± 39.9, days) than did medical cases (4.2 ± 5.8, days; p < 0.001).

4.2. Feeding practices

4.2.1. Feed hygienic quality and quantity

Each presumptive feed sample was confirmed by microbiological investigation (See part 3.2.2.). Bran samples, which constituted just 6 samples from colic horses, and mash samples, which constituted one sample from colic horses and one sample from non-colic horses, were excluded from the study due to insufficient number of samples.

As shown in table 18, none of the feedstuffs' quantities of nutrients, which consisted of hay, straw, oats, pellet and cereal, was associated with colic (table 18).

Results of the hygienic quality of feedstuffs are represented in table 19. The hygienic quality of straw, oats, pellet and cereal was not significantly associated with colic, whereas the hygienic quality of hay was significantly associated with an increased colic risk (p = 0.027). A hay sample had poor hygienic quality due to one or several reasons. Colchiculum autumnale (picture 8.) was found in 15 % (two samples) of poor quality hay samples from colic horses. Yeast was detected in 46 % of poor quality hay samples from colic horses, higher than benchmark, and bacteria was detected in 30 % of the poor quality hay samples from colic horses, higher than benchmark. Mold counts were not over benchmark values in any of these samples. The rest of the hay samples from colic cases were poor quality due to other reasons such as pests, mites, being clammy, strange odor, etc. Poor quality hay samples
from non-colic horses were not highly contaminated with yeast, mold or bacteria. Poor quality in these samples was due to other reasons.

Table 18 Distribution of the quantity of nutrients outcomes in feed samples (n = 213)

<table>
<thead>
<tr>
<th>Feed types</th>
<th>n</th>
<th>Good</th>
<th>Poor</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>24</td>
<td>45</td>
<td>91.6</td>
<td>71.1</td>
</tr>
<tr>
<td>Straw</td>
<td>14</td>
<td>35</td>
<td>92.9</td>
<td>82.8</td>
</tr>
<tr>
<td>Oat</td>
<td>9</td>
<td>21</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Pellet</td>
<td>6</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cereal</td>
<td>12</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

n = number of samples
NC represents samples from non-colic horses
C represents samples from colic horses
p < 0.05 represents significant

Table 19 Distribution of the hygienic quality outcomes in feed samples (n = 213)

<table>
<thead>
<tr>
<th>Feed types</th>
<th>n</th>
<th>High quality</th>
<th>Low quality</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay*</td>
<td>24</td>
<td>45</td>
<td>95.8</td>
<td>71.2</td>
</tr>
<tr>
<td>Straw</td>
<td>14</td>
<td>35</td>
<td>64.3</td>
<td>51.5</td>
</tr>
<tr>
<td>Oat</td>
<td>9</td>
<td>21</td>
<td>88.9</td>
<td>81.0</td>
</tr>
<tr>
<td>Pellet</td>
<td>6</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cereal</td>
<td>12</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

NC represents samples from non-colic horses
C represents samples from colic horses
n = number of samples
* significant association (The non-moderate hygienic quality of hay was significantly associated with colic.)
p < 0.05 represents significant

As described in table 20, the assessments of the owner and the valid assessments for mold contamination in straw demonstrated slight agreement (Kappa= -.136).
Table 20 The percentage of the valid assessment and assessment of the owner concerning straw contamination by mold from 20 horses (include colic and non-colic horses which has both straw sample and owners assessment in the questionnaires)

<table>
<thead>
<tr>
<th>Occurrence of mold (%)</th>
<th>Valid assessment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Highly contaminated (%)</td>
<td>Not contaminated (%)</td>
</tr>
<tr>
<td>No occurrence of mold (%)</td>
<td>100.0</td>
<td>88.2</td>
</tr>
</tbody>
</table>

Highly contaminated represent mold count > $70*10^3$ KBE / g

Intake of foreign material such as earth, wood etc. was recorded in 22 questionnaires from non-colic horses and 52 questionnaires from colic horses. According to these questionnaires, 81.8 % (18/22) of the non-colic horses and 86.5 % (45/52) of the colic horses did not consume foreign material, and there was not a significant association with colic (p = 0.602).

4.2.2. Feeding and watering management

No association was detected between colic and the amount of hay intake or particular type of concentrate intake (kg/day) (oat, squeezed oat, corn, cereal and pellet), as shown in table 21. However, total concentrate intake (kg/day) was significantly associated with an increased risk of colic compared to non-colic horses. The mean value of concentrate intake (kg/day) for colic horses was higher than that of non-colic horses. The number of meals of concentrate per day for colic horses was one for 2.8 % (1/35), two for 48.5 % (17/35), three for 37.14 % (13/35), four for 8.5 % (3/35) and six for 2.8 % (1/35) of them. The number of meals of concentrate per day for non-colic horses was two for 30 % (3/10) of them and three for 70 % (7 / 10) of them. In fact, the number of meals a day (p = 0.452) and concentrate intake per meal (kg / meal; p = 0.138) were not significantly associated with colic. Furthermore, of the 53 cases of colic reported concerning feed intake. Of these 33 (62.2 %) were originally large intestine colic, 9 (16.9 %) were originally stomach colic, 11 (20.7 %) remained undiagnosed, and none of them were known to be small intestinal origin. The possible association between high concentrate intake and colic originating in the large intestine was examined but was not proven (p = 0.414).

Information about change in diet (type or amount) during the two-week period prior to examination was not significantly associated with colic compared with no change in diet (table 23).

Change in water consumption was recorded in the medical records. It was found that decreased water intake was significantly associated with an increased risk of colic (table 23). However, information on the watering method was not available in the medical records. Therefore, type of watering, which was categorized as automatic waterer or bucket, was only given by the care takers in the questionnaires of 52 colic horses and 23 non-colic horses. Thus, 78.8 % of the colic horses and 95.7 % of the non-colic horses drank water from an automatic waterer. Water type showed no significant association with colic (p = 0.067).
Table 21 Association between amount of feed intake and colic according to valid information in 53 questionnaires from colic horses and 25 questionnaires from non-colic horses

<table>
<thead>
<tr>
<th>Feed intake</th>
<th>Non-colic horses</th>
<th>Colic horses</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of horses</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Hay (kg/day)</td>
<td>17 (68)</td>
<td>7.59</td>
<td>3.60</td>
</tr>
<tr>
<td>Oats (kg/day)</td>
<td>5 (20)</td>
<td>0.90</td>
<td>0.67</td>
</tr>
<tr>
<td>Squeezed oats (kg/day)</td>
<td>1 (4)</td>
<td>1.00</td>
<td>*</td>
</tr>
<tr>
<td>Corn (kg/day)</td>
<td>1 (4)</td>
<td>0.50</td>
<td>*</td>
</tr>
<tr>
<td>Cereal (kg/day)</td>
<td>13 (52)</td>
<td>1.27</td>
<td>0.94</td>
</tr>
<tr>
<td>Pellet (kg/day)</td>
<td>4 (16)</td>
<td>1.50</td>
<td>1.69</td>
</tr>
<tr>
<td>Concentrate (kg/day)</td>
<td>19 (76)</td>
<td>1.64</td>
<td>1.16</td>
</tr>
<tr>
<td>Concentrate (kg/meal)</td>
<td>10 (40)</td>
<td>0.75</td>
<td>0.52</td>
</tr>
</tbody>
</table>

p < 0.05 represent significant association, * represent no standard deviation

4.3. Housing and pasture practices

No significant association was detected between pasture duration and colic (p = 0.599) and also between type of stable and colic (table 22; p = 0.268). Furthermore, change in stable conditions was also not significantly associated with colic (table 22; p = 0.327).

Table 22 Valid information concerning housing and pasture practices in 53 questionnaires from colic horses and 25 questionnaires from non-colic horses

<table>
<thead>
<tr>
<th>Pasture duration</th>
<th>Number of non-colic horses (%)</th>
<th>Number of colic horses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>By the hour</td>
<td>16 (64 %)</td>
<td>28 (52.8 %)</td>
</tr>
<tr>
<td>Whole day</td>
<td>5 (20 %)</td>
<td>16 (30.1 %)</td>
</tr>
<tr>
<td>Day and night</td>
<td>1 (4 %)</td>
<td>2 (3.7 %)</td>
</tr>
<tr>
<td>Type of stable</td>
<td>Stable without paddock</td>
<td>6 (24 %)</td>
</tr>
<tr>
<td></td>
<td>Stable with paddock</td>
<td>8 (32 %)</td>
</tr>
<tr>
<td></td>
<td>Stable and some hours pasture</td>
<td>9 (36 %)</td>
</tr>
<tr>
<td>Change in stable conditions</td>
<td>yes 4 (16 %)</td>
<td>13 (24.5 %)</td>
</tr>
<tr>
<td></td>
<td>no 19 (76 %)</td>
<td>38 (71.6 %)</td>
</tr>
</tbody>
</table>
4.4. Other management factors

When compared with the hospital horse population during the same period of time, both administration of an anthelmintic and a history of recent anthelmintic administration (two weeks prior to examination) were significantly associated with colic (table 23).

Duty of the horse was categorized as follows: Hobby, working, breeding, sport, young (foal), and other duties (appendix 88, table 27). When compared with the hospital horse population during the same period of time, hobby horse and working horse were at an increased risk of colic, whereas breeding horses were at a decreased risk (table 23).

4.5. Individual factors

Age of colic horses (median, 11 years; range 3 months to 36 years) was not significantly different from that of hospital horse population with non-colic conditions (median, 10 years; range, 9 months to 32 years). Furthermore, there was no significant association between colic and age when age was considered as a categorical variable (i.e. < 2 years, 2 to 10 years, and > 10 years) (table 23).

Considering gender, when compared with the hospital horse population during the same period of time, geldings were more prone to colic although mares and stallions appeared to be less prone (table 23).

Breed was categorized as follows: Warm-blood, thoroughbred, cold-blood pony, and mixed breed (appendix 87, table 26). Mixed bred horses had an increased risk of colic compared with other breeds (table 23).

4.6. Multivariate analysis

A multivariate logistic regression model fitting each of the variables that were compared between colic and non-colic horses of the hospital horse population and found to be significantly associated with colic (i.e., gender, breed, duty of the horse, decreased water intake, anthelmintic administration, recent deworming; table 25) was created, and decreased water intake remained significantly associated with an increased risk of colic (p < 0.001, OR = 5.025), although the CI was wide (2.1 - 12.3) due to the small number.
Table 23 Variables considering intrinsic factors (gender, breed, age) and the most important management risk factors which have been proved to be associated with colic in univariate analysis.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of cases</th>
<th>OR (Odds ratio)</th>
<th>95% CI for odds ratio</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-colic horses (%)</td>
<td>Colic horses (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mare</td>
<td>1129 (49.0)</td>
<td>151 (41.2)</td>
<td>0.776</td>
<td>0.621</td>
</tr>
<tr>
<td>Gelding</td>
<td>760 (33.0)</td>
<td>178 (48.6)</td>
<td>2.014</td>
<td>1.613</td>
</tr>
<tr>
<td>Stallion</td>
<td>414 (17.9)</td>
<td>37 (10.1)</td>
<td>0.533</td>
<td>0.374</td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm-blood</td>
<td>1467 (61.8)</td>
<td>238 (65.2)</td>
<td>1.153</td>
<td>0.916</td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>485 (20.4)</td>
<td>63 (17.2)</td>
<td>0.811</td>
<td>0.607</td>
</tr>
<tr>
<td>Cold-blood</td>
<td>336 (14.1)</td>
<td>40 (10.9)</td>
<td>0.745</td>
<td>0.526</td>
</tr>
<tr>
<td>Pony</td>
<td>61 (2.5)</td>
<td>15 (4.1)</td>
<td>1.623</td>
<td>0.912</td>
</tr>
<tr>
<td>Mixed-bred</td>
<td>22 (0.9)</td>
<td>9 (2.4)</td>
<td>2.699</td>
<td>1.233</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 years old</td>
<td>1164 (50.7)</td>
<td>217 (59.6)</td>
<td>0.682</td>
<td>0.055</td>
</tr>
<tr>
<td>2-10 years old</td>
<td>921 (40.1)</td>
<td>131 (35.9)</td>
<td>0.500</td>
<td>0.040</td>
</tr>
<tr>
<td>&lt;2 years old</td>
<td>208 (9.0)</td>
<td>16 (4.3)</td>
<td>6.000</td>
<td>0.365</td>
</tr>
<tr>
<td><strong>Duty of the horse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hobby</td>
<td>677 (33.9)</td>
<td>162 (46.9)</td>
<td>1.994</td>
<td>1.593</td>
</tr>
<tr>
<td>Breeding</td>
<td>422 (21.1)</td>
<td>10 (2.8)</td>
<td>0.13</td>
<td>0.069</td>
</tr>
<tr>
<td>Sport</td>
<td>380 (19.0)</td>
<td>72 (20.8)</td>
<td>1.287</td>
<td>0.973</td>
</tr>
<tr>
<td>Working</td>
<td>354 (17.7)</td>
<td>82 (23.7)</td>
<td>1.65</td>
<td>1.259</td>
</tr>
<tr>
<td>Young</td>
<td>97 (4.8)</td>
<td>9 (2.6)</td>
<td>0.593</td>
<td>0.297</td>
</tr>
<tr>
<td>Other Duties</td>
<td>63 (3.1)</td>
<td>10 (2.8)</td>
<td>1.032</td>
<td>0.525</td>
</tr>
<tr>
<td><strong>Water intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unchanged</td>
<td>30 (66.6)</td>
<td>45 (28.4)</td>
<td>0.199</td>
<td>0.098</td>
</tr>
<tr>
<td>Changed</td>
<td>15 (33.3)</td>
<td>113 (71.5)</td>
<td>5.022</td>
<td>2.47</td>
</tr>
<tr>
<td>Decreased</td>
<td>7 (46.6)</td>
<td>78 (69.0)</td>
<td>5.293</td>
<td>2.23</td>
</tr>
<tr>
<td><strong>Recent change in diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>150 (78.5)</td>
<td>258 (80.1)</td>
<td>1.101</td>
<td>0.709</td>
</tr>
<tr>
<td>Yes</td>
<td>41 (21.4)</td>
<td>64 (19.8)</td>
<td>0.908</td>
<td>0.584</td>
</tr>
<tr>
<td><strong>Deworming</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>512 (40.3)</td>
<td>95 (29.5)</td>
<td>0.620</td>
<td>0.476</td>
</tr>
<tr>
<td>Yes</td>
<td>758 (59.6)</td>
<td>227 (70.4)</td>
<td>1.614</td>
<td>1.239</td>
</tr>
<tr>
<td>Recent deworming</td>
<td>64 (8.4)</td>
<td>28 (12.3)</td>
<td>1.795</td>
<td>1.131</td>
</tr>
</tbody>
</table>

"Recent" refers to the two week period prior to examination
CI = Confidence interval. p< 0.05 was considered significant
OR > 1 implies increased risk, OR< 1 implies decreased risk
4.7. Weather-related factors

Temperature (°C) on the day of colic horses’ arrival is presented in figures 5 to 16. The mean temperature on the day of more than one colic case arriving was higher (15.35 ± 6.4) than that of just one colic case arriving (13.27 ± 7.67), and both were higher than on the day of no colic cases arriving (11.92 ± 7.72). Consequently, high temperature on the day of arrival was significantly associated with an increased risk of colic (p = 0.003). Therefore, the risk of colic was highly observed in the summer period (p = 0.002) compared to other seasons. However, none of the specific months were significantly associated with colic (p = 0.061).

Barometric pressure (hPa) on the day of colic horses’ arrival is presented in figures 17 to 26. No association was found between the barometric pressure on the day of arrival and colic (p = 0.750). The mean barometric pressure was similar on the day of arrival for more than one colic case (1016.09 ± 7.51), on the day of arrival for just one colic case (1016.93 ± 8.61) and on the day of no colic case arrival (1016.62 ± 8.59).

Variation in temperature in the one- (p = 0.678), two- (p = 0.511) and three-day (p = 0.323) periods prior to examination and variation in barometric pressure in the one- (p = 0.418), two- (p = 0.895) and three-day (p = 0.597) periods prior to examination were not significantly associated with colic.

4.8. Feces and blood analysis

4.8.1. Clostridium perfringens, Clostridium difficile and Salmonella spp. in feces

As mentioned above, “first feces samples” were collected from colic horses on the day of arrival, and “second feces samples” were collected three days later. Some horses were discharged before second feces samples could be collected.

Considering Clostridium perfringens, results are given in table 24. The calculated odds ratio for C. perfringens in first feces samples from colic horses was 1.86, which indicates only a light effect on occurrence of colic. It was confirmed by a Chi-square test, and no significant association between the presence of C. perfringens in first feces specimens with colic and non-colic horses feces specimens was identified (p = 0.325). Furthermore, only 8 second feces samples were collected from the 27 horses testing positive for Clostridium perfringens on the day of arrival. Clostridium perfringens was identified in 2 of those samples.

Clostridium difficile was also tested for. None of the investigated feces samples, which constituted from 177 first feces samples and 35 second feces samples from colic horses and 34 feces samples from non-colic horses, contained Clostridium difficile.

Salmonella spp. a third bacteria that was examined, was not isolated from feces samples (n = 93) collected from horses admitted because of colic on the day of admission or feces samples (n = 34) collected from non-colic horses.
Table 24 Presence of *Clostridium perfringens* in samples taken from horses suffering from colic on the day of admission and three days after admission, and from horses without colic

<table>
<thead>
<tr>
<th></th>
<th>Colic horses first feces samples</th>
<th>Colic horses second feces samples</th>
<th>Non-colic horses feces samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. perfringens not isolated</td>
<td>150 (84.7%)</td>
<td>68 (97.1%)</td>
<td>31 (91.2%)</td>
</tr>
<tr>
<td>C. perfringens &lt;100 CFU / g</td>
<td>13 (7.3%)</td>
<td>1 (1.4%)</td>
<td>3 (8.8%)</td>
</tr>
<tr>
<td>C. perfringens &gt;100 CFU / g</td>
<td>14 (7.9%)</td>
<td>1 (1.4%)</td>
<td></td>
</tr>
<tr>
<td>Total number of samples</td>
<td>177</td>
<td>70</td>
<td>34</td>
</tr>
</tbody>
</table>

4.8.2. Fecal pH

The outcomes of the fecal pH measurements are presented at table 25. The pH from first feces samples ($p = 0.139$) and the pH from second feces samples ($p = 0.06$) from colic horses were not significantly different than the pH from non-colic horses.

Feces samples were taken from the same 70 horses on the day of admission and three days after the admission. Fecal pH, after three days hospitalization, was decreased in 60% (42/70) of the feces samples, was increased in 37.1% (26/70) of the feces samples and was consistent in 2.9% (2/70) of the feces samples. Consequently, in these 70 horses the fecal pH of the second feces samples ($7.02 \pm 0.71$) was significantly lower than that of the first feces samples ($7.12 \pm 0.56; p = 0.02$).

Furthermore, fecal pH was categorized as follows: $\leq 6.32$ indicated acidic feces and $\geq 6.32$ indicated "alkaline" feces. 3.4% of the first feces samples, 14.3% of the second feces samples, and 2.9% of the feces samples from non-colic horses had acidic feces. Neither first feces samples ($p = 0.07$) nor second feces samples ($p = 0.66$), considering acidity or alkalinity of the feces, was significantly associated with colic compared to non-colic horses. However, the proportion of acidic second feces samples was significantly higher than the proportion of acidic first feces samples ($p = 0.003$).

Table 25 The number of examined samples, minimum, maximum, mean and standard deviation (SD) for the fecal pH

<table>
<thead>
<tr>
<th>Feces samples</th>
<th>n</th>
<th>Min. pH</th>
<th>Max. pH</th>
<th>Mean pH ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFS</td>
<td>177</td>
<td>5.9</td>
<td>8.8</td>
<td>7.29 ± 0.49</td>
</tr>
<tr>
<td>SFS</td>
<td>70</td>
<td>5.5</td>
<td>8.8</td>
<td>7.02 ± 0.71</td>
</tr>
<tr>
<td>NCFS</td>
<td>34</td>
<td>6.1</td>
<td>8.3</td>
<td>7.41 ± 0.52</td>
</tr>
</tbody>
</table>

FFS represents feces samples that were collected on the day of admission from colic horses
SFS represents feces samples that were collected three days after the admission from colic horses
NCFS represents feces samples that were collected from non-colic horses
4.8.3. Biogenic amines in feces and blood

The plasma amines serotonin, the aromatic monoamines, (tryptamine and tyramine),
the diamines (putrescine, cadaverine, histamine, spermidine and spermine), and the
catecholamines (dopamine) were measured but not detected in the plasma samples from colic
horses \( n = 50 \) in the study.

The same biogenic amines were also measured in fecal samples of the same colic
horses \( n = 50 \) and non-colic horses \( n = 34 \), with the following results:

- Serotonin, spermine and dopamine were not detected in the samples from any of the horses.

- Fecal spermidine was not significantly associated with colic \( (6.20 \pm 17.44 \text{ nM/g}) \) compared
to non-colic horses \( (2.29 \pm 5.00 \text{ nM/g}; p = 0.145) \).

- 15.54 nM/g histamine was detected in only one feces sample from a horse with colic,
although histamine was not found in any of the feces samples from non-colic horses.

- At the level of 10.84 nM/g tryptamine was found in only one fecal sample from colic
horses, although it was found in 11 fecal samples from non-colic horses (range 5.43 nM/g to
10.99 nM / g). Tryptamine levels were significantly higher for non-colic horses \( (2.72 \pm 4.10 \text{ nM/g}) \) than colic horses \( (0.21 \pm 1.53 \text{ nM/g}; p < 0.001) \). The proportion of tryptamine was
significantly different between colic \( (2 \%) \) and non-colic horses \( (32.4 \% ; p < 0.001) \).

- Fecal tyramine was not significantly associated with colic \( (3.06 \pm 5.64) \) compared to non-
colic horses \( (1.64 \pm 2.29; p = 0.086) \).

- Cadaverine and putrescine were identified in the fecal samples from all horses with colic
and without colic. There was no significant difference in the concentration of cadaverine \( (p = 0.131) \) and putrescine \( (p = 0.227) \) when comparing fecal contents from non-colic horses
\( (49.38 \pm 106.79; 18.60 \pm 13.93) \) with those from colic horses \( (55.0 \pm 158.05; 25.5 \pm 41.02) \).

4.8.4. Serum bile acids

The serum bile acids values of medically treated horses, surgically treated horses,
 survived horses and dead horses are represented in figure 3.

- Of these 184 horses with colic, serum bile acids mean value was \( 5.47 \pm 9.36 \) (range 1 to
117\( \mu \text{mol} / \text{L} \) for 184 horses with colic, but only 2.17 \% \( (4 / 184) \) of them had bile acids
values in serum higher than the reference range \( (< 20 \mu \text{mol} / \text{L}) \).

- Of these horses 153 were medically treated horses and 31 surgically treated horses No
significant association was detected considering serum bile acids between medically treated
horses \( (5.12 \pm 4.71 \mu \text{mol} / \text{L}) \) and surgically treated horses \( (7.19 \pm 20.46 \mu \text{mol} / \text{L}; p = 0.13) \).

- Of these horses, 171 horses survived and 13 horses died. Serum bile acids was not
significantly different between horses that survived \( (5.32 \pm 9.32 \mu \text{mol} / \text{L}) \) and horses that
did not survive \( (7.46 \pm 10.07 \mu \text{mol} / \text{L}; p = 0.26) \).

- Serum bile acids were also measured for 26 horses with colic 3 days after hospitalization,
but none of the samples contained bile acids value higher than the reference range.
Moreover, change in the serum bile acids value between the day of arrival (4.15 ± 2.82 μmol / L) and 3 days after admission (6.27 ± 3.44 μmol / L) was examined for 26 horses, and there was a significant association (p = 0.001).

![Serum bile acids levels](image)

Figure 3. Serum bile acids mean values and 95 % CI for colic horses (reference range < 20 μmol/L)

### 4.8.5. Serum alkaline phosphatase activity

Total alkaline phosphatase activity (ALP<sub>t</sub>) was measured for 187 colic horses on the day of arrival (figure 4) and 26 horses three days after the admission. Consequently,

- ALP<sub>t</sub> in serum taken on the day of arrival was not significantly different between medically treated horses and surgically treated horses (p = 0.42; figure 4).

- In the 4-group classification (MA, MD, SA, SD), mean serum ALP<sub>t</sub> activity was significantly lower in SD than in MD (p = 0.02), but the value remained within the reference range (figure 4). Serum ALP<sub>t</sub> activity in MA in comparison was not significantly different than that in MD and SA (p = 0.76; p = 0.10). In addition, serum ALP<sub>t</sub> activity in SA was not significantly different than in SD (p = 0.13).

- Horses were also categorized as all alive (horses that survived and were discharged) or all dead horses, and ALP<sub>t</sub> activity in serum taken on the day of arrival in survived horses was not significantly different than horses that had a poor prognosis (death) (p = 0.39; figure 4).

- Blood samples were taken from 26 horses for ALP<sub>t</sub> activity measurement both on the day of arrival and three days after the admission. Compared to ALP<sub>t</sub> activity in serum on the day of admission, 20 (76.6 %) horses' ALP<sub>t</sub> activity in serum three days after admission was increased, but only 9 (4 horses; > 300 U/L, 5 horses; between 250 – 300 U/L) horses had serum ALP<sub>t</sub> activity higher than the reference range. Six (4 horses; > 300 U/L, 2 horses; between 250 – 300 U/L) among these 9 horses already had high ALP<sub>t</sub> activity in serum on the day of admission. Consequently, no significant difference was detected between mean ALP<sub>t</sub> activity in serum on the day of arrival (175.00 ± 119.78) and ALP<sub>t</sub> activity in serum three days after arrival at the hospital (216.85 ± 114.59; p = 0.28).
Figure 4 Serum alkaline phosphatase activity (ALP\textsubscript{t}) in 187 horses with colic categorized as surgically or medically treatment and alive or dead

Normal ALP\textsubscript{t} represents < 250 U/L, Increased ALP\textsubscript{t} represents > 250 U/L, (Mean ± SD)
5. DISCUSSION

5.1. Clinical history of colic, treatment procedure of colic and mortality rate

Of the 2743 cases, 13.3% had one or more colic episodes between August 1, 2006 and August 1, 2007, treated at the Equine Clinic located at the University of Veterinary Medicine Vienna, Austria. Of the 333 animals with colic in our study, 8.4% had multiple episodes of colic in a year. Similar to our study, KANEENE et al. (1997) reported that 16% of the horses with colic had multiple episodes in a two-year prospective study, but TINKER et al. (1994) reported that 18.9% of the horses with colic had multiple cases of colic in a year-long prospective study.

In the present study, overall case fatality risk was 15.3%, the case fatality rate for non-surgical colic was 13.5%, while the case fatality rate for surgical colic was 22.5%. These results are similar to the findings of KANEENE et al. (1997), which reported that the overall case fatality risk was 13%, the case fatality risk for non-surgical colic was 10%, and the case fatality risk for cases treated surgically was 31%. Moreover, surgical colic cases remained in the hospital longer than medical cases in our study, and these results are similar to those reported by ABUTARBUSH et al. (2005), FREDERICO et al. (2006) and RHOADS et al. (1997). The authors propose that horses with surgical colic may be more likely to have a poorer prognosis than medical cases. However, it is not necessarily that the higher case fatality rate for surgical cases is due to the act of surgery, it is rather more likely the underlying pathological changes of the gut affects the outcome. Also surgical cases usually stayed at the hospital longer than the medical cases, due to continuing treatment, which seems quite logic. Interestingly, ABUTARBUSH et al. (2005) and COHEN (1997) found animals treated by surgery for colic were significantly older compared with those medically treated; in contrast, no association was detected between age and treatment type of colic in our study. This may be due to a different equine population. The horse population around Vienna is quite unbalanced for young horses due to lack of significant breeding and racing activities.

5.2. Feeding practices

In this study, feedstuffs from colic horses and non-colic horses were evaluated by feed sensory evaluation as its goal understanding, whether feedstuffs were generally eligible for feeding to horses or not. Both quantity of nutrients and hygienic quality were scored with a predetermined standard system.

As seen from the results in table 19, poor hygienic quality of hay increased risk of colic; however, hygienic quality of straw or particular type of concentrate were not significantly associated with colic in the present study. The association between yeast, mold or bacteria and occurrence of colic was not directly examined in this study. Microbiological investigation was only conducted in this study for confirmation of yeast, mold and bacteria in presumptive feedstuffs. No association was detected between hygienic qualities of oats and colic in our study; therefore, the results are not similar to that reported by MEYER et al. (1986). MEYER et al. (1986) stated that after the feeding of oats and mixed feeds of poor hygienic quality, in most reviewed cases there was a history of digestive disturbances, and increased yeast concentration was often connected with colic. KAMPHUES (1996) reported that poor hygienic quality of feed frequently were associated with contamination with mites and molds (e.g., in hay and straw) and to a lesser extent with yeast contamination (e.g., in silages, molassed oats or, less frequently, in concentrates). Oats were especially contaminated with high numbers of bacteria. Significant contamination of the feed is indicative for poor hygienic
quality and may cause digestive disorders (enhanced gastrointestinal gas forming by yeasts and further gas producing microorganisms, reduced fiber digestion in the hindgut due to dysbiotic changes in the flora).

In this study, no association was found between intake of foreign material such as sand, wood or litter shavings and colic. The authors did not expect to find any association concerning sand and occurrence of colic, because Austria's soil and pastures do not include a high amount of sand.

To date, the association of feeding practices with the development of digestive disorders in horses has long been studied. No significant association was found between occurrence of colic and the amount of hay or particular type of concentrate fed daily (oats, squeezed oats, corn, cereal and pellets) in our study. However, colic horses' total concentrate intakes ($2.74 \pm 2.09$) were significantly higher than the non-colic horses' total concentrate intakes ($1.64 \pm 1.16$). Thereby, high total concentrate intake was significantly associated with increased risk of colic. These findings are not in agreement with COHEN and GIBBS (1999), who found no association between total concentrate intake and colic. However, TINKER et al. (1997a) reported that the risk of colic increased for horses at the highest concentrate intake levels over the horses on pasture ($> 2.5$ kg / day dry matter, OR = 4.8, $> 5$ kg / day dry matter, OR = 6.3) compared to horses that received no concentrate. The association between occurrence of colic and high amount of total concentrate intake may be explained by reduction in the luminal pH, alteration of the intestinal flora and production of endotoxins in the colon (CLARKE et al., 1990; COHEN and PELOSO, 1996). This may also be explained by increased daily intake of grain, which decreases the water content in the colon due to less fiber, which binds to water, and also increases gas production and creates the environment needed for tympany and displacement (NATHANIEL, 2005).

Despite the increased risk of colic due to the high amount of concentrate intake, the numbers of meals of concentrate a day and concentrate intake in a meal were not significantly associated with colic in our study. Our finding was in agreement with HUDSON et al. (2001), which recorded no association between feeding frequency of concentrates and colic. The same authors mentioned that to prevent digestive dysfunctions, grain intake in a horse fed two to three meals daily should be limited to about 0.5 kg / 100 kg body weight per feeding. When grain ingestion exceeds these amounts, there is a dramatic increase in the amount of starch that escapes digestion and absorption from the small intestine, which greatly increases the risk of problems such as excessive gas production and colic (LEWIS, 1995b). Therefore, the authors think that high concentrate feed intake could be related to originally small intestinal colic, but interestingly none of the horses that were examined for feed intake had originally small intestinal colic in this study. The reason is unknown.

However, one should note that the amount of concentrate given is also directly correlated with the level of exercise or training undertaken (ARCHER and PROUDMAN, 2006; TINKER et al., 1997b). Unfortunately, the interaction of these two variables was not examined in this study due to the extent of the difference in the number of valid data.

A recent change in diet (either type or amount) up to the 2-week period prior to examination was not associated with development of colic in this study. That was partially consistent with results of a similar study (HUDSON et al., 2001) that found no association between the recent change in the type of hay and colic. However, the same authors also found that horses that had a recent change in amount of hay (either an increase or decrease) fed were at a greater risk of colic. Moreover, several studies (COHEN et al., 1995; COHEN and
GIBBS, 1999; TINKER et al., 1997a) were not in agreement with our study; that change in either type or amount of diet increased risk of colic. Consequently, the absence of statistical association between recent change in diet and colic in our study could mean that this variable does not have an important influence on development of colic, or it could be attributable to a lack of statistical power, because the number of horses, which had valid data (n = 105) was too small.

An adequate supply of good-quality water is essential for horses. The amount of water needed varies primarily with the amount of water lost from the body, which is altered by the amount, type, and quality of the feed consumed, the ambient temperature and humidity, and the health, physiological state, and physical activity of the horse (LEWIS, 1995b). REEVES et al. (1996) found that insufficient water supply in outdoor enclosures increased the risk of colic. With respect to the water source, KIPER et al. (1990) found that horses that drank from an automatic waterer were less likely to have colic than horses that drank water from a bucket. This association may have occurred because horses that drink water from buckets may have extended periods without water unless the buckets are refilled frequently. In addition, water in buckets may freeze over and deprive the horse of a fresh water source; however, KANEENE et al. (1997) did not find an association between the use of water heaters in cold weather to prevent freezing in tanks or buckets and risk of colic. In our study, bivariate analysis indicated that horses that had decreased water intake were five times more likely to develop colic compared with horses that had no decrease in water intake. However, in our study, watering type according to information of the owner (e.g., bucket and automatic waterer) was not significantly associated with the development of colic, and that was consistent with results of a similar study (HUDSON et al. 2001). The information concerning change in water intake was obtained from medical records, while watering types were obtained from questionnaire in our study. Possibly, information concerning these two related parameters was not observed from the same horses, or was observed only for a small number of the same horses, causing these conflicting results.

5.3. Housing and pasture practices

Husbandry conditions like time spent on pasture, stable provision and recent change in stabling provision were evaluated in this study, mainly for understanding whether exercise or physical activity of horses could have an independent effect on occurrence of colic in horses. However, these parameters were not significantly associated with colic.

Considering change in stable, the data did not agree with that found by COHEN and GIBBS (1999) and TINKER et al. (1997a), which found that a change in stabling within the previous two weeks was significantly associated with increased risk of colic, although these studies did not examine which type of change in stabling was found to be a risk factor for colic. In addition, COHEN et al. (1995) also found that recent change in stabling was a risk factor for colic, but this association disappeared in a multivariable analysis.

The studies concerning housing and pasture practices, which do not agree with our results were found by COHEN and GIBBS (1999), which stated that horses at pasture year-long were at lower risk for colic than horses living indoors, and by HILLYER et al. (2002), which reported that increasing the hours stabled and decreasing the hours at pasture were both associated with an increased risk of simple colonic obstruction. Another study (HUDSON et al. 2001) did not agree with our results, either. HUDSON et al. (2001) found that horses that spend 100 % of their time in the stable have been reported to be at increased risk of colic when compared to horses that spent no time in a stable. We could not correlate duty of the
horses with housing and pasture practices, because answers and data were too imprecise. Further studies could be designed to completely evaluate activity of the horse with regard to housing and pasture practices.

5.4. Other management factors

Considering the duty of the horse, hobby horses and working horses were found to be at increased risk of colic, whereas breeding horses had a decreased risk of colic in bivariate analysis, but in the final multivariate analysis horse activity could no longer be proved to be associated with the risk of colic in our study. These results are supported by COHEN et al. (1995), COHEN and GIBBS (1999), and REEVES (1996), who found no relation between the activity of the horse and colic, and also by KANEENE et al. (1997), who found that breeding activity was not a risk factor for colic. The data do not agree with that found by TINKER et al. (1997b), who reported that intense activity increased the risk of colic, and by MORRIS et al.(1989), who reported horses in racing the day before colic were associated with large colon impaction. Activity of the horses was obtained from the medical records, giving valid information for 2338 horses, although duration of activities (hour/day) was obtained from questionnaires, giving valid information for only 25 horses. Because of this, duration of activities was not examined in this study. Ensuing studies could be designed to evaluate these two related variables in the same population.

Considering deworming, horses that had anthelmintic administration and horses that had recent deworming (two-week period prior to examination) were more likely to develop colic compared to non-colic horses (by 1.6 times and 1.7 times, respectively) in univariate analysis, but in the multivariate analysis the association was not verified in our study. The results are similar to the findings of COHEN et al. (1995) and MORRIS et al. (1989), who reported no association between the frequency of anthelmintics administration, the recent administration of anthelmintics, the type of anthelmintics and colic. Our results were in disagreement with HUDSON et al. (2001), which found that recent deworming decreased risk of colic and also with COHEN and GIBBS (1999), who found that recent deworming increased the risk of colic.

5.5. Individual factors

Considering gender, geldings were two times more likely to develop colic compared with non-geldings, but this association disappeared in the multivariate analysis in our study. The results are similar to findings of REEVES et al. (1996), TINKER et al. (1997a) and TRAUB-DARGATZ et al. (2001), which found no significant difference between sexes and occurrence of colic. However, the results are in a disagreement with ABUTARBUSH et al. (2005), COHEN and PELOSO (1996), which recorded that geldings were more susceptible to colic than stallions. That may be due to geldings are kept under worse conditions. In urbanized areas with predominantly riding horse’s populations, geldings are more common than in areas with a large race horse population. In the latter high price money of stallion stakes is a protecting factor against castration.

Mixed-breed horses were found to be two times more likely to have colic than other breeds, but this association could not be verified in the multivariate analysis. Our findings are in agreement with the results of ABUTARBUSH et al. (2005) and KANEENE et al. (1997), who reported no breed predisposition to colic. The data do not agree with that found by COHEN et al. (1995), COHEN and PELOSO (1996), and TINKER et al. (1997a), who recorded that Arabian horses have a higher risk than others, and by HUDSON et al. (2001),
who stated that thoroughbreds were at an increased risk of colic compared with Arabians, Quarter horses and horses of other breeds. Moreover, REEVES et al. (1996) found that Arabian horses were more than twice as likely to get colic compared to other horses, whereas standardbred horses were nearly half as likely to get colic compared with thoroughbreds. MEHDI and MOHAMMAD (2006) reported that crossbred horses had the highest susceptibility to colic among thoroughbred, Turkmen and Arabian breeds.

Considering age, no significant association was detected between colic and age when age was considered as a categorical variable (e.g., < 2 years, 2 to 10 years, and > 10 years old) in the current study. Our findings were confirmed by COHEN et al. (1995) and HUDSON et al. (2001), who also stated that there was no significant association between colic and age. The data do not agree with that found by TINKER et al. (1997a; 1997b), who recorded that horses aged 2 to 10 years had a higher risk of occurrence of disease, and by KANEENE et al. (1997), who stated that increased age increased risk for colic. Moreover, MORRIS et al. (1989) found that horses 15 years or older were most represented in small colon obstruction and gastric rupture, and COHEN and GIBBS (1999) reported that horses older than 10 years old had increased incidence of colic, and also, the incidence of certain forms of colic such as strangulated lipomas is more common among older horses. This relationship can also be explained by physiologic and anatomic abnormalities associated with age (COHEN and PELOSO, 1996) and more opportunity to develop colic.

The authors believe that this study is limited to comparisons of the gender, breed and age for colic cases with the proportions for the total hospitalized population at the same centre, and overall there is no clear association between individual factors and colic. This was supported by the conclusion of two reviewed articles (ARCHER and PROUDMAN, 2006; COHEN, 2003), which stated that the reason for conflicting results in studies considering individual factors may be the variety of the number of horses included in studies and different geographical regions of the studies. For example, COHEN (2003) stated that there were a few standardbred horses in Texas. Therefore, it is unlikely that standardbred horses would be identified as being at increased risk for colic in studies conducted in Texas.

5.6. Weather-related factors

The mean temperature on days that more than one colic case was admitted higher than that of just one colic case arriving and both were higher than on the day of no colic case arriving in our study. Therefore, high temperature or rapid change in temperature appears a predisposing factor for development of colic symptoms. In addition, the risk for colic was in the summer period, but not in any specific month. The data do not agree with the observation of HILLYER et al. (2001) and PROUDMAN (1991), which recorded that seasonal incidence of horses with colic increased during the spring and autumn, of TRAUB-DARGATZ et al. (2001), which reported a higher percentage of colic cases in spring compared to summer or autumn, and of ARCHER et al. (2006), which reported that the highest incidences were in the months of April / May and again in October / November / December. The authors believe that the increased incidence of colic that appeared in the summer period and the high temperature on the day of arrival were not directly related to the weather, but rather to management changes (e.g., decreased water intake) caused by the weather. This is an opinion similar to the opinion of ARCHER et al. (2006) and NATHANIEL (2005), not a systematic result.

Barometric pressure on the day of arrival was not significantly associated with incidence of colic in the present study. In addition, change in temperature and barometric pressure on days one, two and three before hospital admission were not significantly
associated with colic incidence. These results are supported by FORAMEN and WHITE (1986), which found that a change in ambient temperature and a change in barometric pressure during the 24-hour period prior to development of colic were not associated with increased frequency of colic. However, COHEN and GIBBS (1999) did not agree with our findings, and recorded that weather changes during the three days prior to examination increased the risk of colic. That study recorded substantial change in weather conditions according to owners of colic horses' opinion; however, temperature and barometric pressure were recorded day by day from a public web site in our study.

5.7. Summarize of individually, environmentally and nutritionally mediated risk factors of colic

Colic risk factors are complex, and the data is difficult to interpret due to many parameters and possible interactions, confounding and bias. Therefore, the author gave an overview concerning generally argued alterable risk factors of colic, which includes our results and some of the other studies results, see below.

<table>
<thead>
<tr>
<th>Possible feeding and management risk factors</th>
<th>Cohen et al., 1995</th>
<th>Reeves et al., 1996</th>
<th>Kaneene et al., 1997</th>
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<td>1 y</td>
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<td>Recent deworming</td>
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<tr>
<td>Duty of the horse</td>
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<tr>
<td>Activity level of the horse</td>
<td>0</td>
<td></td>
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<td>0</td>
</tr>
</tbody>
</table>

Recent change means two weeks prior to examination,
# increased risk of colic, * decreased risk of colic, 0 = no association
The author concludes that high temperature on the day of arrival, decreased water consumption, low hygienic quality of hay, and high amount of concentrate intake increase the risk of colic in a hospital-based study in Vienna, Austria. Weather related factors (temperature, raining, etc.) may not be altered but a better knowledge of their role should enable horse owners and veterinarians to pay more attention (GONCALVES et al., 2002). However, it should be carefully considered that the determined risk factors are valuable for prevention of colic if these risk factors can be controlled. Although location and alterable risk factors may be related to each other, alterable risk factors should be identified for each location separately. Consequently, horse caretakers should pay considerable attention to these alterable factors. Attention to these factors may not stop occurrence of colic but may decrease occurrence of colic in Austria. Those conducting further colic studies in Austria should have a better understanding of risk factors for colic and eventually provide prevention programs.

5.8. Specific bacteria in feces and fecal pH

The results of our study detected fewer colic horses positive for Clostridium perfringens in feces (15.2 %) than similar studies conducted by GRIFFITHS et al. (1997) and JOBST et al. (2002), which documented that 30.3 % of the feces samples belonging to surgical colic horses were positive for Clostridium perfringens, and 52.2 % of the feces samples from horses undergoing medical or surgical treatment for colic were positive for C. perfringens, respectively. Geographic and seasonal variations in environmental concentrations of C. perfringens (DONALDSON and PALMER, 1999) and different populations may explain the variation in prevalence reported these studies. As mentioned above, this is the first study in Austria to consider C. perfringens in feces from colic horses. Therefore, comparison of the current study with another study from the same environment was not possible. However, no significant association was detected considering the presence of C. perfringens in feces specimens collected on the day of admission of colic horses compared to non-colic horses' feces specimens. It is possible that the C. perfringens detected in horses is a constant contaminant from the animals' environment (GRIFFITHS et al., 1997). Proportion of C. perfringens positive feces decreased during hospitalization that may be due to the treatment of colic during the 3 day period. The authors believe that nosocomial acquisition of C. perfringens was not possible in the current study, as only 2 horses were positive for C. perfringens on both the day of admission and three days after the admission. No horses testing negative for C. perfringens on the day of admission acquired the bacteria during the 3 days hospitalization.

The second bacteria examined, Clostridium difficile, is not commonly found in feces of healthy adult horses (PERK et al., 1993). The current investigation further confirms that C. difficile was not isolated from feces samples of horses with colic (BAVERUD et al., 2003). In addition, C. difficile was not isolated from the feces samples collected three days after the admission of colic horses in the present study; in contrast, a similar study (JOBST et al. 2002) isolated C. difficile in feces samples of horses with colic during hospitalization. In other studies (ARROYO et al., 2006; JOBST et al. 2002; PERK et al. 1993), the isolation frequency of C. difficile from public parks and playgrounds varied between different sampling, and there was a tendency towards more positive soil samples in connection with water-filled ditches (BAVERUD et al., 2003). The absence of C. difficile in this study may be explained by this is an uncommon bacteria specie in Austria.

The third bacteria Salmonella spp. was not isolated either from feces samples from colic horses or non-colic horses. The results of our study are therefore in accordance with the results of DONALDSON and PALMER (1999) recorded that Salmonella spp. was not
isolated from fecal samples (n = 30) collected from horses that developed diarrhea as a complication of colic. However, our findings are not in agreement with the results of HIRD et al. (1986), who found that horses admitted because of colic were more likely to have Salmonella isolated as those, admitted for other reasons. Colicky horses may have a tendency to acquire Salmonella spp. at the time of hospitalization (PALMER et al., 1985), especially during the period from the fourth to the seventh day (DUNOWSKA et al., 2004). It was detected in feces at least once in 9-13 % of horses with colic during the hospitalization in two studies (ERNST et al., 2004; KIM et al., 2001) due to stress associated with abdominal pain, hospitalization may compromise the immunologic status of carrier horses (PALMER et al., 1985). However, we did not examine any samples from colic horses that were collected during hospitalization.

Considering fecal pH, the pH of feces collected from colic horses on the day of arrival as well as the pH of feces collected from the same horses three days after their arrival was not significantly associated with colic compared to non-colic horses in this study. However, the mean fecal pH of feces samples collected from colic horses on the day of arrival was significantly higher than that of feces samples collected from the same horses three days after their arrival. In fact, mean fecal pH was 7.0 to 7.2 in all of the samples that were acquired from colic horses, and 7.4 in samples that were acquired from non-colic horses. Besides the propionate, fecal starch concentration was a further potential pH-influencing factor (RICHARDS et al. 2006); therefore, grain supplementation decreased the fecal pH (HUSSEIN et al. 2004). Because horses did not receive additional grain supplementation during the three days of hospitalization in this study, that can not be an explanatory factor for lowered fecal pH. Mostly they were fasted and treated with laxatives. The lowered fecal pH may be attributable to the long duration of digestion in horses, because digest reaches the cecum approximately 3 hours after a meal, and remains in the large intestine for 36-48 hours (PILLINER and DAVIES, 2004). Therefore only the fecal pH of the samples acquired three days after the admission may indicate actual fecal pH related to colic.

5.9. Biogenic amines in feces and blood

Amines, if released into the circulation following carbohydrate overload, may contribute to digital vasoconstriction, having a selective effect on the digital vasculature over the systemic circulation (BAILEY et al., 2004) and this may predispose for laminitis.

In this study, histamine was detected in just one feces sample from colic horses, but serotonin, spermine and dopamine were not detected in any of the feces samples from either colic horses or non-colic horses. In addition, tyramine was detected in some of the feces samples from colic horses and non-colic horses, although there was no significant association with colic. Cadaverine and putrescine were found in the feces samples from all of the horses, but there was also no significant association with colic. In a study (ELLIOTT et al., 2003) tryptamine, tyramine, putrescine, cadaverine, histamine, spermidine and spermine were measured in feces and were identified in samples from all ponies (normal ponies = 6 and laminitis prone ponies = 6). The same authors also found that tryptamine and tyramine were potent constrictors of arteries and veins. Another study (ELLIOTT and BAILEY, 2006) mentioned that tryptamine is the most potent cecum-derived amine. In our study, fecal tryptamine levels (nM/g) in colic horses (0.21 ± 1.53) were significantly different from those in non-colic horses (2.72 ± 4.10; p < 0.001). However, this outcome may not be reliable due to the extent of differences in the number of tryptamine detected feces samples of horses between colic and non-colic horses, because tryptamine was found in only one fecal sample from colic horses (n = 50), consistent at 10.84 nM/g, although it was found in 11 fecal
samples from non-colic horses (n = 34). Therefore, the statement “A significant difference was found between the proportions of tryptamine detected feces samples from colic horses (2 %) and non-colic horses (32.4 %)” is more reliable than the statement “Tryptamine levels were significantly associated with colic”. The role of fecal tryptamine levels in this study could not be established.

In our study, amines (serotonin, tryptamine, tyramine; putrescine, cadaverine, histamine, spermidine, spermine and dopamine) were not found in plasma samples from colic horses. This is contrast to another study (HODSON et al., 1989), which found different results from our study, found that histamine was detected in plasma, although the plasma histamine levels were not significantly different between controls and colic horses. Furthermore, BAILEY et al. (2003a) identified the following amines methylamine, ethylamine, propylamine, isoamylamine, isobutyramine, tryptamine, tyramine, kynuramine, phenylethylamine, putrescine, cadaverine, histamine, diaminohexane, spermidine and spermine in equine caecal and colonic contents. However, the plasma concentrations of these amines are below the limits of detection. It is not yet known to what extent these amines may cross the mucosal barrier into the circulation. This may be an explanatory factor of detection of some biogenic amines (cadaverine, putrescine and tyramine) in feces samples, but not in plasma samples of the same horses, in our study. The intestinal wall is obviously a barrier to the diffusion of amines into the circulation (ELLIOTT and BAILEY, 2006).

5.10. Serum bile acids

In our study, only 2.17 % of the horses with colic had serum bile acids values higher than the reference range. Therefore, our study was highly in agreement with WEST (1989), who found that there was no increase in total plasma bile acid concentrations outside the normal range in horses with gastrointestinal disease in all except two horses (n = 26). Of two interesting studies, one (ARGENZIO and WHIPP, 1983) found that if bile acid absorption is decreased, bile acids entering the colon induce diarrhea by causing increased permeability of the colon thorough mucosal damage, and the other one (LITTLE et al. 2005) found increased fecal bile acids in horses without a functional ileum and following jejunal or ileal resection, explaining the normal range of serum bile acids values in horses with colic found in our study. To elaborate, it seems likely that if the small intestine has damage that may cause decreased bile acids absorption; therefore bile acids enter the colon and may be lost in feces. If the large intestine has damage, that may not have an effect on serum bile acids values, because it is not related to the enterohepatic cycle. Thus bile acids values may not be an important parameter for determination of colic or probability of surgery and prognosis. Measurement of the plasma bile acid concentrations improved the diagnostic value of routine hepatic tests in the detection of hepatobiliary disease (WEST, 1989) and acute or chronic liver diseases, because bile acids are known to be produced only by the liver, present in high concentrations in portal circulation, extracted by the liver at a high level of efficiency and normally transported away from the liver through the biliary circulation (ENGELKING, 1989).

5.11. Serum alkaline phosphatase activity

The diagnostic and prognostic relationships of total alkaline phosphatase activity (ALP) in serum of horses with colic were examined in our study. Only 11.2 % of horses with colic had higher serum ALP activity than the reference range, and therefore, ALP activity appears to be of no diagnostic value. In our study, ALP activity was not significantly different between medically and surgically treated horses, and therefore, ALP activity was not useful for predict the probability of surgery. A similar study (KLUCKNER, 2005) was
conducted at the University of Veterinary Medicine Vienna in 2005 and also found that no significant differences in alkaline phosphatase activity in serum between medically ($n = 40$) and surgically ($n = 38$) treated horses with colic. Moreover, our study is in agreement with two studies (SAULEZ et al., 2004; FROSCHER and NAGODE, 1981) in finding ALP$_i$ activity in serum, was not useful for probability of surgery and prognosis. SAULEZ et al. (2004) found that neither serum ALP$_i$ nor ALP$_j$ activity was useful in classifying type or severity of intestinal damage, but higher ALP$_i$ and ALP$_j$ activities in peritoneal fluid were associated with greater intestinal damage, increased probability of surgery and a worse prognosis. Another study (MILNE et al., 1990) revealed that surgical cases were unique in having bloodstained peritoneal fluid with a high alkaline phosphatase activity. From these, it seems that if analysis for ALP isoenzymes can be of diagnostic use in acute abdominal disease of the horse, it will probably result from tests on peritoneal fluid rather than on serum, because the ALP isoenzyme most responsible for the observed increase in peritoneal fluid is granulocytic in origin (FROSCHER and NAGODE, 1981). These may be the explanatory factors considering absence association for the diagnostic and prognostic relationships of alkaline phosphatase activity in serum in our study. Furthermore, unlike the other studies, the change in value of alkaline phosphatase activity in serum on the day of arrival in comparison to after 3 days hospitalization was examined, but no significant difference was detected.

ALP activity decreased with age, especially during the first year of life, according to a study of LEPAGE et al. (1990), because during the first year of life the bone isoenzyme fraction slowly disappears and in normal cases only liver ALP could be detected after the first year of life (THOREN-TOLLING, 1988). In our study, the data was not statistically examined for possible association between age and the level of alkaline phosphatase activity, but age may not have a strong influence on the results, because only 3 horses of the 187 examined horses were less than 1 year old.
6. SUMMARY

In order to identify important factors that influence the development of colic, the investigation of many parameters is essential. The aim of this study was to determine risk factors for colic in Austria. Therefore, nutritional factors such as hygienic quality and its nutritional value, individual factors, environment and management factors were studied. Furthermore, the predictive value of the serum alkaline phosphate activity, serum bile acids levels and concentrations of biogenic amines (serotonin, tryptamine, tyramine, putrescine, cadaverine, histamine, spermidine, spermine dopamine) in both feces and blood and fecal pH were studied. Finally the presence of *Clostridium perfringens*, *Clostridium difficile*, and *Salmonella spp* was studied as factor for developing colic.

A hospital-based study was conducted in this study, and 2743 horses arrived at the University of Veterinary Medicine Vienna during the 1 year study period. Of these horses, 366 had colic. A questionnaire was developed for the supplemental data, which were not valid in the hospital electronic data (TIS). A total of 221 feed samples, which consisted of 155 samples from 51 colic horses and 66 samples from 26 non-colic horses were examined by a sensory evaluation for both hygienic quality of the food and quantity of nutrients. Feces and blood samples were taken from colic horses on the day of admission (first sample) and three days after admission (second sample); thereby 177 first feces samples, 70 second feces samples, 187 first blood samples and 26 second blood samples were collected. In addition, 34 feces samples were collected from non-colic horses.

All of the feces samples were examined for *C. perfringens*, and all of the feces samples except half of the second feces samples were examined for *C. difficile*. 93 first feces samples from colic horses and all feces samples from non-colic horses were investigated for *Salmonella spp*. 177 first feces samples, 67 second feces samples and 34 control feces samples were examined for fecal pH. Biogenic amines in feces and blood were investigated by rapid high performance liquid chromatography. The SPSS 14.0 (Statistical Package for the Social Sciences) was used to perform statistical analysis. For all statistical tests, a value of \( p < 0.05 \) was considered significant.

Considering feeding and management related factors the following factors were significantly associated with colic: decreased water intake, feeding with poor hygienic quality of hay, feeding high amounts of concentrate. Although poor hygienic quality of hay increased risk of colic, remarkably, the hygienic quality of straw or any particular type of concentrate was not significantly associated with colic. The association between the level of feed contamination with yeast, mold or bacteria and occurrence of colic was not tested in this study. Qualitative microbiological investigation was performed to confirm yeast, mold and/or bacteria in presumptive feedstuffs. Colic horses' total concentrate intakes \((2.74 \pm 2.09 \text{ kg})\) were significantly higher than the non-colic horses' total concentrate intakes \((1.64 \pm 1.16 \text{ kg})\). Thereby, high total concentrate intake was significantly associated with increased risk of colic. Moreover, considering environmental factors, high temperature on the day of arrival at the clinic was found to be risk factor related to colic, which seems logic since colic prevalence was highly in the summer period.

In regard to selected bacteria cultured from feces: *C. perfringens* was identified in 15.2 % of first feces samples, 2.85 % of the second feces samples of horses with colic and in 8.8 % of the feces samples from non-colic horses. *C. difficile* and *Salmonella spp*. were identified in none of the investigated feces samples. Fecal pH of the second samples \((7.02 \pm 0.71)\) was significantly lower than that of the first samples \((7.12 \pm 0.56)\).
Considering biogenic amines, fecal tryptamine levels were significantly higher for non-colic horses ($2.72 \pm 4.10$) than colic horses ($0.21 \pm 1.53$). Cadaverine and putrescine were identified in the fecal samples from all horses with colic and without colic. None of the amines were likely absorbed from the gut, or at least did not enter the systemic circulation since they were not identified in blood samples.

Both serum alkaline phosphatase activity and serum bile acids concentrations were not significantly different between medically treated horses and surgically treated horses or between survived horses and non-survived horses.

The result of this study is that consuming high hygienic quality of feed and lower amount of concentrate feed intake can decrease the risk of occurrence of colic in horses.
7. ZUSAMMENFASSUNG


In 15,2 % der untersuchten Kotproben vom 1. Tag und in 2,85 % der Kotproben, die am 3. Tag von den Kolikpatienten gesammelt wurden sowie in 8,8 % der Kotproben der Kontrollgruppe wurden C. perfringens nachgewiesen. C. difficile oder Salmonella spp. wurden in keiner der untersuchten Kotproben gefunden. Der pH-Wert des Kotes vom 3. Tag war signifikant niedriger (7,02 ± 0,71) als jener der Kotproben des ersten Tages (7,12 ± 0,56), allerdings sind die Unterschiede sehr gering.

Der Tryptamin gehalt im Kot von nicht an Kolik erkrankten Pferden war signifikant niedriger (0,21 ± 1,53) als im Kot der Kolikpatienten (2,72 ± 4,10). Cadaverin und Putrescin wurde in allen Kotproben gefunden. In den Blutproben wurden keine der untersuchten biogenen Aminen nachgewiesen. Die Aktivität der alkalischen Phosphatase und der Gehalt an Gallensäuren im Serum waren weder zwischen den chirurgisch und konservativ behandelten Kolikfällen noch zwischen den überlebenden und nicht überlebenden Tieren unterschiedlich.
Die Ergebnisse dieser Studie zeigen, dass eine hygienisch einwandfreie Qualität des Futters sowie eine artgerechte Fütterung mit nicht zu hohen Kraftfuttermengen das Kolikrisiko vermindern können.
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8. APPENDIX

8.1. Questionnaire

Name des Patienten (Patients name):

<table>
<thead>
<tr>
<th>UNTERSUCHUNG VON KOLIKURSACHEN</th>
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<tbody>
<tr>
<td>(INVESTIGATION OF COLIC CAUSES)</td>
</tr>
</tbody>
</table>

Sehr geehrte/r Patientenbesitzer/in, für eine Studie zur Untersuchung von Kolikursachen bitten wir um Ihre Mithilfe.

Erstens bitten wir Sie, uns den ausgefüllten Fragebogen zurückzugeben.
Zweitens bitten wir Sie, Futter (Heu, Silage, auch Stroheinstreu, Kraftfutter) zu bringen. Dieses wird GRATIS einer grobsinnlichen Beurteilung unterzogen, in fraglichen Fällen folgt eine mikrobiologische Untersuchung (Pilz- und Bakterienkeimzahlen)!!!!!!

Den Befund senden wir Ihnen selbstverständlich gerne zu (bitte Adresse angeben).

(Dear patient owner, we need your help about investigation of colic causes study. First, please fill out the questionary. Second, please bring some fees samples (Hay, Silage, Straw, Concentrated Feed). Feed samples will examine by microbiological investigation (Yeast-Mold and Total Microbial Content) and that is FREE. The result will be send to your address which is standing below.)

Name und Adresse zur Befundübermittlung (Name and Address for Delivery):

FRAGEBOGEN zu Futter/Fütterung (Questionnaire for Feed/Feeding):

<table>
<thead>
<tr>
<th>Hay (Heu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjektive Beurteilung (Subjective Evaluation):</td>
</tr>
<tr>
<td>sehr gut (very good)</td>
</tr>
<tr>
<td>Menge/Tag (Amount/day): ____ kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentrated Feed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menge/Tag (Amount/day): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Menge/Mahlzeit (Amount/meal): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Art (Others):</td>
</tr>
<tr>
<td>Hafer (Oat): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Quetschhafer (Squeezed Oat): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Gerste (Bran): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Mais (Corn): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Müsli (Cereals): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Pellets (Pellets): ____ kg oder (or) ____ Liter</td>
</tr>
<tr>
<td>Trockenschnitzel: wie viele Stunden vor der Fütterung wurde eingeweicht? (Dry beet pulp:</td>
</tr>
</tbody>
</table>
**Hat ein Futterwechsel stattgefunden (Any feed changes):**
- nein (No)
- ja (Yes)

Wenn ja (If yes): wann (when) ________

Welche Änderungen wurden vorgenommen? (Which feed has been started?)
- neue Heulieferung (New hay)
- neue Kraftfutterlieferung (New Concentrated Feed)

**Andere (Others):**

**Wurde im Stroh (Einstreu), im Heu oder im Kraftfutter Schimmelbefall bemerkt?**
(Have you noticed a mold sign in Straw, Hay or Concentrated Feed?)
- nein (No)
- ja (Yes)

Wenn ja (If yes): :
In welchem Futter (In which feed):
Ausmaß des Schimmelbefalls nach Ihrer Meinung (Do you think, how much mold it includes?):

**Ist das Pferd auf der Weide (Is horse at the pasture?):**
- stundenweise (by hours)
- ganztags (full time)
- auch nachts (also at night)

**Wasserversorgung mit (Water provision):**
- Selbsttränke (automatic waterer)
- Eimertränke (bucket) ___ mal / Tag (times / day)

**Haben Sie bemerkt, dass das Pferd fremde Materialien aufgenommen hat?**
(Is your horse consumed any foreign material?)
- nein (No)
- ja (Yes)

Wenn ja, was (If yes, what):
- Erde (Soil), Sand (Sand), Holz (Wood), Späne aus der Einstreu (shavings litter),
### Haltung des Pferdes (Horse Stable Provision):
- Box ohne Auslauf (*Stable without paddock*)
- Box mit Paddock, Einzelhaltung (*Stable with paddock, private paddock*)
- Box und stundenweise Weidehaltung in der Gruppe (*Stable and paddock in a group by hours*)

Sonstiges (Others): :

### Wurden die Aufstallungs-/Haltungsbedingungen im letzten Monat geändert?
(*Have stable provision or individual staff changed in last months?*)

- nein (No)
- ja (Yes)

Wenn ja beschreiben Sie bitte, was sich geändert hat:
(*if yes, please write what is changed*)

### Das Pferd ist ein (This horse is a):
- Hobbyreitpferd, Freizeitpferd (*Hobby horse*)
- Vielseitigkeitspferd (??????)
- Springpferd (*Jumper*)
- Dressurpferd (*Trainer*)
- Fiakerpferd (*Worker*)

Gesamt ____ Stunden/Tag im Einsatz (*Length of activities a day*)

Sonstiges (Others): :

---

Bitte rücksenden an oder beim Portier hinterlegen für:
(*Please send it here or to doorman for*)

Prof. Christine Iben
Diss. Gülsah Kaya
Institut für Ernährung, Veterinärplatz 1, 1210 Wien
Tel. 01 25077 3213
Fax: 01 25077 3290
8.2. Report to owners

Institut für Ernährung
Department für öffentliches Gesundheitswesen in der Veterinärmedizin
Veterinarmedizinische Universität Wien

Prof. Christine Iben, Diss. Gülsah Kaya
Veterinärplatz 1, A-1210 Wien, Austria
www.vu-wien.ac.at/i124

Tel. ++43 1 25077 3213
Fax: ++43 1 25077 3290

Herr/Frau (Mr/Ms)........... Datum (Date):

Ergebnis der Futtermitteluntersuchung (Report considering feed investigation)

Name und Nummer des Patienten (Name and number of the patients): ____________

Art des Futtermittels (Type of feed): ________________

Beurteilung des Nährstoffgehalts (Quantity of nutrients in feed):

- Sehr gut (Very good- good)
- Befriedigend (Satisfactory)
- Mäßig (Moderate)
- *Sehr gering (Poor)

Beurteilung des hygienischen Status (Hygienic quality of feed):

- Einwandfrei (Good)
- Leichte Mängel (Slightly good)
- Deutliche Mängel (not good)
- *Massive Mängel (poor)

*Ergebnis der mikrobiologischen Untersuchung (Results of microbiological investigation):-

Zusammenfassung (Comments):

__________________________________________________
### 8.3. Tables

#### Table 26 Breed categorization

<table>
<thead>
<tr>
<th>Categorization</th>
<th>Breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold-blood</td>
<td>Abtenauer, Friesian, Haflinger, Haflinger-mix, Cold-blood, Noric, Quarter horse, Shire horse</td>
</tr>
<tr>
<td>Cold-blood / Thoroughbred</td>
<td>Arabohaflinger, Haflo-Araber</td>
</tr>
<tr>
<td>Cold-blood / Warm-blood</td>
<td>Haflinger- Warmblood mix, Quarter-Paint-Mix, Quarter-Tinker-Mix</td>
</tr>
<tr>
<td>Others</td>
<td>Dutch breed, Mongolíšches Horse,</td>
</tr>
<tr>
<td>Pony</td>
<td>Argentinisches Polo-Pony, Connemara Pony, Deutsches Reitpony, Freiberger, Pony</td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>Achal tekkiner, American miniature horse, Berber, British miniature horse Anglo-Araber, Araber, Partbred Araber, Shagya arabic, Gidran, Trotter horse, Thoroughbred, Thoroughbred Arabic, Thoroughbred Britisch, Thoroughbred French</td>
</tr>
<tr>
<td>Thoroughbred / Thoroughbred / Cold-blood</td>
<td>Araber-Berber-Haflinger</td>
</tr>
</tbody>
</table>

Cold-blood / Thoroughbred, Cold-blood / Warm-blood, Thoroughbred / Thoroughbred / Cold-blood were categorized as mixed breed
Table 27 Duty of the horse categorization

<table>
<thead>
<tr>
<th>Categorize</th>
<th>Duty of the horse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding horse</td>
<td>Breeding horse</td>
</tr>
<tr>
<td>Hobby horse</td>
<td>Free time horse, Walking horse, Charity horse, Retired horse, Show horse, Free time horse,</td>
</tr>
<tr>
<td>Others</td>
<td>Exercise horse, others</td>
</tr>
<tr>
<td>Sport horse</td>
<td>Galloper, Riding horse, Jumper horse, Trotting horse, Variety horse, Vaulting horse,</td>
</tr>
<tr>
<td>Working horse</td>
<td>Training horse, Cab horse, Horse for production, Sumter, Therapy horse, Carriage horse</td>
</tr>
<tr>
<td>Young horse</td>
<td>Foal, Young horse</td>
</tr>
</tbody>
</table>

8.4. Pictures

Picture 1. Identification of Yeast and Mold Colonies on the RBC Agar

Picture 2. Bacteria from the total plate count Petri dishes
Picture 3. Presumptive *Clostridium perfringens* colonies on TSC Agar

Picture 4. API20A Strip

Picture 5. Presumptive *Clostridium difficile* colonies on the C.difficile Agar
Picture 6. Escherichia coli colonies on BPLS Agar (adapted from JERGENSEN et al., 2000)

Picture 7. Salmonella spp. colonies on BPLS Agar (adapted from JERGENSEN et al., 2000)

Picture 8. Poisonous plant, Colchicum autumnale in hay
8. 5. Figures

**August**

Figure 5 Daily temperature and prevalence of colic cases in August 2006

**September**

Figure 6 Daily temperature and prevalence of colic cases in September 2006

**October**

Figure 7 Daily temperature and prevalence of colic cases in October 2006
Figure 8 Daily temperature and prevalence of colic cases in November 2006

Figure 9 Daily temperature and prevalence of colic cases in December 2006

Figure 10 Daily temperature and prevalence of colic cases in January 2007
Figure 11 Daily temperature and prevalence of colic cases in February 2007

Figure 12 Daily temperature and prevalence of colic cases in March 2007

Figure 13 Daily temperature and prevalence of colic cases in April 2007
Figure 14 Daily temperature and prevalence of colic cases in May 2007

Figure 15 Daily temperature and prevalence of colic cases in June 2007

Figure 16 Daily temperature and prevalence of colic cases in February 2007
Figure 17 Daily barometric pressure and prevalence of colic cases in August 2006

Figure 18 Daily barometric pressure and prevalence of colic cases in September 2006

Figure 19 Daily barometric pressure and prevalence of colic cases in October 2006
Figure 20 Daily barometric pressure and prevalence of colic cases in November 2006

Figure 21 Daily barometric pressure and prevalence of colic cases in December 2006

Figure 22 Daily barometric pressure and prevalence of colic cases in January 2007
Figure 23 Daily barometric pressure and prevalence of colic cases in February 2007

Figure 24 Daily barometric pressure and prevalence of colic cases in March 2007

Figure 25 Daily barometric pressure and prevalence of colic cases in April 2007
Figure 26 Daily barometric pressure and prevalence of colic cases in May 2007

Figure 27 Daily barometric pressure and prevalence of colic cases in June 2007

Figure 28 Daily barometric pressure and prevalence of colic cases in July 2007