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# Outbreak of equine grass sickness in Denmark – 4 cases

*Nina Resetic, Ainoa Richter Jorgensen and Louise Husted*

Højgård Hestehospital, Morud, Denmark

**Summary:** Equine grass sickness (EGS) is a rare and often fatal disease of the grazing equids, with the majority of clinical signs associated with failure of normal gastrointestinal function. EGS has the highest incidence in Great Britain but is also frequently reported in other northern European countries. A very similar condition called mal seco has been recognised in South America. The precise aetiology of the disease is unknown, but is thought to be associated with potent neurotoxins, produced in the gastrointestinal system by *Clostridium botulinum* type C, a bacteria commonly found in the soil, under certain environmental conditions. EGS occurs almost exclusively in horses that have an access to grass and it is acknowledged that certain premises are associated with a higher occurence of EGS. Most often only one animal is affected, however, outbreaks in a larger number of horses in a limited period of time are not uncommon. The disease can affect any age of the horse but is most commonly diagnosed in younger animals between two and seven years of age. Older horses may develop resistance to the causative agent and therefore not get affected. It was found that periods of dry, sunny and frosty weather result in an increased number of cases and occurence of the disease is most prevalent in the spring months between April and June, with a peak in May. The risk of developing EGS is higher if the affected horses have recently had a change of feeding regimen, been moved to a new pasture or treated with anthelmintics. EGS occurs in three different forms; acute, subacute and chronic. Clinical signs are a result of extensive neuronal degeneration of autonomic and enteric nervous system, while also affecting brain and spinal cord. Symptoms in the acute and subacute cases are severe and occur rapidly. Gastrointestinal paralysis results in signs of acute colic, accumulation of large amounts of gastric reflux, gasseous distention of the large intestine and lack of fecal output. Muscle tremors, ptosis and patchy sweating are often observed. The prognosis is usually bleak and most of the time the horses diagnosed with EGS are subjected to euthanasia. Chronic form is less severe and develops more slowly. The symptoms are those of a mild intermittent colic, reduced appetite, difficulty in swallowing and rapid weight loss. Rhinitis sicca can also be observed. Some of these cases may survive under certain conditions and with an intensive supportive care. It is often difficult to distinguish EGS from other causes of colic. A definite diagnosis is confirmed with histopathologic examination of myenteric or submucosal plexus of the ileum. Loss of neurons can be observed in all parts of the gastrointestinal system. Commonly used ancillary diagnostic procedure is a ptosis test, which involves topical application of phenylephrine eye drops to the cornea, causing reversal of the drooping eyelids. This report describes three acute cases and one subacute case of EGS which were presented to Højgård Hestehospital, Denmark in May 2020. Time between onset of clinical signs between the first and the last case was 15 days, following a 14-day period of dry, cold and sunny weather conditions. Age of admitted horses ranged from two to seven years and all of them were treated with ivermectin two days before the onset of clinical signs. The first three cases had been living together on the same grass field and were presented to the hospital with nearly identical acute colic signs. They were tachycardic, had decreased peristaltic, developed a lack of fecal output and accumulated large amounts of gastric reflux. An exploratory laparotomy was performed on the first admitted case where moderate distention of small intestine and signs of enteritis were found, with proximal enteritis being considered the most likely diagnosis at the time. Postoperative treatment consisted of antimicrobial and antiinflammatory therapy, administration of intravenous fluids, prokinetics and frequent gastric decompression. There was no significant improvement after six days of supportive care and the horse was subsequently euthanised. Post mortem samples of small intestine were collected and pathohistological findings revealed autonomic neuronal depletion and neuronal degeneration of ileum, which confirmed diagnosis of an acute case of EGS. Second and third cases were kept on medical treatment and were euthanised after four and three days, respectively, after their condition had progressively worsened. The fourth case was turned out daily on the paddock next to the grass field where the first three horses were being kept and was referred to the hospital after being unsuccesfully treated for an impaction by the referring vet. Dullness, droopy eyelids and muscle tremors were noted upon presentation, along with other clinical signs, consistent with an impaction of large intestine. No improvement was observed after medical treatment and the decision for surgical intervention was taken later in the day, being aware of the fact that the horse had a high probability of being affected by EGS as well. Cecum and large colon impaction was found and pelvic flexure enterotomy was performed to empty the contents of the intestine. The horse continued on supportive therapy after. Patchy sweating over shoulder and gluteal regions was noted on the second day after the surgery. On the third day the horse was euthanised due to lack of clinical improvement and poor prognosis. Based on the clinical appearance and findings, the first three presented cases were examples of an acute form of EGS, while the last case was a subacute form. The last official report of EGS in Denmark goes back to 1948, however, many anecdotal reports of the disease appearing in certain regions of the country exist. The main purpose of this case report is to draw more attention to EGS as it appears to occur more often than being noted, in order to set a correct diagnosis, avoid unnecessary treatment and therefore not prolong animal suffering. Suspicion of EGS should be considered whenever a horse is presented with clinical signs of acute colic, nasogastric reflux, intestinal hypomotility and decreased fecal output, especially in the spring months, with high suspicion when muscle fasciculations, patchy sweating and bilateral ptosis are observed four to five days after the onset of the disease.

**Keywords:** horse, EGS, grass sickness, dysautonomia, colic, Denmark

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**Correspondence:** Nina Resetic, DVM, Rugardsvej 696, 5462 Morud, Denmark; nina.resetic@gmail.com

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## Introduction

Equine grass sickness, or equine dysautonomia, is a neurodegenerative disease that predominantly affects the neurons of the autonomic and enteric nervous system of all equidae. Neuronal degeneration also occurs in the brain and spinal cord, so it can be classified as a polyneuropathy (*Reed* et al. 2018). It was first reported in 1909 in Scotland and has since mainly been seen in northern Europe. Severity of the disease varies and is determined by the extent of neuronal damage in myenteric and submucosal plexuses of the enteric nervous system. Acute and subacute forms of EGS are characterized by extensive neuronal degeneration, which results in intestinal dysmotility, and are mostly fatal, while a proportion of chronic forms with less severe neuron damage and slower development of the disease will survive (*Pirie* et al. 2014). Aetiology remains unknown, however, as the name implies, there is a strong association between EGS and grazing. It affects mostly young horses between two and seven years of age with access to pasture, especially in the spring (*Reed* et al. 2018).

This case report describes a series of three acute cases and one subacute case of grass sickness presented to Højgård Hestehospital, Denmark, in May 2020. The first three horses were living on the same grass field and the fourth horse was turned out daily in a paddock next to it. Time between onset of clinical signs between the first and the last case was 15 days.

## Case 1

*Case history*

A four-year old Warmblood gelding was referred to the equine hospital in May 2020 with acute colic signs. The horse was dewormed with an oral paste formulation of Ivermectin (0.2 mg/kg BW) two days before onset of clinical signs and developed a colic with a lack of fecal output. The referral vet obtained 14 L of gastric reflux on nasogastric intubation but could not perform a rectal examination as it was too painful for the horse. Consequently, the horse was referred promptly due to suspicion of a surgical lesion.

*Clinical findings*

Upon presentation the horse was quiet, had a rectal temperature of 37.5 °C, heart rate of 52 bpm and a respiratory rate of 12 bpm. Mucous membranes were pink and dry, with a capillary refill time (CRT) of 2 seconds. Metamizole (25 mg/kg) and butylscopolamine (0,2 mg/kg) were administered to perform rectal examination, where cecum and small intestine were moderately distended with gas. 4 L of dark green and foul-smelling gastric reflux were obtained via nasogastric intubation. A complete blood cell count revealed lymphopenia ($0{,}69 \times 10^9$/L; normal 1.50–5.10 $\times 10^9$/L) and eosinophilia ($1{,}37 \times 10^9$/L; normal 0.00–0.80 $\times 10^9$/L). Blood lactate was 4,7 mmol/L (normal < 2 mmol/L). No clinically significant abnormalities were detected on serum chemistry. On the abdominal ultrasound distention and hypomotility of small intestine could be observed while the wall of small intestine appeared thickened subjectively. Abdominocentesis was performed, with total protein count being 5 g/dL (normal 0–2.5 g/dL), lactate 7,5 mmol/L (normal 0.3–1 mmol/L) and total nucleated cell count 73.0 $\times 10^9$/L (normal < 5 $\times 10^9$/L).

Based on the findings, a surgical small intestinal lesion could not be ruled out, therefore the horse underwent general anaesthesia for midline exploratory laparotomy. Moderate distention of the ileum and distal part of the jejunum with gas and fluid was observed, with normal mobility of the remaining small intestine and signs of enteritis (serosal edema, petechiae) present. Proximal enteritis was considered the most likely differential diagnosis at the time.

*Treatment and outcome*

Postoperatively, with proximal enteritis suspected, the horse was treated with Na-penicillin (22 000 IU/kg i.v. q 6 h); gentamicin (6.6 mg/kg i.v. q 24h.); metronidazol (25 mg/kg p.o. q 12 h); flunixin meglumine (Flunixin Vet. 1.1 mg/kg i.v. q 12 h); and Ringer Acetate (60 ml/kg/day i.v.). During the day the horse remained quiet, with increased heart rate (88 bpm) and decreased abdominal borborygmi in all four abdominal quadrants. A nasogastric tube was inserted and left in place to monitor the accumulation of gastric reflux. In 14 h after the surgery there was a net amount of 22 L of gastric reflux and a constant rate infusion (CRI) of lidocaine (0,05 mg/kg/min) was added to the treatment plan for pain relief and antiinflammatory effect.

Day 2: The horse was quiet, with a slightly elevated heart rate (48 bpm). Other vital parameters were within normal limits. A total of 38 L of gastric reflux were obtained over the last 24 h and the horse had not passed any manure.

Day 3: Gut sounds were absent and the horse continued to accumulate large amounts of gastric reflux. Heart rate remained increased (56 bpm) while mucous membranes became paler, with prolonged CRT (3 sec). Complete blood count revealed leukopenia ($2.98 \times 10 \times 10^9$/L; normal 4.90–11.10 $\times 10^9$/L), neutrophilia ($0{,}88 \times 10^9$/L; normal 2,50–6,90 $\times 10^9$/L), lymphopenia ($1.03 \times 10^9$/L; normal 1.50–5.10 $\times 10^9$/L) and monocytosis ($1.06 \times 10^9$/L; normal 0.20–0.60 $\times 10^9$/L). Biochemistry revealed elevated levels of alkaline phosphatase (404 U/L; normal 10–326 U/L) and total bilirubin (142 mmol/L; normal 0–60 mmol/L).

Day 4: Intestinal sounds were still not audible during auscultation and there was no faecal output since the horse had arrived. 3–8 L of gastric reflux were obtained every three hours and motility of the small intestine was monitored with abdominal ultrasound.

Day 5: Muscle fasciculations in the hindlimb area started to appear. Heart rate remained elevated and intestinal sounds were absent. Toxic line could be observed on the mucous membranes. Net amount of gastric reflux during the day was 50 L. Blood serum triglycerides were elevated (> 4.25 mmol/L; normal 0.12–0.77 mmol/L); lymphopenia ($0.55 \times 10^9$/L; normal 1.50–5.10 $\times 10^9$/L) and monocytosis ($1.43 \times 10^9$/L; normal 0.20–0.60 $\times 10^9$/L) persisted.

Day 6: Muscle fasciculations were still present and there was 50 L of gastric reflux obtained over the night. Signs of rhinitis *sicca* were noticed on the left nostril. The abdominal ultra-

sound showed normal motility of the small intestine, including the duodenum, with no signs of thickening. Gastroscopy was performed; pylorus and duodenum were large and flacid but no other abnormalities could be found. The horse continued to accumulate large amounts of gastric reflux and was subsequently euthanised due to animal welfare concerns.

*Pathological findings*

Post mortem examination was performed. The position of organs in the abdominal cavity was anatomically correct and the organs were roughly normal. The small intestine was distended with fluid and the intestinal wall was thickened over the whole length, especially in the ileum.

Five sections of small intestine were collected, fixed in formalin and sent to Rossdales Laboratories for further analysis due to suspicion of grass sickness. The histopathological examination revealed marked autonomic neuronal depletion and occasional active neuronal degeneration of ileum. Mild inflammation and moderate villous blunting were present, which was likely to be secondary to altered intestinal motility. Many of the villi were blunted and there was some vilous fusion, with the luminal epithelium formed by attenuated low cuboidal epithelial cells. Within the lamina propria, there were moderate infiltrates of lymphocytes, plasma cells and very occasionally eosinophils, with a few macrophages and sparse neutrophils and nuclear debris in the remaining tips of villi. Some of the sections had lymphoid aggregates at the base of the mucosa. In the submucosa, the outline of the autonomic ganglia was discerned with almost complete neuronal depletion, there were only small amounts of nuclear debris detected in some ganglia and very occasional swollen neuronal remnants with pale cytoplasm. In the myenteric layer, there was a similar paucity of neurons, with rare cells with darker cytoplasm. One of the sections had a small lymph node associated with it and there were slightly increased numbers of macrophages within the subcapsular sinuses, with low numbers of small reactive lymphoid follicles. These findings confirmed the diagnosis of acute grass sickness.

## Case 2

*Case history*

A two-year old mare from the same stable started to become quiet and was lying down and drinking more than usual. The heart rate was increased and rectal examination by the referring veterinarian revealed gaseous distension of cecum and there was lack of fecal output. Despite medical treatment no clinical improvement was observed and the mare was referred to the hospital, six days after the first case had been presented.

*Clinical findings*

At the arrival the horse was alert and nervous, with a heart rate of 80 bpm. Mucous membranes were icteric, with prolonged CRT (3 sec). Gut sounds were decreased in all four quadrants. Other clinical findings were normal. Nasogastric intubation yielded 20 L of spontaneous reflux. Rectal examination and abdominal ultrasound revealed distention of small intestine and cecum, with no movements present.

Haematology and a serum biochemical profile showed leukopenia ($4.49 \times 10^9$/L; normal 4.90–$11.10 \times 10^9$/L) with lymphopenia ($0.80 \times 10^9$/L; normal 1.50–$5.10 \times 10^9$/L), elevated urea (11.8 mmol/L; normal 3.6–8.9 mmol/L), total protein (84 g/L; normal 56–79 g/L), globuline (53 g/L; normal 24–47 g/L), AST (1002 $\mu$/L; normal 100–600 U/L), total bilirubin (98 $\mu$mol/L; normal 0–60 $\mu$mol/L) and CK (593 $\mu$/L; normal 10–350 $\mu$/L) levels. Haematocrit was within normal limits (43.9 %; normal 30.0–47.0 %).

*Treatment and outcome*

Treatment was focused on stabilizing the cardiovascular system and providing anti-inflammatory effect, using CRI of lidocaine (0,05 mg/kg/min) and i.v. fluid therapy (Ringer acetate). Nasogastric tube was placed for decompression of the stomach. Metronidazol (20 mg/kg orally q 12 h.) and oxytetracycline (7 mg/kg i.v. q 12 h) were used for antimicrobial therapy.

Day 2: The mare was bright and alert but nervous, with elevated temperature (38.5 °C) and heart rate (76 bpm). Abdominal borborygmi and fecal output were decreased and 5–6 L of gastric reflux could be obtained every four hours.

Day 3: Vital parameters were within normal ranges. The mare became quiet, had no fecal output and accumulated 30 L of gastric reflux in total.

Day 4: Over the next 24 h the clinical condition of the horse notably deteriorated; heart rate was elevated (80 bpm), CRT prolonged (3 s) and no abdominal borborygmi were audible. Because of persisting large amounts of gastric reflux, dullness, extreme signs of pain and poor response to treatment, the horse was subjected to euthanasia.

## Case 3

*Case history*

A four-year-old gelding was hospitalized eight days after the first case because he developed similar symptoms to those of the two previous cases. The owner had reported halitosis earlier in the day.

*Clinical findings*

On admission to the hospital the horse was bright and alert, heart rate was elevated (56 bpm). Mucous membranes were pink and dry, with CRT of 2–3 sec. Gut sounds were decreased but not absent. Rectal palpation identified cecum distension and a large impaction on the right side along with a moderately distended part of a small intestine that could be felt in the region of pelvic flexure. No gastric reflux could be obtained. The horse was primarily treated for impaction and was given fluids with epsom salt via a nasogastric tube.

Haematology parameters were within normal limits. Biochemistry analysis showed elevated glucose (8.74 mmol/L; normal 3.56–8.34 mmol/L), total protein (88 g/L; normal 56–79 g/L) and globulin (56 g/L; normal 24–47 g/L) levels, while calcium (2.57 mmol/L; normal 2.60–3.22 mmol/L) and chloride (95 mmol/L; normal 97–10⁹ mmol/L) were decreased.

*Treatment and outcome*

Flunixin meglumine (1.1 mg/kg i.v.) along with metamizolnatriummonohydrat (25 mg/kg) and hyoscinbutylbromid (0,2 mg/kg) were administered for its analgesic and spasmolytic effects, respectively. Food was withheld and presence of gastric reflux was regularly monitored.

Day 2: The horse was quiet and uncomfortable, with sighing often observed. Heart rate was elevated (60 bpm) and the mucous membranes were dry. No manure had been seen since the arrival. Gastric reflux started to accumulate during the day. Treatment with i.v. fluids (Ringer acetate), oxytetracycline (6.6 mg/kg i.v. q 12 h) and metronidazole (20 mg/kg orally q 12 h) was started. Haematology revealed lymphopenia ($0.9 \times 10^9$/L; normal 4.90–$11.10 \times 10^9$/L).

Day 3: Signs of pain, elevated heart rate and decreased gut sounds persisted. Every three hours 8–10 L of serosanguinous fluid was obtained via nasogastric intubation. As the horse did not respond to our treatment and with similarities being drawn to the previous two cases, a presumptive diagnosis of acute grass sickness was made and the horse was subjected to euthanasia.

## Case 4

*Case history*

A seven-year old gelding started to become dull, dysphagic and had difficulties passing manure that contained fibrin. One of the signs observed by the owner was also playing with water quite often. It was treated for an impaction for several days by a referring veterinarian and after five days of administering oil via nasogastric tube and no signs of improvement, the owner decided to bring the horse to the hospital, 15 days after the first case had been presented.

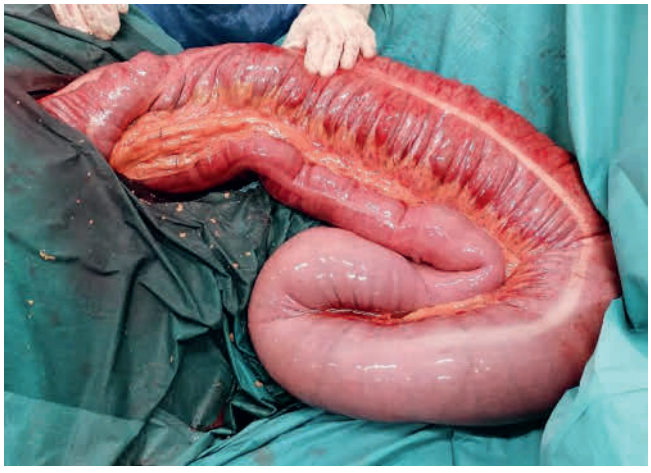

**Fig. 1** Appearance of an impacted large colon following exploratory laparotomy in a horse with a subacute form of EGS. | *Aussehen eines betroffenen Dickdarms nach explorativer Laparotomie bei einem Pferd mit einer subakuten Form von Graskrankheit.*

*Clinical findings*

Clinical examination revealed elevated heart rate (60 bpm), drooping eyelids, tucked-up appearance and mild muscle tremors. On the rectal examination moderate impaction of pelvic flexure was identified along with a very tight cecal band on the right side. Some soft manure were present in the ampulla. Blood analysis showed stress leukogram, mild haemoconcentration (47.1 %; normal 30.0–47.0 %) and elevated bilirubin levels (66 µmol/L; normal 0–60 µmol/L).

*Treatment and outcome*

Treatment was focused on resolving the impaction. The horse was withheld from food and put on fluid therapy (i.v. and oral) in attempt to soften the impaction in the large colon and cecum. Repeat abdominal ultrasound and rectal examination identified further gaseous distention and the persistence of impaction. Surgical intervention was performed under general anesthesia, with the high suspicion for being EGS case as well being taken into consideration.

The ascending colon and cecum were in normal anatomic position. Cecum could only be partly taken out and was strongly impacted with feed material. Over the whole surface it was covered by ecchymoses. Sternal flexure was impacted as well. Enterotomy was performed with a 15 cm long incision on the antimesenteric side at pelvic flexure and the content of large intestine was flushed out. Ileum and jejunum were thickened and had normal motility. Stomach was slightly distended and nasogastric tube was placed to relieve the distention. Postoperative recovery went without any complications.

Horse received antibiotic therapy - Na-penicillin (20 000 IU/kg i.v. q 6 h;) and gentamicin (6.6 mg/kg i.v. q.d.).

Day 2: The horse was somnolent with elevated heart rate (52 bpm). Gut sounds were decreased and there was no faecal output. Blepharospasm was present in both eyes along with muscle fasciculations and patchy sweating in the shoulder and gluteal areas. Haematology showed lymphopenia ($1.46 \times 10^9$/L) with band neutrophils suspected. Metronidazole was added to the therapy (20 mg/kg orally b.i.d.) and the horse continued to receive i.v. fluids (Ringer acetate). Feed was withheld. There was a net amount of 24 L of gastric reflux obtained over the day.

Day 3: Muscle fasciculations and patchy sweating continued. Heart rate was elevated (60 bpm), there were no audible gastrointestinal sounds and toxic line was present on mucous membranes, with CRT of 3 seconds. The horse continued to accumulate gastric reflux (20 L in total) and passed one small fecal pile during the day.

Bloodwork revealed leukopenia ($3.52 \times 10^9$/L; normal 4.90–$11.10 \times 10^9$/L), neutropenia ($1.66 \times 10^9$/L: normal $2.50–6.90 \times 10^9$/L),

lymphopenia ($0.94 \times 10^9$/L; normal 1.50–$5.10 \times 10^9$/L) and monocytosis ($0.91 \times 10^9$/L; normal 0.20–$0.60 \times 10^9$/L). Calcium levels were lowered (2.54 mmol/L; normal 2.60–3.200 mmol/L) while AST (1082 U/L; normal 100–600 423 U/L), total bilirubin ($10^9$ µmol/L; normal 0–60 µmol/L) and CK (1462 U/L; normal 10–350 U/L) values were elevated.

The horse was euthanised later during the day due to lack of clinical improvement and high probability of EGS. Spontaneous reflux occured when the nasogastric tube was removed.

## Discussion

EGS is most common in the UK, but is also known in mainland European regions (*Wylie* and *Proudman* 2009). This is only the second officially reported case of the disease in Denmark after Bendixen had reported of a case of EGS occurring in 1946. However, a lot of anecdotal reports of EGS exist from certain regions in Denmark, especially from an island called Saltholm, where horses often develop the disease if left there at certain times during the year.

EGS is in most cases a fatal neurodegenerative disease of the grazing equids. The majority of clinical signs is associated with failure of normal gastrointestinal function. The literature describes three clinical presentations of the disease: acute, subacute and chronic. This classification is based on duration of illness. The acute form develops rapidly (in one to two days) and is in all cases fatal, with dysphagia, tachycardia, muscle fasciculations, ptosis, gastric reflux and decreased gut motility being the most obvious clinical signs. The subacute form presents with colonic impactions instead of small intestine distension and moderately severe colic signs that last more than two days. It is difficult to distinguish it from the acute form, however. The chronic form develops more gradually and usually lasts more than seven days. The most common clinical signs are rapid and severe weight loss along with mild dysphagia. Rhinitis sicca is also often observed in this group. With intensive care, survival may be possible (*Wylie* and *Proudman* 2009, *Hahn* et al. 2001). Based on the clinical presentation, the first three cases presented were acute forms of EGS, while the last case was a subacute form.

Diagnosis can be difficult as different diseases with clinical symptoms of intermittent colic and cachexia can mimic EGS (gastric ulcers, endoparasitism, intestinal neoplasia, inflammatory bowel disease, ileus, peritonitis, oesophagitis, intestinal obstruction, dental disease etc.). A definite antemortem diagnosis is based on histopathologic examination of specific lesions in the neurons within the myenteric or submucosal plexus of the ileum (*Scholes* et al. 1993). Postmortem diagnosis can be confirmed by examining ileal sections, the peripheral sympathetic celiacomesenteric or cranial cervical ganglia (*Wylie* and *Proudman* 2009). Characteristic lesions include chromatolysis, loss of Nissl substance, neuronal swelling, vacuolation, intracytoplasmic eosinophilic inclusions, nuclear pyknosis and neuronophagia (*Hahn* et al. 2001, *Piccinelli* et al. 2019). *Hudson* et al. (2001) reported of significantly decreased interstitial cells of Cajal in myenteric plexus and circular muscle regions in ileum and pelvic flexure in horses diagnosed with EGS, compared to normal animals. These interstitial cells serve as a pacemaker for smooth muscle contraction in the gastrointestinal system and their loss can be a significant factor in the development of intestinal stasis (*Hudson* et al. 2001). Loss of neurons can be found throughout all the gastrointestinal system, from the oesophagus to the rectum (*Milne* et al. 2010). Small intestinal samples were in our case collected only from the first horse, in order to confirm the suspected diagnosis. Further sampling in the next three cases was not deemed necessary due to similarities of their history and symptoms with the first case and because of financial constrictions as well. Ancillary diagnostic tests to help with diagnosis are a topical administration of 0.5% phenylephrine to the cornea, to confirm the presence of ptosis (*Hahn* and *Mayhew* 2000), fluoroscopic observation of esophageal motility, esophageal endoscopy (*Greet* and *Whitwell* 1986), and in chronic cases, identification of rhinitis sicca (*Lyle* and *Pirie* 2009). However, they do not provide definitive confirmation of EGS. A Pposis test could not be performed as it was not possible to get phenylephrine eye dropsin the acute situation.

While multiple epidemiological studies have identified many risk factors associated with EGS, the precise aetiology of the disease remains unknown (*Pirie* et al. 2014). Evidence suggests EGS may be the result of potent neurotoxins produced within the gastrointestinal system by *Clostridium botulinum* type C under certain environmental conditions and after dietary changes (*Wylie* and *Proudman* 2009). *McCarthy* et al. (2004) proved that low antibody levels to *C. botulinum* type C, *C. novyi* type A surface antigens and a *C. botulinum* type C toxoid are associated with increased risk of EGS.

The main risk factor is thought to be grazing. However, reports of EGS cases exist where horses had not been exposed to pasture before onset of clinical signs. Horses that have been grazing together with a surviving case of EGS are reported to have a decreased risk of being affected, which suggests that there could be some sort of acquired immunity to the causative agent (*Pirie* et al. 2014). Other risk factors include a previous occurence of EGS in the same area, increased nitrogen levels in the soil, recent movement to a new field, recent construction and soil disturbance, a high number of young horses on the same field, presence of Ranunculus species and high levels of iron and heavy metals found in herbage on EGS sites (*Pirie* et al. 2014, *Wylie* and *Proudman* 2009, *Edwards* et al. 2010, *McCarthy* et al. 2004). Horses that had moved to a new field within the previous two weeks or had a change of feed regimen are also at a higher risk (*Wood* et al. 1998). It is suggested that a change in feeding regimen can cause a change of the gut microbiota, which can cause proliferation of *Clostridium* sp. and toxin production. Horses that are fed hay or haylage also have higher antibody levels against *C. botulinum* and *C. novyi* cell surface antigens in comparison to horses that are kept solely on pasture (*McCarthy* et al. 2004). *Newton* et al. (2004) reported about an increased risk of disease recurrence connected to mechanical removal of faeces from the field, while manually removal was associated with a decreased risk, along with grass cutting or co-grazing with ruminants, if pastures were not cut. The first three horses presented in this report had lived outside and shared the same field for the last two years. They had hay at free disposal. The same field was previously inhabitated with horses for at least three years with no problems being encountered. There was no change in feeding regimen or training and the field was not cut or harrowed; feces was removed manually daily. Thus, from previously described risk factors, the only one fitting is the fact

that this was a group of young horses living together. Five days after the first case had been admitted to the hospital, a 5-year old mare spent one day on the same field but did not show any clinical signs of disease. The fourth case was turned out daily in the paddock next to the grass field mentioned beforehand but was stabled overnight, together with another horse that was not affected. After the first horse had been euthanised, all of the remaining horses were moved to another field. Days earlier, four Icelandic horses on a farm a few kilometres away had died suddenly due to an unspecific cause.

EGS is significantly associated with age. Young horses were found to be at the highest risk of disease, especially those between two and seven years of age, with the risk in older horses declining progressively. It is theorized that older animals develop tolerance to the etiological agent, while very young animals are not affected due to the presence of maternal antibodies and lack of grass ingestion (*Wylie* and *Proudman* 2009). The mentioned age category fits our cases, with the ages of presented horses ranging from two to seven years.

Weather conditions also may result in increased disease incidence. The peak time occurs predominantly during spring and early summer. *Wood* et al. reported that the majority of cases were presented after periods of dry weather (*Wood* et al. 1998), while *Wylie* and *Proudman* reported that the risk is increased with more sun hours and frost days (*Wylie* and *Proudman* 2009). However, *McCarthy* et al. did not identify weather conditions to be associated with EGS occurrence (*McCarthy* et al. 2004). All of the described cases were presented in the first half of May. The weather at that time was sunny and dry, with no rain in the week before the first case had been presented. Daily temperatures ranged from 5–15 °C. This is consistent with the findings of previously mentioned reports.

All four horses were treated with ivermectin at the same time, two days before the onset of clinical signs in the first case, while another three-year old horse that shared the same field as the first three described cases was not treated and did not develop any symptoms of EGS. This brings up a familiar question whether anthelmintic treatment with ivermectin is associated with an increased risk of EGS. *McCarthy* (2004) reported that frequent administration of ivermectin can be a risk factor, while a high strongyle burden is associated with a decreased risk. Their study has revealed a significant, dose-dependent protective effect of strongyle infection, measured by FEC (*McCarthy* et al. 2004). Ivermectin is a macrocyclic lactone which acts by opening glutamate-gated chloride channels found only in neurons and myocytes of invertebrates. This allows chloride to enter into these cells and cause neuromuscular paralysis (*Edwards* 2003). Ivermectin toxicity in equids is not common. Transient injection-site reactions and clostridial myositis have been reported after intramuscular administration. Mydriasis, lethargy, inferior lip droop, muscle fasciculations, ataxia and other neurologic symptoms have also been associated with ivermectin usage. Most reports involve young animals that received an overdose of the antihelmintic. A case report also exists of three adult quarter horses that developed neurological signs after receiving the recommended dosage (*Swor* et al. 2009). Unlike in intervertebrates, glutamate-gated chloride channels are only present in the central nervous system in mammals. Capillary endothelial cells in a blood-brain barrier express P-glycoprotein. This protein, encoded by multidrug resistance (MDR1) gene, is responsible for limiting the brain penetration of a range of compounds. Therefore, mammals cannot be affected by the neurological effects of the drug. Incomplete expression of the MDR1 gene in young animals, mutation of the gene or an overdose of ivermectin could lead to elevation of the drug concentrations in the central nervous system, leading to severe neurotoxicity (*Edwards* 2003). Ingesting a specific, and for now unknown, substance that inhibits a normal function of P-glycoprotein and causes damage to the enteric nervous system could be a potential factor in developing EGS. Toxicological studies have not identified any clinical signs of gastrointestinal dysfunction after administration of supratherapeutic doses of ivermectin. Recently, antimicrobial activity of ivermectin has been demonstrated against *S. aureus* clinical isolates including methicillin-resistant strains, *Mycobacterium tuberculosis* and *Mycobacterium ulcerans* (*Miró-Canturri* et al. 2019). Further research is needed to investigate whether administration of ivermectin can alter the equine gut microbiota and potentially facilitate pathogenicity of the etiologic agent of EGS.

Treatment is usually not considered in acute and subacute cases of EGS. The diagnosis of EGS in our first case was confirmed after the first three cases had already been admitted and therefore treatment was performed with no comprehension of poor prognosis. The decision to proceed with surgery and treatment of the fourth case was taken due to the owners wishes to do so. Furthermore, clinical signs were more consistent with a subacute form of EGS and as the horse was older (seven years), there was a higher possibility of survival. Chronic cases can occasionally survive, however, they require intensive care with offering a highly palatable, nutritious food, frequent grooming and rugging to prevent hypothermia. Medical treatment consists of analgesics, appetite stimulants, probiotics, antibiotics and fluid therapy. Major complications include aspiration pneumonia, diarrhoea and choke.

Summarizing, reasonable suspicion of EGS should be considered whenever a patient is presented with clinical signs of colic, reflux, stasis of cecum and decreased fecal output, especially in the spring months, with high suspicion when muscle fasciculations and ptosis are observed additionally four to five days after onset of disease.

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