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Prevalence of Equine Gastrointestinal Ulcers in Freely Roaming Przewalski Horses in Hungary

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List of Abbreviations

HCl: Hydrochloric acid

EGGD: Equine Glandular Gastric Disease

EGUS: Equine Gastric Ulcer Syndrome

ESGD: Equine Squamous Gastric Disease

GIT: Gastrointestinal Tract

NSAIDS: Non-Steroidal Anti-inflammatory Drugs

PPIS: Proton Pump Inhibitors

VFA: Volatile Fatty Acids

Chapter 1

Introduction

Peptic ulcers are open sores located in the esophagus, stomach, and duodenum. Ulcers that occur in the stomach are usually identified as gastric ulcers and in the case of horses we refer to them as Equine Gastric Ulcer Syndrome (EGUS). The stomach is divided into 2 distinct parts separated by a margo plicatus, the lower glandular part that makes up two thirds of the stomach, and an upper non-glandular part, making up the remaining third of the stomach, that is less protected. If the lesion occurs in the non-glandular part and more specifically on the lesser curvature of the stomach is also referred to as Equine Squamous Gastric Disease (ESGD), while if the lesion occurs in the glandular region we refer to it as Equine Glandular Gastric Disease (EGGD) (Sykes et al., 2015).

Gastric Ulcers are common in horses and in foals, and according to researchers 60% of performing horses develop EGUS while this number increases to over 90% in racing horses and is less than 60% in freely roaming horses. Even foals are said to have a 50% chance to develop EGUS until they are several weeks old because of an underdeveloped gastric mucosa, and out of these numbers between 4-10% of ulcers heal naturally however medical treatment is required especially in cases where the horses are maintained the same environment that has caused EGUS (Bell et al., 2007).

The Equus Przewalski is of Asian origin and is the only extant wild horse. This breed of horse went extinct in the wild in 1960s. Since the 1980s, several captivity programs worked on trying to rebreed the Przewalski horses by bringing horses form some zoos in China. Since then, the Przewalski population has increased, and the horses are only kept in reserves where they can roam freely without any constraints however still under supervision and observation (Ryder, 1993). Many studies around the world are currently being done on this breed of horse because scientists are interested in their behavior and their lifestyle, and how that affects their internal condition, for instance since they are a free roaming breed they are constantly grazing, how would that have an effect on the stomach? Will they develop EGUS? If so, is it in a specific location in the stomach? As well as many more questions can be asked, and only by studying them and observing them can we fully understand the way this breed functions and get answers to our questions.

Chapter 2

Review Of The Literature

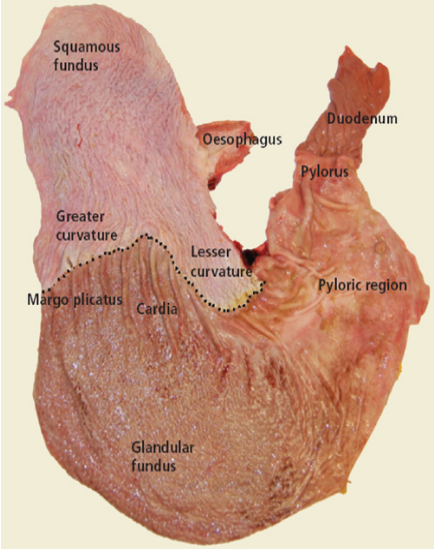
2.1 Gastric Ulcers in Horses

2.1.1 Anatomy and Histopathology of the Equine Stomach

Unlike other animals, horses have a smaller stomach, with a capacity of 5-15 liters, compared to the stomach of other species. The stomach is split into 4 parts: cardia, large fundus, body, and a pylorus. The fundus and part of the body belong to the non-glandular part of the stomach while the rest belong to the glandular part. The stomach consists of 4 layers: tunica mucosa, tunica submucosa, tunica muscularis which is also divided into lamina propria, lamina epithelialis, lamina muscularis, and the last layer of the stomach is tunica serosa (Konig amd Liebich, 2009). The non-glandular part is lined by stratified squamous epithelium, while the glandular part has a folded mucosa and is lined by simple columnar epithelium that secretes the mucus. We can further divide the glandular part into cardia, proper (fundic), and pyloric regions (Bacha and Bacha, 2012).

In the fundic region we can find parietal and chief cells that secrete HCl and pepsinogen respectively, as well as other hormone secreting cells. In the pyloric region, we can find gastrin and somatostatin secreting cells (Bacha and Bacha, 2012)

Image 1: A picture illustrating the anatomical parts of the stomach



Picture taken from: Equine Gastric Ulcer Syndrome (EGUS), The Atlanta Equine Clinic Strides Ahead, (Online)
http://www.atlantaequine.com/pages/client_lib_EGUS.html. Web. 27 January 2015.

2.1.2 Ulceration of the Gastrointestinal Tract (GIT)

Gastric ulcers in both foals and adults are commonly found in the non-glandular part of the stomach and in severe cases they can spread to the glandular part or even into the esophagus and duodenum (Nieto, 2012).

The small stomach of horses makes it difficult for them to handle large amount of feed therefore they keep on grazing constantly especially in free roaming horses. This is why they constantly secrete acid at a steady flow to help with digestion. The amount of acid secreted can add up to thirty four liters per day even when the horse is not eating, and that would lower the stomach pH leading to the damage of the stomach mucosa. The glandular part has the lowest pH because of its secretory glands and the pH can drop to 1.4-4. However, foals have a uniform pH since they feed on milk (Nieto, 2012).

The upper, non glandular, part of the stomach is more prone for gastric ulcers, not just because it is less protected from the acid, but also because this part of the stomach is where

mixing of stomach content takes place. In addition, since the stomach is constantly producing acid, when the animal stops eating, the squamous mucosa is exposed to acid several hours leading to the erosion of the lining of this region (Sykes et al., 2015).

According to several studies done on the methods and scoring of EGUS, around 90% of these lesions are found in the non-glandular (esophageal part) of the stomach and more specifically on the lesser curvature of the stomach, also referred to as ESGD, and this is because of the low pH of the fluid found in the glandular region of the stomach as well as the increasing pressure in the abdomen forcing the gastric content upwards leading to inflammation and erosions in the non-glandular squamous mucosa. However in some cases, gastric ulcers can develop in the glandular region of the stomach and we refer to it as EGGD (Sykes et al., 2015).

2.1.3 Protective and Offending Factors

In order to prevent EGUS from developing or to help in healing, in "Equine Gastric Ulcer Syndrome", it is stated how the body itself has several mechanisms, one of which is the mucosal blood flow, which plays an important role in the glandular part; it provides oxygen and nutrients to the cells to help remove excess hydrogen ions (Andrews, 2003). The mucosal glands, due to the increase in acidic concentration in the stomach or due to mechanical irritation, secrete the bicarbonate. Prostaglandin is also a defense mechanism because it inhibits acid secretion and increases mucosal perfusion. Opposite to the glandular part that is well protected from acid effect, the non-glandular part is more vulnerable to low pH. This part is mainly provided by horse's saliva, which is produced during constant chewing, is alkaline, and provides a chemical buffer. There is also the intercellular tight junction in the squamous mucosa that acts as a barrier to prevent EGUS (Table 1).

Table 1: Physiologic Factors affecting Ulcer development (Buchanan and Andrews).

Aggressive Factors	**Protective factors of non-glandular region**	**Protective Factors of glandular region**
HCL secretion	Epithelial restitution	Bicarbonate and mucus layer secretion
Organic acid production	Mucosal blood flow	Epithelial restitution
Pepsin conversion from pepsinogen		Mucosal blood flow
Duodenal reflux of bile acids		Prostaglandin E production

2.1.4 Causes of EGUS

Many factors help in the development of EGUS and are divided into two major groups, the first group is the *intrinsic factors* like hydrochloric acid, Volatile Fatty Acids (VFAs), and lactic acid, and the second group is the *extrinsic factors* also known as risk factors such as exercise, housing and transport, diet and feeding, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), temperament, stress, and infective agents (Andrews et al., 1999).

Andrews (2003) explains about VFAs and their effect on the stomach. The three most important VFAs are acetate, propionate, and butyrate. VFAs are known for their high lipid solubility, so at low pH these VFAs can easily penetrate the squamous mucosal cell where they cause acidification and inflammation, which then leads to ulceration. Therefore a diet high in VFAs led to a lower stomach pH (lower than 4) affecting the sodium transport chain and led to the disruption of the mucosal protective factors hence affecting the mucosal lining. Also bile acids, from duodenal reflux and pepsin, are said to cause gastric ulcers. In combination with pepsin, bile acids act to increase the permeability of the esophageal mucosa to hydrogen ions.

Nutrition of horses also plays a really important role in developing EGUS. Horses with access to some turnout were less likely to develop ESGD, which even decreased even

more if these horses were turned out with other horses irrespective of the pasture quality or time at pasture (Lester et al., 2008). In addition, free access to fibrous feed or frequent grazing seemed to reduce the risk of developing EGUS, although strong evidence supporting this belief is also lacking (Sykes et. al., 2015). For instance, feeding alfalfa hay to exercising horses reduces the severity of gastric squamous ulceration (Lybbert et. Al, 2007). An alfalfa hay grain diet may buffer stomach acid in horses. ESGD developed in all horses within 14 days of their removal from pasture, stabling (fed 6 kg concentrate feed/day) and entering a stimulated training regimen (Vatistas et al, 1999). On the other hand there were no differences observed on intragastric pH in horses fed ad libitum grass hay and grain twice a day (1kg/100kg/day) when they were housed in a grass paddock, in a stall on their own or in a stall with adjacent companion, suggesting that pasture turnout on its own might not affect gastric pH per se (Husted et. al, 2008). Intermittent access to water increases the chances to develop EGUS in all parts of the stomach. However, these studies showed that despite the absence of other risk factors, reduction of EGUS might not be as great as previously believed (Sykes et al., 2015).

As stated in the paper: “Gastric Ulcers in Foals and Adult Horses”, the prevalence of EGUS increases with increase workload and duration that is why racehorses are most often affected especially as they get closer to the competition (Andrews, 2002). In addition, the chance of developing EGUS increases when horses have lesions in their bowls, liver, or esophagus.

Transportation of horses also has a negative effect on them especially if the transport period is long. Transportation can result in dehydration and increase chances of respiratory diseases such as pneumonia. In addition water and feed consumption of the horse decreases and therefore can lead to ESGD (Buchanan and Andrews, 2003).

Ulcers can also develop in the glandular part of the stomach but the possibility of that developing is very little compared to ulcers in the non-glandular portion of the stomach. These ulcers develop due to stress that should be taken into consideration when treating gastric ulcers and that explains the increase in percentage in gastric ulcers in racehorses. Some studies done explain that this because of the low velocity muscular contractions of the stomach with decreased mucosal capillary blood flow resulting in acid injury. Additionally,

because of exercise, there is a delay in gastric emptying and/or increasing gastric acid secretion, which lead to gastric ulcerations. Another factor is the use of NSAIDs which, secondary to reduced prostaglandin secretion, can lead to ulcerations in both portions of the stomach, including: decreased mucous secretion, increased acid secretion from the glands leading to inflammation and erosions as well (Murray, 1994). Intermittent access to water can also be a cause of ulcers in all parts of the stomach. To most of these clinical signs it is could be unclear whether EGUS itself caused these signs or if they are symptoms of other diseases, that is why it is always important to perform other diagnostic tests to identify the cause of the symptoms.

Fifty percent of foals develop mild gastric ulcers within the first five months of life and mainly because of the physiologic stress caused by weaning. However these ulcers tend to heal by themselves as the foals grow and adapt to their new lifestyles (Picavet, 2002). However foals can also develop gastric ulcers because of septic diarrhea and other infections in the period when the mother's immunity starts to decrease, and in these cases treatment is required (Lewis, 2003).

Table 2: Summary of Clinical signs and Risk Factors of EGUS in Adults and foals [1]

Clinical Signs in Adults	**Clinical Signs in Foals**	**Risk Factors**
Acute colic	Diarrhea	Stress
Recurring Colic	Abdominal Pain	Transportation
Excessive recumbency	Restlessness	High-grain diet
Poor body condition	Rolling	Stall confinement
Partial anorexia	Lying in dorsal recumbancy	Intermittent feeding
Poor appetite	Hyper salivation	Intense exercise
Poor performance	Bruxism	Racing
Attitude Changes	Intermittent nursing	Illness
Stretching often to urinate	Poor appetite	NSAID use
Inadequate energy		Management Changing

[1] Taken from the research paper done by Benjamin Buchanan, DVM, and Frank M. Andrews, DVM, MS.

2.1.5 Clinical Signs of EGUS

It is hard to diagnose gastric ulcers in foals because they do not show any clinical signs at the beginning, and only when the ulcers are severe and widespread the foals start showing signs such as diarrhea, poor nursing, dorsal recumbency, ptyalism, and grinding of teeth however these signs are not specific to EGUS only. As for adult horses, non-specific signs can observed: abdominal discomfort, colic, poor appetite, weight loss, poor body condition, poor coat condition, poor performance and behavioral changes (Table 2). In addition, it is important to keep in mind that it is possible for horses with EGUS not to show any clinical signs, and are considered to have “silent” or “non-clinical gastric ulceration” (Nieto, 2012).

2.1.6 Diagnostic Methods

Diagnosis of EGUS is often based on history, response to treatment, clinical signs observed, however, they are not specific to EGUS but rather common with other diseases as well. The most effective diagnostic method is gastroscopy, but to do so it is referable of the horse has been fasting for at least 12 hours and no water should be given to him for at least 4 hours and the horse should be sedated to minimize the stress with a short acting tranquilizer (Nieto, 2012).

Assessment of the severity of the lesions is done by giving a grade that describes the appearance of the mucosa, the most common form of the grading is the 0-4 grading system which is given according to the severity, depth, size and number of ulcers. The table below will show the grading system recommended by the EGUS council.

Table 3: Grading system of gastric ulcers in non glandular part	
Grade	
0	the mucosa is intact, no reddening
I	the mucosa is intact but there are reddened areas
II	small single, or multifocal ulcers
III	large single, or multifocal ulcers
IV	multiple lesions that might merge forming deep ulcerations

Table 4: Another grading system which is more commonly used by Picavet

Lesion number score	**Description**
0	No lesions
1	1-2 localized lesions
2	3-5 localized lesions
3	6-10 lesions
4	>10 lesions or diffused lesions
Lesion severity score	**Description**
0	No lesion
1	Appears superficial (only mucosa missing)
2	Deeper structures involved
3	Multiple lesions and variable severity
4	Same as 2 and has active appearance
5	Same as 4 plus hemorrhagic or adherent blood clot.

2.1.7 Treatment of EGUS

Treatment for EGUS is important since only 4-10% of gastric ulcers heal naturally. Many articles talk about treatments including the one written by Nieto (2012), and they all state that the treatment could either be management modification on which we decrease stress, change in feed, ventilation, or medical therapy were we give drugs to control gastric production or mucosal protectant. These drugs could either be H2-receptor antagonists that elevate gastric pH treating ulcers in both adults and foals or Proton Pump Inhibitors (PPIs) (omeprazole) that irreversibly bind to H+/K+ ATPase proton pump of parietal cells and block the secretion of hydrogen ions. Mucosal protectants such as sucralfate that stimulates mucus secretion and inhibits pepsin could also be used. It is also very important to treat the signs as well, and to keep in mind that it is not recommended to treat horses with EGUS continuously because lifelong treatment is costly and another important thing is that the acid is found in the stomach for a reason and that completely eliminating the acid secretion in the stomach will also have a negative effect on the stomach and food digestion, not to mention the effect it will have on the normal microbial flora of the GIT and the disturbance in normal ion concentration. (Nieto, 2012).

Misoprostol is a synthetic prostaglandin E1 analogue used in the treatment of EGUS, and more precisely EGGD. It acts on increasing mucosal blood flow, increasing bicarbonate secretion, suppressing acid secretion, and increasing mucosal restitution. However, it also acts on the uterus by increasing uterine contractions and that is why it is contraindicated in pregnant horses (Buchanan and Andrews, 2003).

Out of all the above-mentioned drugs, omeprazole is the only drug shown to allow gastric to heal consistently (Nieto, 2012).

2.1.8 Prevention of EGUS

In cases of gastric ulcers, prevention is always preferable over treatment. You can do so by trying to minimize stress as much as possible, and also by watching out for their diet by making sure they get good quality hay and that not too high amounts of concentrates are fed to the horse especially in horses that are prone to EGUS in other words, the timing and feed provided to the horses should mimic that of a free roaming horse which enables a continuously higher pH in the stomach. As well as avoid or decrease the usage of anti-inflammatory drugs (Nieto, 2012).

2.2 Przewalski Breed

Not only does the Przewalski breed differ from domestic horses by appearance but also genetically, this wild horse has 66 chromosomes and has a limited coat color variation, states Oliver Ryder in his paper on the prospects for reintroduction of Przewalski horses into the wild. As for the appearance the Przewalski has an erect mane, lacks a forelock, and has a tail with no long hairs. They have shorter legs than domesticated horses and have a length of approximately 2 meters and weigh around 300 kg (Ryder, 1993).

These horses are not solitary but rather juvenile male horses form bachelor herds when they are separated from their original herd (Boyd and Houpt, 1994). The oldest and most dominant of the male horses is the first to acquire a mare. In general the males don't obtain any female until they are 4 years of age unlike the female who is 1 or 2 years of age and that's is why stallions are dominant over their mares. The normal band consists of one stallion and his harem, which includes 3-5 mares, while a bachelor group consists of only males. If the harem is larger than 5 mares then the stallion is at risk of becoming apathetic or aggressive towards the mares (Boyd, 1991).

Image 2: A herd of Przewalski breed at Hortobagy

Although this breed was said to go extinct, now because of breeding programs and captivities, where cooperative breeding programs are applied between different countries and captivities to prevent inbreeding, research shows that the yearly population growth is 9%. Their reproduction period is the same as domestic horses, about 11-12 months, and it is a seasonal process (Ryder, 1993).

As for their diet, one of the studies showed that it consists mostly of vegetation. Even though they eat several different species of plant they tend to prefer one species depending on the season they are in for example in spring time, they prefer Elymus repens and festuca valesiaca (Slivinska and Kopij, 2011). Some studies done on this breed showed that during winter they experience hypodermis due to slower metabolism compared to other times of the year. Like any other specie or breed, water is and it is documented that Przewalski horses consume around 8.6 liters of water on a daily basis.

Chapter 3

Hypothesis and Objectives

3.1 Hypothesis

Before conducting the experiment, we expect not to have any EGUS symptoms or pathological alterations of EGUS in the Przewalski breed unlike domesticated horses. The reason for our hypothesis was that in domesticated horses development of ulcer is usually related to feeding and stabling management and the stress that is caused by the surrounding. These stressors include transportation, preventing continuous grazing like in wild horses, competition, heavy exercise, and other human and animal interactions related to stabling. Being kept in the stall for a long period, also increases the stress level of the horse because of the decrease in opportunities to self-exercise as well as limit the grazing period. In addition, use of NSAIDs is not a scenario in wild horses so it cannot be a risk factor in this breed but commonly used in show, pleasure, and racehorses to treat different types of inflammatory processes.

Since the Przewalski horses are kept in the wild, they are freely roaming, they continuously graze which is beneficial in EGUS prevention since the acidic content in the stomach is being diluted by the basic pH entering the stomach from the saliva due to the continuous chewing, and unless there are any problems within the herd due to certain cases, the stress levels should somewhat be low and therefore rarely have horses who develop ulcers.

Our hypothesis was that EGUS is a disease of civilization and only affects domesticated equidae.

3.2 Objective

The purpose of this study is to determine the prevalence of gastric ulcers in wild horses. Specific objectives for the conduct of this study included:

1. To examine of the stomach.
2. To find out whether EGUS is common in Przewalski breed, in other words is it common in wild horses?
3. To identify the exact location of the ulcers in affected horses.
4. To identify whether EGGD or ESGD is more prevalent in freely roaming horses.
5. To grade the ulcers found if any.
6. To possibly identify the risk factors of EGUS in Przewalski breed.

Chapter 4

Materials and Method

This study is an observational cross sectional study, which is a type of observational study that involves the analysis of data, collected from a population at a specific time. The study was done on a two days period (October 2-3, 2014) along with other research groups of departments of the university and from Australia and Germany conducting different studies on this special breed.

The whole experiment took two days, the first day 7 horses were shot and dissected and at the end of the day our observations were recorded, and the next day 4 other horses were shot and dissected and also our observations were recorded.

In the Hortobagy reserve, the current population of Przewalski breed is 309 horses divided into several herds all kept in the wild freely roaming the reserve, and they are still expecting 20 new foals. The average age of the horses on the reserve is relatively young as the population is growing very fast and they have more and more foals every year. At the moment the average age in the population is 5 years.

The horses fed on plants, mainly grasses which they can find in the area, no additional feedings like hay, silage or grains is given to them. The Przewalski horses have seasonal food preferences, meaning they tend to prefer certain plants over others depending on the time of the year.

The horses were not randomly chosen for the experiment. We think it has to be stressed that the horses were shot due to their previous medical, behavioral or social conditions and not for the purpose of our EGUS prevalence study. This situation only gave us an exceptional possibility to perform a study that has never been done before. This selection could unintentionally bias our results.

Image 3: A herd of Przewalski breed at Hortobagy on the run during the shooting of selected horses for the experiment.

Data of the selected 11 horses are shown in table 5. The average age of selected horse was 5.4 years and a standard deviation 6.06 years. We have to note here that two horses showed lameness, one had tumor-like growth on the check and one horse showed poor body condition. Other horses looked healthy on observation.

This experiment required a licensed experienced shooter with a perfect aim because the herds where on the run constantly and the horses where very close to each other. The shooter was located on a separate vehicle roaming the area and following the herd at a distance as not to raise suspicion and to agitate the herd making it easier for him to mark his target and shoot without the risk of hitting another horse by mistake and only after shooting and a signal from the shooter himself were we aloud to approach the horse. The shooter aimed directly to the head of the horse on one hand not to cause any unnecessary suffering and on the other hand not to cause damage to other parts of the body which were examined by researchers doing their studies on the Przewalski breed. The stomachs of all horses stayed intact during the whole procedure of shooting and dissection.

At the site of the shooting, first we evaluated body condition according to Henneke et al. (1983). Gross-pathological examination was done within 30 minutes. The procedure of gross pathology was performed as described by Vetesi and Meszaros (1994). The stomach was dissected along its major curvature into the esophagus and along the duodenum. As the next step, washing with tap water was done to wash away the feed content in the stomach to observe any lesions through visual examination and taking photos of what we have found. The following picture shows how the horses were dissected and lesions that were found in some of the horses that were shot.

Image 4: Dissection of the Przewalski horse upon shooting

Table 5: Identification of the shot horses

ID	Gender	Passport ID	Date Of Birth	Position in the Harem	Anamnesis
1	Male	4415	23/7/2004	harem stallion	the right front leg lameness, 2014 September 5 it was injured during combat
2	Male	5454	5/10/2009	bachelor	2013 August tumor-growing formula on the right cheek
3	Male	4730	8/5/2006	harem stallion	domestic horse phenotypic characteristic appearance
4	Male	2652	30/5/1994	bachelor	From the summer of 2014, behavioral changes observed, aggression towards stallions and foals
5	Female	5211	8/8/2008	member of harem, no foal	Since the summer of 2012, a chronic problem was observed leading poor condition
6	Female	2300	5/5/1992	member of harem, no foal	lameness and bone proliferation on the left knee since summer of 2012
7	Male	3903	28/6/2003	harem stallion	surplus, 29 live offspring and in 2014 covered 10 mares
8	Male	4995	24/5/2007	harem stallion	domestic horse phenotypic characteristic appearance
9	Male	5324	20/7/2006	harem stallion	infertile, severely inbred lines of blood, typical homemade horse look
10	Female	4360	30/5/2004	member of harem, no foal	Unknown
11	Male	6073	13/8/2012	bachelor	genetically loaded stallion, infected wound in the left buttock

Chapter 5

Results

The whole procedure went smoothly, quickly, and as planned. The shootings were performed after a short period of chasing. Horses were stressed by the abnormal situation and heavy exercise of fleeing no longer than 45 minutes.

All of the horses had stomach of normal size and content. We have found ulcerations in the stomach in three horses. The 3 horses affected were all males and with the ages of 9, 12, and 3. The older 2 were harem stallions while the third belonged to a bachelor group.

The affected horses had normal body condition compared to their herd mates. Horses selected for euthanasia had body condition scores 5-7/9, while all three EGUS affected horses were given score of 6/9 on inspection. Teeth seemed to be normal for their age and for the horses living in the wild. We could observe macroscopically that they were all infected with Gastrophilus intestinalis in their stomachs and 5 of them with tapeworm (Anoplocephala perfoliata) in the ileo-caecal area. When examining these three horses, except the 7 cm wide open sore on the left gluteal are of the horse No. 11, no further specific diseases could be observed on gross pathology. The following table 6 shows which horses had gastric ulcers and the location of these ulcers.

Table 6: Results obtained from the pathological examintation.

ID	Gender	Passport ID	Date Of Birth	Position in the Harem	Location of ulcer	Grading	Severity
3	Male	4730	8/5/2006	harem stallion	Pars glandularis	4	2
7	Male	3903	28/6/2003	harem stallion	Pars glandularis	2	1
11	Male	6073	13/8/2012	bachelor	Pars glandularis	2	2

As seen in the table above, only 3 out of the 11 horses were diagnosed with EGUS all of them showing lesions in the glandular part hence being diagnosed with EGGD. None of the horses had any lesions neither in the squamous, non-glandular part of the stomach, nor in the esophagus. This meant that these horses were not affected by ESGD. As seen in the following pictures, horse No.3 showed more lesions in the glandular part giving it a lesion number score of 4 and a severity score of 2. Horse No. 7 showed lesions in the glandular part of the stomach, and was given a number score of 2 and severity score of 1 as well. Where as horse No.11 who also showed 3-5 localized lesions in the glandular part was only given a number score of 2 and severity of 2. None of the lesions were found around the pyloric are and no lesions were found in the duodenum of any of these horses. Secondary signs of delayed gastric emptying, like dilated stomach, impacted stomach or esophageal ulceration were not observable. The severity of ulcers ranged between superficial, where only mucosa was missing to some, where deeper structures were also affected and these erosions were non-bleeding. When ulcers were of different severities in the same animal, finally the higher grade was given as a general evaluation.

Image 5-10:Lesions in the Stomach of Horses 3 (images 5,6), 7 (images 7), and 11 (images 8,9).

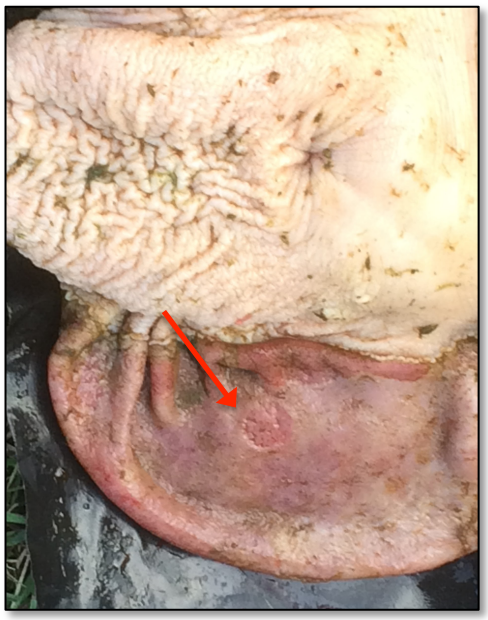


Image 5

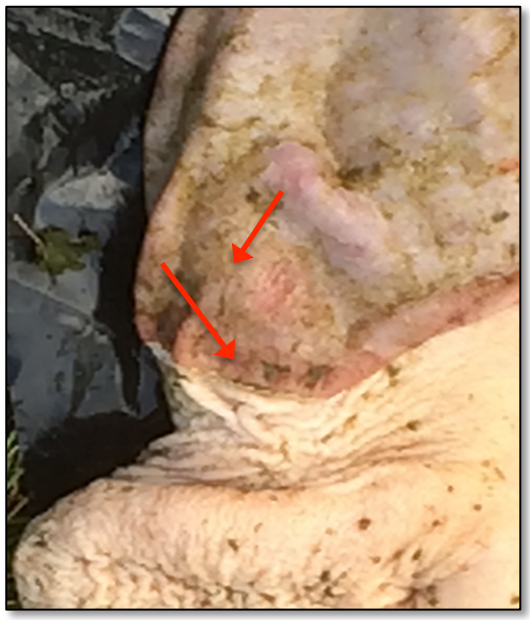


Image 6

Image 7

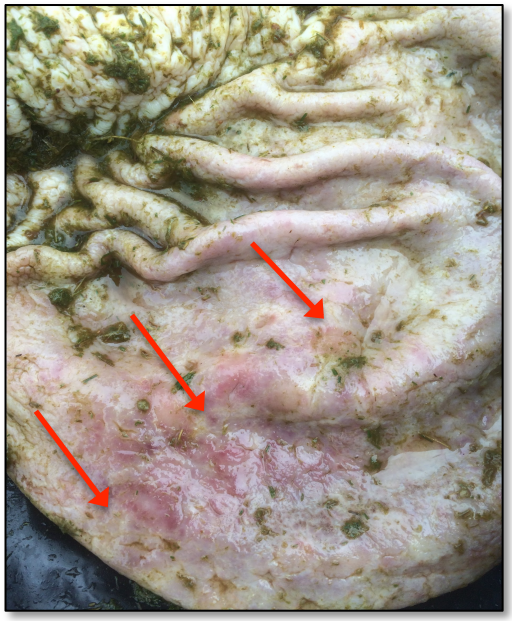


Image 8

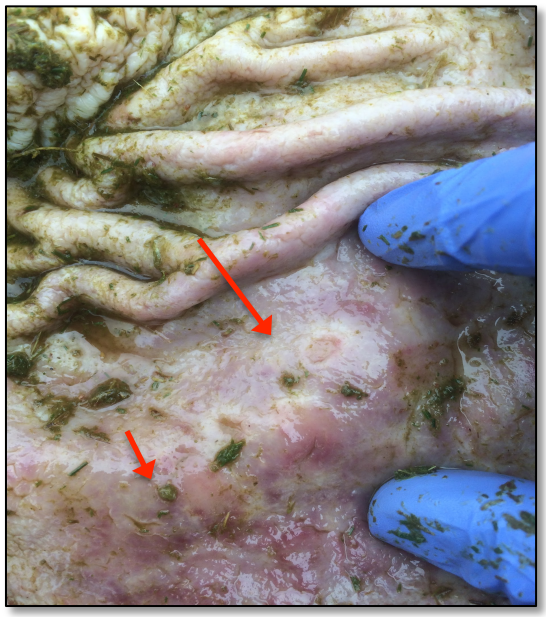


Image 9

Chapter 6

Discussion

The study we have done is a unique study not performed before, according to our knowledge there is no similar reports about feral horses in the international literature. We could demonstrate the presence of EGUS in freely roaming, wild horses.

The study we have done helped us to understand more EGUS however there were a few setbacks that limited our research and so affected our interpretation of the results obtain. The first setback was the limit number of horses that we were able to examine. A larger scale study would reflect general EGUS prevalence better but it was not possible to perform due to ethical issues. The second setback was the selection. These horses were pre-picked due to their medical or ethological history and not randomly chosen.

After analyzing the results obtained from the experiment, our study showed that a small amount of our population to be positive for EGUS (3 out of the 11 horses) all of which were males, all 3 were cases of EGGD with no case of ESGD and they seemed to vary in their number score but none of them were more severe than 2.

Although Sykes and colleagues (2015) performed a similar study comparing prevalence of EGUS in feral and domesticated horses but that study was heavily influenced by different confounding factors. They have found ESGD was present in 22.2% and 60.8% and EGGD was present in 29.6% and 70.6% of feral and domesticated horses, respectively. The results of their study suggest that the prevalence of both ESGD and EGGD are higher in domesticated horses than feral horses. Unfortunately, their study was conducted in an abattoir and the horses were transported there for slaughter. While arriving to the slaughterhouse, these animals, including feral horses, had experienced long transportation and food deprivation which are well-known causes of gastric ulceration. Ulcers can quickly develop when horses are starving and are under stress (Murray, 1994). Since the Przewalski horses of our study were examined in their original environment, they were not transported and fasted so we could avoid these confounding factors.

The only link found between the three horses that showed EGUS was their sex, all three of them were male horses. In a large scale cross sectional study a higher prevalence was reported in stallions than mares and geldings (Sandin et al., 1999), which was consistent with our results. In contrast, studies of Thoroughbred racehorses documented no significant effect of sex on the likelihood of having ESGD (Vatistas et al., 1999, Lester et al., 2008). However, there were only 3 mares in the examined group, so males were clearly overrepresented in the study group. Another thing that we noticed was the position of the horses in the harem. Two of them were harem stallions, while the third was a bachelor.

Although we did not have enough samples to draw concrete conclusion about prevalence according to age but it seemed that age was no factor for EGUS since the three horses that had ulcers were of different ages. The youngest horse examined was 2 years old and was affected. This study did not aim to examine prevalence according to age and there were no foals, weanling and yearlings involved. In a previous report by Luthersson et al. (2009), older horses were not more likely to be affected by EGUS but they were more likely to have both ESGD and EGGD.

The Przewalski breed doesn't have the same genetics as the domestic horse, which raises the question if the difference in prevalence in EGUS is due to the difference in genetics. Although the prevalence of gastric ulceration varies with breed, use, level of training, as well as between ESGD and EGGD (Sykes et al., 2015)., breed seems to be a less important predisposing factor than training level and feeding management. Based on the pathophysiology of this disorder and previously identified risk factors, prevalence in Przewalski is supposed to be independent from the genetic differences.

Most of the studies on EGUS are done based on clinical cases were the diagnosis is established on the history of the horses, by elimination technique or by clinical examination and gastroscopy (Nieto, 2012). However in our experiment, the aim was to study the prevalence of EGUS in Przewalski horses, which is a breed of wild horses living in the reserve of Hortobagy. It was not possible to observe the horses individually and monitor possible clinical signs or their actions and behavior, as well as it was impossible to perform diagnostic examination on this breed. On the other hand, all of these horses showed good clinical conditions on post mortem evaluation, and based on these findings we can suspect that these horses had good appetite and continued grazing despite having ulcers in the

stomach. Here we have to note that body condition evaluation based on Henneke scoring system might not be perfectly applicable to feral Pzewalski horses since it was established for domesticated mares (Henneke et al., 1983).

The prevalence and severity of ESGD in horses with clinical signs (poor appetite, poor bodily condition, and abdominal discomfort) was demonstrated to be significantly greater than in horses without clinical signs. Silent gastric ulceration also does exist (Murray et al., 1989). In our cases these ulcers might not be severe or painful enough to stop horses grazing. The other possibility that wild horses have less tendency to care about pain and are less sensitive to pain sensation than domestic animals. This suspected decreased pain sensation might be necessary for surviving in the wild although individual differences to pain sensation might exist in feral and domestic horses as well. It is widely accepted that individual differences in nociception are observed (Kavaliers, 1988, Elmer, 1998).

Concurrent diseases are causing chronic stress and possibly disturbed perfusion of the mucous membrane and might predispose the animal to ulceration of the stomach. Interestingly, it seemed that previous medical conditions, like chronic lameness (Chronic pain) did not cause the occurrence of EGUS in these horses. Only one of the affected animals had a non-significant medical condition, the other two horses were generally healthy. The horse with poor body condition and the two chronically lame horses were free of ulcerations. There were Gastrophilus intestinalis larvae in every horse's stomachs independently of the presence of EGUS. In a study by Sweeney (1990), it was also concluded that bot larvae are not important in the etiopathogenesis of gastric ulceration or erosion.

In our study we have found lesions diffusely in the glandular fundus and below the margo plicatus in the cardia, but none in the pyloric area. This finding is opposite to results of a previous studies were the majority of EGGD lesions were found within the pyloric antrum (Sykes et al., 2015). Differences in locations might result from differences in the predisposing factors when comparing feral horses to domesticated animals. Breed specificity cannot be fully excluded as a reason for the unusual locations.

When we were looking for the risk factors in ulcer development of feral horses we have found some interesting points. First of all, domesticated horses are more prone to develop ESGD since their grazing is limited and therefore not enough saliva is produced and

swallowed to buffer the continuously secreted acidic content. However, based on Sykes' (2015) study and our results, EGGD seems to be more prevalent in freely roaming horses. We think that the ulcers found in our study were not diet related. Since the food of stabled horses is granulate and hay, which is given in portions, causing instable and alternatively too low pH and acid production. In our case of the Przewalski breed, they were continuously grazing leading to normal amount of saliva production, no stomach overload and a stable pH in the stomach.

When looking at the different causes of stress, we could talk about exercise, in domestic horses, excessive exercise and training eventually will lead to the increase in stress and the horse causing them to develop EGUS (Andrews, 2002). In our case since the Przewalski horses are freely roaming in the wild, they are not exercised. They regularly spend time with grazing, playing and moving around, but there is no heavy workload done by these animals. They do not have to move long distances for food, there is no predator in the Hortobagy chasing them. The only heavy exercise they performed happened when their selection and hunting started. This hunting took minimum 10 minutes to maximum 45 minutes in each case, which is a fairly short period of heavy exercise and is unlikely to cause ulceration of the stomach, since it just happened right before the examinations. On the other hand, it is important to keep in mind that living in the wild is not as easy as one might think, it can be stressful to the animal and would have a negative effect on the animals' health.

Another important factor that can be observed from the result is all 3 horses are stallions, which narrows down the cause of EGUS for us. In the case of the experiment done on the Przewalski breed, you can't but ask yourself the question of why only male horses showed EGUS upon dissection even though pregnant mares can also develop ulcers due to stress as well. The only reason we could think of is their sexual drive. If you look at the different herds like a normal harem or a bachelor herd, then different reasons can come to mind to why stallions are almost always stressed. In a bachelor herd, the males are always stressed because of their inability to mate, not because they are incompetent but rather because of their inferiority to the harem stallion and this stress increases with time and leads to increased histamine release and other stress related hormones and leads to change in the normal homeostasis of the horse. As for the harem stallion, the reason why he also seemed to be susceptible to develop EGUS is also somewhat similar. Stress secondary to male dominance fights and too large harems can be the cause of glandular ulcers in wild horses.

Previous data about the prevalence according to behavioral type show some discrepancy. Show horses with a nervous disposition are more likely to have ESGD than are quiet or behaviorally normal horses (McClure et al., 1999). In contrast, no effect of nervousness has been shown in racehorses, and in fact in this population, aggression might have an effect on limiting ESGD (Lester et al., 2008).

Another factor that should be taken into consideration is the use of NSAIDs or other ingested toxic materials. Studies done showed that the causes of glandular ulcers were mostly stress-related or secondary to the long-term usage of NSAIDs like phenylbutazone (Nieto, 2012). Looking at our studied population of feral horses, they were not treated with anything and that is why we can eliminate the use of NSAIDs as the reason for the development of EGUS. These horses are kept in the National Reserve of Hortobagy where there are strict environmental control measures implicated, so it is unlikely to have any toxic chemical material uptaken by the animals. Toxic plants causing ulceration of the GIT are not prevalent in this geographical region.

Chapter 7

Conclusion

Although our original hypothesis was that EGUS is a disease of civilization and only affects domesticated equidae, we have found ulcers in the stomach of wild horses. If we look at our results closer we still can accept our hypothesis, since the type of EGUS found was different in these feral horses than those typically diagnosed in domesticated animals. Based on the results that we have obtained from our study along with the results from Sykes and his team's study (2015), which do largely coincide, we can conclude that:

1. EGUS does exist in freely roaming horses as well.
2. Different types of EGUS seem to be more prevalent in feral and domestic horses.
3. EEGD seems to be more prevalent in feral horses and ESGD in domestic horses.
4. Feral horses do not show weight loss with lower levels (up to grade 2) of EGUS.
5. Some risk factors are the same in both feral and domestic horses like stress and possibly strong exercise.
6. Some risk factors are different like feeding, medications, and transport.
7. Based on these, we think that the type of risk factor predestinates for different type of EGUS.
8. Although less likely we still cannot exclude breed differences in the type of EGUS diagnosed in Przewalski breed.
9. ESGD does not occur in Przewalski horses. This study underlines again the significance of feeding and stabling management in the development of ESGD in domestic animals and the role of civilizational environment.

Chapter 8

Abstracts

The Equus Przewalski is of Asian origin and is the only existing wild horse. Once extinct but with the help of breeding management, the population seems to be increasing nowadays. Not only does the Przewalski breed differ from domestic horses by appearance but also genetically, this wild horse has 66 chromosomes.

Gastric ulcers are common in horses and according to previous studies 60% of performing horses develop equine gastric ulcer syndrome (EGUS) while this number increases to over 90% in racing horses and is much less in freely roaming horses. The chances of developing EGUS does change depending on the use of the horse and that is because of the different causative factors that range from nutrition and training to stress levels of individual animals.

The aim of this study was to evaluate prevalence of EGUS in freely roaming adult Przewalski horses.

Stomach of 11 Przewalski horses (8 males, 3 females) with the age of 2-22 years (m:9,9, sd:6) were examined postmortem for EGUS. Their body condition and general health state was evaluated by observation and reviewing their medical history. These horses spent their lives freely roaming in 2361 ha grassland biosphere reserve in the Hortobágy National Park.

We found ulcers in 3/11 (27%) horses. All affected horses were males, there were two harem stallions and a bachelor. Neither the esophagus and the non-glandular squamous epithelial mucosa, nor the margo plicatus area did not show any alterations in any of the examined animals. The glandular part was affected with lesion number score of 2 in each case and lesion severity score was 1 in one animal, and 2 in two others. The pyloric region was always free of ulceration. None of the horses showed signs of severe weight loss.

Opposite to other horse populations, freely roaming Przewalski horses had gastric ulcerations exclusively in the glandular region. Since these horses spend approximately 50% of their time grazing, gastric pH stays more constant in the higher range making peptic injury to the squamous mucosa less likely. Most of the ulceration initiating factors described in domestic horses could be excluded based on the living style of wild horses and their medical records. Glandular mucosal erosion and ulceration can originate from stress-induced impairment in mucosal blood flow and the mucus-bicarbonate barrier. Stress secondary to male dominance fights and too large harems might be the cause of glandular ulcers in wild horses. Unfortunately due to their way of living, an observational study would have been difficult to perform to see if other factors play a role in disease development or how exactly did the EGUS affect their performance or behavior in the group or alone.

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Chapter 9

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Chapter 10

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