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Chronic iron overload causing haemochromatosis and hepatopathy in 21 horses and one donkey

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Summary

Background: Iron toxicosis is rarely reported in horses and chronic excessive oral iron intake has not been reported to cause clinical symptoms in equids.
Objectives: This case series describes 21 genetically unrelated horses and one donkey with chronic iron overload causing haemochromatosis and hepatopathy.
Study design: Case series.
Methods: All equids showing clinical signs compatible with chronic liver disease presented to Utrecht University and diagnosed with iron overload and haemochromatosis based on histopathological evaluation of liver tissue and/or blood transferrin saturation levels of >80% and proof of excess dietary iron intake due to excess iron content in drinking water were included.
Results: This study included 22 equids. All tested animals (n = 19) had transferrin saturation >80% and 21 of 22 had increased gamma-glutamyltransferase (γGT). Ultrasonography revealed rounded liver margins in five out of six horses and increased echogenicity in 4/6. Histological examination of liver tissue of 12 animals showed hepatitis, fibrosis and haemosiderin accumulation in macrophages and hepatocytes. Post-mortem examination also revealed haemosiderin accumulation in other organs in all seven examined animals. High iron content in drinking water was identified as the source of iron overload in all cases. All animals were housed under the same conditions for a minimum of 9 years prior to diagnosis of haemochromatosis. Of 22 animals, 13 survived until 1 January 2018, ranging from 17 to 79 months post diagnosis.
Main limitations: Histology of liver tissue was not available for 10 of 22 cases.
Conclusions: Chronic iron overload can lead to haemochromatosis and hepatopathy in equids. Development of disease is slow and clinical signs are nonspecific. Long-term excessive iron intake in equids should be avoided. If animals drink from natural water sources, it is important to test the water for iron content.

The Summary is available in Spanish – see Supporting Information

Keywords: horse; iron toxicosis; liver failure; iron toxicity; liver disease; iron saturation; haemosiderin

Introduction

Reports on iron toxicosis in horses are rare and all documented cases have occurred after the oral administration of iron-containing feed supplements [1–3]. In all such cases, the clinical signs developed acutely or subacutely. Increased numbers of haemosiderin containing cells were identified in the liver and varying degrees of hepatic disease were seen as a result of iron toxicosis.

Other studies have described horses with haemochromatosis and hepatic fibrosis without identifying an apparent source for any excess dietary intake of iron [4,5]. Because of the unclear connection between excess dietary iron intake and hepatic disease, Pearson and Andreasen conducted a study in 2001 in which ponies were fed excessive iron over a period of 8 weeks [6]. No histologic lesions were observed in liver biopsies collected at the end of the study. The authors concluded that iron toxicosis because of short term (<8 weeks) increased dietary intake of iron was unlikely to develop in horses. They also suggested that previous reports of hepatopathies in animals with haemosiderin accumulation were most likely the result of primary hepatic disease and not of excess dietary iron.

In the Netherlands it is fairly common for animals to drink from available natural surface water, such as ditches that often border grazing pastures. Surface water in the Netherlands regularly contains high iron levels [7]. Horses, like all mammals, do not possess a regulated iron excretion pathway [8]. Therefore, horses that ingest large amounts of iron over a prolonged period of time face a potential risk of iron overload and toxicosis. Nevertheless, no cases of chronic iron overload and toxicosis have been described in equids. The current case series describes 21 genetically unrelated horses and one donkey with iron toxicosis, hepatopathy and haemochromatosis.

Materials and methods

Case selection

This case series started with one case presented to Utrecht University Equine Hospital in 2011, which was diagnosed with haemochromatosis and liver dysfunction of unknown origin. After another genetically unrelated horse from the same farm was also diagnosed with haemochromatosis and liver failure, an investigation into the underlying cause was initiated. This investigation revealed that all other horses (n = 9) kept at that same farm were also affected. An evaluation of seven horses on surrounding farms identified another five cases. After these findings had been made public by a press release in the national media, six more animals were diagnosed with hepatopathy and haemochromatosis due to chronic iron overload, resulting in a total of 21 horses and one donkey from eight different farms.

The inclusion criteria for this case series were evidence of haemochromatosis obtained by histological evaluation of liver tissue and/or blood transferrin saturation levels >80%, clinical signs compatible with chronic liver disease (such as icterus, weight loss, rough hair coat, dullness and increased liver serum biochemistry parameters) and proof of excess dietary

iron intake (due to excess iron content in drinking water). Ideally, a definite diagnosis of iron toxicosis would be based on increased transferrin saturation and evidence of haemochromatosis on histological evaluation of liver tissue. Liver biopsy was not performed in all cases. For eight out of 10 horses on which histology of liver tissue was not available, transferrin saturation was increased (>80%) and histologic evidence of haemochromatosis was proven in a herd mate. The remaining two horses, which did not have a liver biopsy performed either on themselves or on a herd mate, both had highly increased transferrin saturation (>80%), evidence of liver damage and access to iron-rich drinking water and were therefore also included.

Haematology and serum biochemistry

Haematology and serum biochemistry analyses were performed at the University Veterinary Diagnostic Laboratory at Utrecht University. Serum iron, total iron binding capacity (TIBC) and transferrin saturation of 15 equids diagnosed with noniron related subacute to chronic hepatitis were also included for comparison purposes as a control group.

Ultrasonography of the liver

Ultrasonographic evaluation of the liver was performed using a Philips HD11 XE ultrasound machine.[a]

Histology of the liver

Ultrasound guided tru-cut biopsies were taken from seven horses and seven animals underwent a post-mortem examination during which liver tissue samples were obtained. These samples were fixed in 10% neutral buffered formalin, routinely paraffin-embedded and 4 μm tissue sections were stained with haematoxylin and eosin for histopathological evaluation. A Prussian blue stain was performed to additionally visualise the presence of iron. Histology was performed at the Veterinary Pathology Diagnostic Centre at Utrecht University.

Post-mortem examinations

Post-mortem examinations were performed at the Veterinary Pathology Diagnostic Centre at Utrecht University. The histological evaluation of samples collected at necropsy was performed in a way similar to that described above.

Environmental sample analysis

Water samples were analysed for iron content by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by a commercial lab (Gezondheidsdienst voor Dieren, Deventer, the Netherlands). Grass analysis for iron content was performed by a commercial lab (BLGG AgroXpertus, Wageningen, the Netherlands) and iron content in soil samples was determined by another commercial lab (ALcontrol Laboratories, Rotterdam, the Netherlands).

Results

In total, 21 horses and one donkey originating from eight different farms were included in the current study. All animals for which historic information on housing was available (n = 18) had been housed on their respective farms for >9 years under the same conditions before diagnosis of haemochromatosis. Thirteen were mares and nine were geldings and there were five Friesians, eight mini Shetland ponies, four Shetland ponies, one Fjord, one Tinker, one Warmblood and one crossbred horse. The donkey was of unknown breed. Age on presentation varied from 9 to 24 years (mean 15 ± 5). For more detailed information, see Supplementary Item 1. Some details were not recorded and, due to owners' financial constraints, not all tests were performed for all animals and not all information was available for each case.

Clinical presentation

Of 22 animals, 14 were dull on first presentation and one was somnolent. Five of 22 showed signs of hepatic encephalopathy and 9 of 18 had a body condition score of ≤3/9. Rough hair coats were seen in 17 of 21 equids and 2 of 19 animals were icteric. Breathing frequency varied from 8 to 28 breaths per minute (mean: 15 ± 5, n = 17). Heart rate ranged from 28 to 104 beats per minute (mean: 45 ± 18, n = 18). Body temperature ranged from 37.2 to 38.2°C (mean: 37.6 ± 0.3, n = 17). For more detailed information, see also Supplementary Item 1.

Haematology

Haematology tests at initial presentation (n = 16) revealed anaemia in five animals, increased white blood count (WBC) in 10 animals and leucopenia in one animal (Table 1, Supplementary Item 2).

Serum biochemistry

At initial presentation, gamma-glutamyltransferase (γGT) concentrations were increased in 21 of 22 animals. The alkaline phosphatase (ALP) concentrations were increased in all tested equids (n = 16), and 9 of 15 animals had increased aspartate aminotransferase (AST). Increased lactate dehydrogenase (LDH) was found in 14 of 15 equids. Bile acids were increased in 10 of 18 animals. Bilirubin was only increased in 3 of 16 cases. Ammonia was increased in 3 of 5 equids. Hyperproteinemia was seen in 7 of 16 equids and increased β2-globulins were found in 10 of 15 cases. Serum iron was increased in 15 animals (mean 70.7 ± 19.7 μmol/L, n = 19) and TIBC was increased in four (mean 78.0 ± 20.8 μmol/L, n = 19). Transferrin saturation was >80% in all tested animals (mean 90.4 ± 3.7%, n = 19). Serum iron, TIBC and transferrin saturation of a control group of 15 horses suffering from noniron related subacute to chronic hepatitis were as follows: serum iron mean 28.7 ± 8.5 μmol/L; TIBC mean 64.9 ± 12.5 μmol/L; transferrin saturation mean 44.6 ± 11.6%. Serum biochemistry results are presented in Table 1. For more detailed information, see also Supplementary Item 3.

Ultrasonography of the liver

Ultrasonographic evaluation of the liver was performed in six horses and showed rounded liver margins in five horses and increased echogenicity in four horses (Table 1). An image of the ultrasonographic evaluation of the liver of one of the affected horses is presented in Figure 1. For more detailed information, see also Supplementary Item 4.

Histology of the liver

Liver tissue was available for histologic evaluation in 12 of 22 equids. Histology showed varying degrees of hepatitis and fibrosis in all 12 animals, and moderate to severe haemosiderin accumulation was observed in macrophages, as well as in hepatocytes in all samples (Fig 2). For more detailed information, see also Supplementary Item 4.

Post-mortem examination

Nine equids were euthanised, ranging 0–53 months after diagnosis. Seven animals underwent post-mortem examination. The livers of these animals showed a varying reduction in size, often with a nodular aspect, an increased consistency and a 'rusty-brown' discolouration. Besides haemosiderin accumulation in the liver, this accumulation was also seen in the histology of other organs, such as the pancreas, lungs, spleen, brain, ganglion, muscles, kidneys, intestines, synovial membranes, lymph nodes, thyroid glands and adrenal glands. Images of a post-mortem examination are presented in Figure 3. For more detailed information, see also Supplementary Item 4.

Water analysis

All animals included in the current study had access to natural surface water. For most of these animals, this was the primary source of drinking water. Water samples collected from the ditches from which the horses were drinking were evaluated for iron content. All tested samples had increased iron levels, varying from 0.74 to 72.5 mg Fe/L and were deemed to be unsuitable as drinking water for animals (maximum acceptable level for iron in drinking water: 0.3 mg/L) [9].

Grass analysis

Grass from the farm from which the first 11 cases originated was collected for further analysis. The iron content of the grass was mildly increased, 227 mg/kg (reference value 215 mg/kg) but the grass was deemed suitable for consumption by animals.

TABLE 1: Diagnostic test results from equids diagnosed with chronic iron toxicosis

Blood analysis	n	Range	Mean	s.d.	Reference range
Haematocrit (L/L)	16	0.23–0.47	0.35	0.07	0.32–0.42
WBC ($\times 10^9$/L)	16	3.3–19.5	11.1	3.5	7.0–10.0
γGT (IU/L)	22	29–2940	818	826	<34
ALP (IU/L)	16	179–2378	635	515	71–153
AST (IU/L)	14	259–9414	1129	2388	224–492
LDH (IU/L)	15	481–5278	1150	1178	197–550
Bile acids (μmoL/L)	18	5–86	24	22	<12.8
Bilirubin (μmoL/L)	16	6.8–87.9	22.4	22.9	<35
Ammonia (μmoL/L)	5	17–247	100	95	<30
Fibrinogen (g/L)	4	1.6–2.1	1.8	0.2	1.0–4.0
Serum iron (μmoL/L)	19	48.8–121.5	70.7	19.7	18.0–54.0
TIBC (μmoL/L)	19	49.8–132.6	78.0	20.8	54.0–90.0
Transferrin saturation (%)	19	83.7–98.0	90.4	3.7	21–48
Total protein (g/L)	16	68–91	78	5	52–79
Albumin (g/L)	17	25–39	32	4	26–37
α1 globulins (g/L)	15	1–2	1	0	1–7
α2 globulins (g/L)	15	9–21	12	3	3–13
β1 globulins (g/L)	15	5–15	8	3	4–16
β2 globulins (g/L)	15	3–15	11	4	3–9
γ globulins (g/L)	15	8–22	14	4	6–19
Ultrasonography	**n**	**Present**	**Not present**		
Rounded liver margins	6	5	1		
Increased echogenicity	6	4	2		
Histology	**n**	**Present**	**Not present**		
Fibrosis	12	12	0		
Haemosiderin in hepatocytes	12	12	0		
Haemosiderin in macrophages	12	12	0		
Necropsy	**n**	**Present**	**Not present**		
Haemosiderin accumulation in other organs	7	7	0		
Outcome	**n**	**Alive**	**Euthanized**		
(1 Jan 2018)	22	13	9		

WBC, white blood count; γGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TIBC, total iron binding capacity.

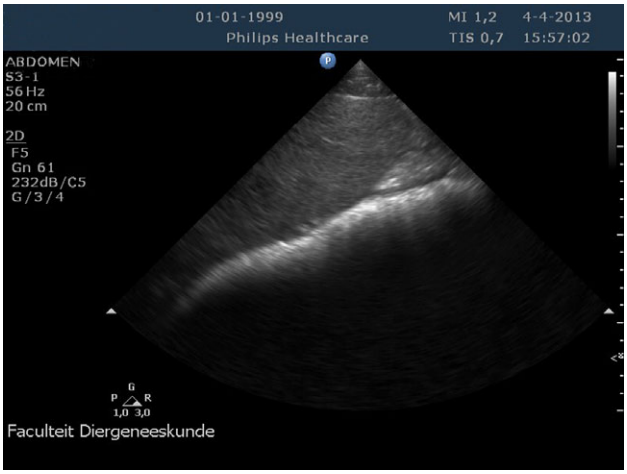

Fig 1: Ultrasonography of the liver in a patient with chronic iron overload. Increased echogenicity and rounded liver margins can be observed.

Soil analysis

Multiple soil samples were also collected from the farm from which the first 11 cases originated. Their total iron content ranged from 38,000 to 57,000 mg Fe/kg dry matter soil. The vast majority of iron, >99.9%, was ferric iron (Fe^{3+}). Ferrous iron (Fe^{2+}) was only present in very limited quantities, <0.1%.

Treatment

Supportive therapy was administered to affected animals based on their clinical symptoms at time of presentation including balanced electrolyte infusions, corticosteroids, antibiotics, nonsteroidal anti-inflamatory drugs, opioids, vitamin E supplementation and/or omeprazole. Chelation therapy and therapeutic phlebotomy were not attempted due to financial constraints and the fact that several animals were already slightly anaemic and that their general condition was already poor, indicating that adverse effects could be anticipated.

Follow-up

Five animals were euthanised immediately after the diagnosis of haemochromatosis and liver failure was made. Four horses progressed slowly, necessitating euthanasia 9–53 months post diagnosis because of the development of clinical signs of liver failure, such as hepatic encephalopathy. Of 22 animals, 13 survived until 1 January 2018 (17–79 months post diagnosis). Most surviving horses were reported to be relatively well at the end of the study but signs of chronic disease such as poor body condition and poor hair coats were present. Follow-up blood samples (collected 8–30 months after diagnosis) in six horses revealed stable blood transferrin saturation levels, which thus did not decrease after excessive oral iron intake was halted.

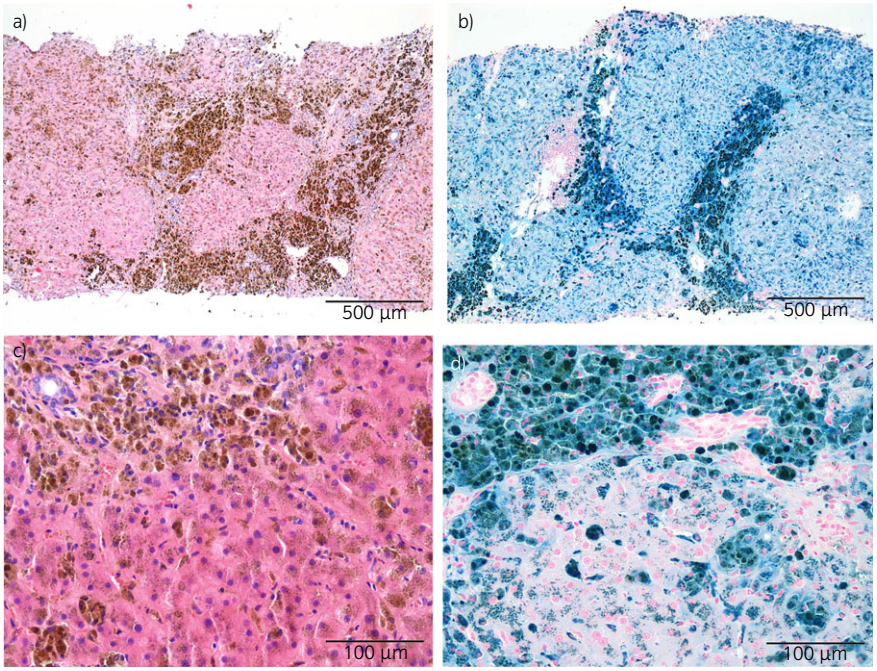

Fig 2: Histology of a liver biopsy by Haematoxylin & Eosin staining a), b) and Prussian Blue staining c), d). Excessive haemosiderin deposition can be observed in hepatocytes and macrophages. Bridging fibrosis is also present.

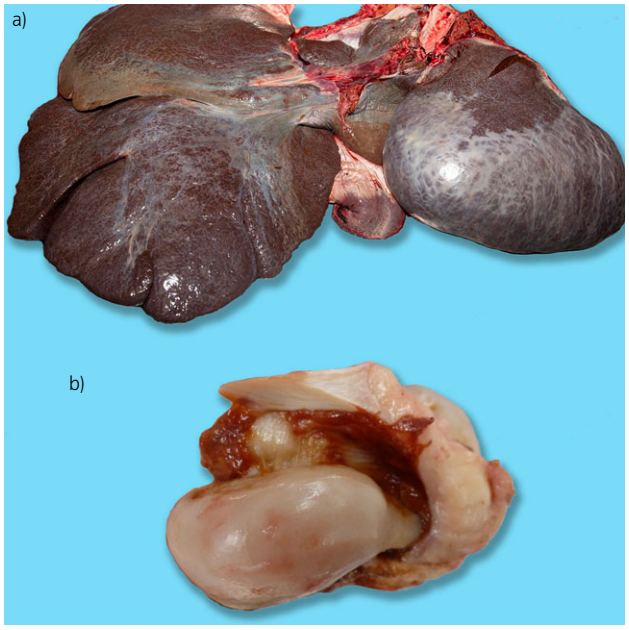

Fig 3: a) Fibrosis observed in the liver at post-mortem examination of a horse diagnosed with chronic iron toxicosis. b) Macroscopically visible haemosiderin deposition in the synovial membrane of a joint of a horse diagnosed with chronic iron toxicosis.

Discussion

This case series describes 22 equids with chronic iron overload leading to haemochromatosis and liver disease. Diagnosis was based on evidence of liver disease (n = 22) in combination with at least one of the following: 1) histopathological evidence of haemochromatosis in liver tissue (n = 12) and/or 2) blood transferrin saturation >80% (n = 19). A hereditary cause of the observed haemochromatosis in the equids included in the current study was considered unlikely, because they were genetically unrelated. Therefore, an acquired form of haemochromatosis was suspected. Chronic excessive iron intake originating from drinking water containing high iron levels was proven for all cases.

The primary biological function of iron is to be incorporated in haemoglobin, contributing to oxygen delivery to tissues. Iron is also a cofactor in several enzymes [8]. Iron uptake takes place mainly in the small intestine. After iron is taken up by enterocytes, the iron is transferred into the circulation where it is bound to transferrin. Transferrin distributes iron throughout the body. Iron in excess of need is stored intracellularly in hepatocytes and reticuloendothelial macrophages as ferritin and haemosiderin (aggregated ferritin without protein). Hepcidin is the hormone that regulates iron uptake and distribution. Upregulation of hepcidin reduces iron absorption from the gastrointestinal tract and increases the sequestration of iron in macrophages and organs such as liver and spleen. There is no mechanism for the active excretion of excess iron, so excessive iron is stored in the body. The organs most affected by iron overload are the liver, heart and pancreas.

Iron can act as a pro-oxidant through the catalysis of the formation of reactive oxygen species, thereby damaging cells and causing organ damage [8]. This oxidative stress is the underlying mechanism by which iron overload causes liver damage and potentially damages other organs. Iron overload also increases susceptibility to infections, as bacteria need a constant iron supply for proliferation. In humans, iron overload is also associated with neoplasia, cardiomyopathy, hepatitis C infection, arthropathy, hyperpigmentation and endocrine disorders [10].

Haemochromatosis is seen in many mammalian species, including Salers cattle, red deer, lemurs, rhinoceros and humans [11]. Most known forms of haemochromatosis in humans and animals are hereditary, caused by genetic mutations in iron regulatory genes [10]. Some forms of haemochromatosis are acquired, caused by excessive iron intake. 'African iron overload' in humans has been attributed to consumption of large quantities of traditionally fermented home-brewed beer rich in iron (although a genetic mutation may also be implicated) [12]. The pattern of histologically visible iron deposition in haemochromatosis caused by iron overload is different from hereditary forms of haemochromatosis. In iron overload haemochromatosis, iron accumulation is seen in both

hepatocytes and macrophages, whereas in hereditary haemochromatosis, iron accumulation is primarily seen in hepatocytes [10]. In the current study haemosiderin deposition was visible in hepatocytes and macrophages, supporting the theory that iron overload rather than a genetic mutation was the primary cause of haemochromatosis in these cases, although iron uptake by macrophages after haemosiderin release as a result of hepatic cell death cannot be excluded in severe cases.

In a previous study in donkeys, accumulation of hepatic iron was often seen in old donkeys (aged 21–57 years) but was not related to other pathological changes in the liver and was therefore most likely an incidental finding [13]. The donkey included in the current study was of middle age, 17 years old, and did show significant pathological changes in the liver, such as active hepatitis and extensive fibrosis. Furthermore, haemosiderin deposition was also seen in other organs. Given the fact that the donkey was drinking water with high iron content and also had a high transferrin saturation, we concluded that the donkey in the current study was suffering from iron toxicosis rather than showing hepatic iron accumulation related to old age.

In previous case reports on haemochromatosis in horses, no source of excess dietary iron was identified [4,5] and it was concluded that haemochromatosis was either of primary origin (hereditary) or secondary to severe hepatic damage. In an experimental study in which ponies were fed excessive amounts of iron, no histological lesions were seen in liver tissue after 8 weeks, although hepatic iron concentrations, serum iron concentrations, transferrin saturation and serum ferritin had increased [6] suggesting that iron toxicosis relating to short term increased dietary intake of iron was unlikely in horses. This conclusion was supported by previous studies suggesting that haemosiderin accumulation in horses with hepatopathies was most likely the result of primary hepatic disease and not the result of excess dietary iron [4,5]. The duration of the excessive iron intake in that study was, however, relatively short: 8 weeks in total. Given the fact that all affected animals in the current study were exposed to high dietary iron intake (due to excess iron content in drinking water) for prolonged periods (>9 years), we suspect haemochromatosis develops very gradually in equids and that the time frame in the previously mentioned study may have been too short for haemochromatosis to develop [6]. The primary source of the excessive iron in the current study was drinking water, whereas in the study by Pearson *et al.* ferrous sulphate was added to feedstuff [6]. The source of excess iron might also affect bioavailability of iron and therefore could have influenced development of disease. Previous reports only describe cases of acute or subacute iron toxicosis [1–3]. The current study shows that chronic iron toxicosis also occurs in equids after several years of exposure to high dietary iron (due to excess iron content in drinking water). Therefore, long-term excessive iron intake in equids should be avoided. A typical equine diet easily meets the daily iron requirement for horses [14]. Unless iron deficiency is diagnosed in a horse, iron supplementation should be avoided, especially long-term.

Due to its very high iron content, drinking water was identified as the main source of dietary iron for all cases in the current study, although the unintended ingestion of iron rich soil might have contributed to development of disease as well. It is important to note that high iron levels in water are not always visible by simple observation. Two different forms of iron can be found in water: ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}). Fe^{3+} is visible as an orange brown discoloration of the water, whereas Fe^{2+} is colourless when dissolved in water. Fe^{2+} is more bioavailable than Fe^{3+}, but due to the low pH in the stomach of equids most Fe^{3+} will be converted to Fe^{2+}, so both forms of iron pose a threat to the animal if taken up in large quantities.

The origin of the iron in the ditch water that was responsible for these equids' condition is currently unknown. It might be a natural phenomenon relating to iron deposition by ground water rising to the surface in the Dutch polders (reclaimed land from the sea), which is caused by continuous upward water pressure in this low-elevation land (below sea level). Iron contamination of surface water by human activity is another possibility.

Increased serum iron and increased transferrin saturation (>80%) are indicative of iron overload. These parameters were more severely increased in horses suffering from hepatopathy caused by iron overload compared to horses suffering from hepatitis by other causes. Hepatic enzymes, especially γGT, can be used to assess liver damage and functional parameters, such as bile acids, can be used to monitor liver function. A definite diagnosis of haemochromatosis can only be made by histological evaluation of liver tissue obtained by performing a liver biopsy or at a post-mortem examination.

The treatment of equids diagnosed with haemochromatosis is mainly supportive in nature. Chelation therapy can be considered but is costly. Phlebotomy can also be considered as treatment to remove excessive iron from the body but there is a potential risk of clinical deterioration if animals are chronically ill and already anaemic. In humans with chronic anaemia and iron overload, therapeutic phlebotomy is also controversial and phlebotomy treatment in humans does not improve already established liver cirrhosis [10,15]. More research into the effect of phlebotomy in equids is needed to determine the usefulness of this technique. Identifying the source of the excessive iron and limiting further intake is an important aspect of clinical management in affected animals. The removal of iron-rich feedstuff and removing access to water containing high levels of iron is critical.

All 22 cases were diagnosed between 1 June 2011 and 31 August 2016. Of 22 equids, 13 were still alive on 1 January 2018, 10 of which had been diagnosed more than 3 years earlier. In a study on the prognostic value of liver biopsies, severe haemosiderin accumulation was negatively associated with survival [16]. Based on the cases included in the current study we concluded that, although iron toxicosis can be fatal, progression of disease is slow and, if further iron intake is minimised, horses may survive for several years after diagnosis.

Limitations

Our study has some limitations. Ideally, a definite diagnosis of iron toxicosis is based on increased transferrin saturation and evidence of haemochromatosis on histological evaluation of a liver biopsy. A liver biopsy, however, is an invasive and costly procedure. Therefore, this exam was not performed in all cases. All horses for which histological evaluation of liver tissue was not available, had increased transferrin saturation (>80%) and biochemical evidence of liver damage. We did not measure iron content of liver tissue in any animals but histology demonstrated excessive iron deposition in all cases. It would also have been interesting to measure hepcidin levels in the affected equids.

Conclusions and clinical relevance

The current study shows that chronic iron overload can lead to haemochromatosis and liver disease in equids, which can be fatal if not detected at an early stage. The development of disease is slow and clinical signs are subtle until haemochromatosis is severe. A definite diagnosis of iron toxicosis can only be made by histological evaluation of a liver biopsy. Long-term excessive iron intake in equids, either from the administration of supplements or from natural sources, should be avoided. It is therefore also important to test water for iron content, especially for animals drinking from natural water sources.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight not required by this journal: descriptive clinical report. Explicit owner informed consent for inclusion of animals in this study was not stated.

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Authorship

All the authors have made substantial contributions to this study. M. Theelen, M. Beukers, G. Grinwis and M. Sloet van Oldruitenborgh-Oosterbaan were all involved in the clinical diagnostic workup and/or treatment of the patients included in the study and the acquisition of the data. All the authors have contributed to drafting the article and revising it critically for important intellectual content and all the authors approved of the final version to be published.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Summary in Spanish.

Supplementary Item 1: General information, clinical examination and outcome of equids diagnosed with chronic iron toxicosis.

Supplementary Item 2: Haematology results from equids diagnosed with chronic iron toxicosis.

Supplementary Item 3: Clinical chemistry results from equids diagnosed with chronic iron toxicosis.

Supplementary Item 4: Additional diagnostic test results from equids diagnosed with chronic iron toxicosis.