LIVER DISEASE IN THREE HORSES

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Signalment:

Patient 1: Pony, 18 years old, sex not reported

Patient 2: Haflo-Arab, 14 years old, sex not reported

Patient 3: Islandic Horse, 18 years old, male

Clinical findings: as far as clinical findings were reported or asked from the clinicians they included: anorexia, abdominal pain and jaundice of mucous membranes.

Lab diagnosis:

A number of biochemical parameters and a CBC were performed in all three patients (**Tables 1, 2**).

Table 1: Haematology results (Advia 2120i, Siemens Healthcare Diagnostics, Tarrytown, USA)

Analytes (units)	Patient 1, Day 1	Patient 2, Day 1	Patient 2, Day 10	Patient 3	Reference Intervals
RBC (T/L)	8.7	10.5	8.2	7.1	6.5-11
HGB (g/L)	169	176	142	116	10-18
HCT (L/L)	0.51	0.51	0.41	0.34	0.32-0.53
MCV (fL)	↑ **58.5**	48.3	49.7	47.6	37-55
MCH (pg)	↑ **19.4**	16.8	17.3	16.3	13-19
MCHC (g/L)	↓ **332**	↓ **348**	↓ **348**	↓ **342**	360-400
WBC (G/L)	↑ **12.4**	4.2	5.8	5.6	5-10
NEUTROPHILS (G/L)	↑ **10.29**	3.32	4.86	2.84	3-7
LYMPHOCYTES (G/L)	1.98	↓ **0.48**	↓ **0.71**	2.11	1.5-4.0
MONOCYTES (G/L)	0.12	0.39	0.2	0.13	-0.5
EOSINOPHILS (G/L)	0	0	0.01	0.52	-0.35

BASOPHILS (G/L)	0	0.01	0.02	0	-0.15

Table 2: Biochemistry results (Dimension EXL 200, Siemens Healthineers, Frimley, Camberely, UK).

Analytes (units)	**Patient 1**	**Patient 2, Day 1**	**Patient 2, Day 10**	**Patient 3**	**Reference Intervals (SI)**
Glucose (mmol/L)	↓ **2.71**	↑ **6.66**	n.t.	5.00	3.05-5.27
Urea (mmoL/L)	↓ **2.16**	↓ **2.33**	n.t.	5.1	3.33-6.66
Creatinine (µmol/L)	141.4	106	n.t.	11.9	-176.8
Bilirubin, total (µmol/L)	43	↑ **62**	↑ **55**	23.4	12.0-53.0
Bilirubin, direct (µmol/L)	↑ **6**	↑ **10**	↑ **13.2**	0.23	1.7-5.1
Bilirubin, indirect calc,(µmol/L)	37.3	↑ **51.3**	42	1.14	10.3-47.9
ALP (U/L)	44	↑ **1456**	↑ **1358**	↑ **244**	-350
AST (U/L)	4	↑ **693**	↑ **847**	↑ **549**	-500
GGT (U/L)	↑ **271.4**	↑ **435**	↑ **486**	↑ **101**	-30
LDH (U/L)	↑ **587**	↑ **1629**	↑ **1464**	↑ **741**	-450
CK (U/L)	17	↑ **453**	n.t.	↑ **233**	-200
Total protein (g/L)	68.4	↑ **77.4**	↑ **85.9**	69.1	55-75
Triglycerides (mmol/L)	↑ **0.66**	↑ **9.27**	n.t.	0.23	- 0.57
Cholesterol (mmol/L)	↑ **4.66**	↑ **4.66**	↑ **4.99**	2.5	0.5-3.1
Calcium, total (mmol/L)	2.6	2.7	n.t.	3.0	2-3.20

Phosphorus (mmol/L)	1.2	0.8	n.t.	0.9	0.5-1.3
Magnesium (mmol/L)	↓ **0.5**	↓ **0.5**	n.t.	0.8	0.7-1.2
Iron (µmol/L)	23.2	n.t.	n.t.	n.t.	11.8-37.8
Sodium (mmol/L)	144	n.t.	n.t.	n.t.	126-157
Potassium (mmol/L)	4.2	n.t.	n.t.	n.t.	3.5-4.5

Questions:

1. What are the major differentials for liver disease in horses?
2. How could you reach a final diagnosis?

Diagnosis: Poisoning with common ragwort (*Senecio jacobaea*), confirmed in 1 case, suspected in 2 others.

Haematology interpretation:

Patient 1: The complete blood count (CBC) revealed an increased mean cell volume (MCV) 58.5 fL, (RI: 37-55 fL); increased mean corpuscular haemoglobin (MCH) 19.4 pg, (RI: 13-19 pg); decreased mean cell haemoglobin concentration (MCHC) 33.2 g/dL, (RI: 36-40 g/dL). The increased MCV is possibly due to recent regenerative responses as the horse was not anaemic and are no signs of regeneration in the blood smear such as polychromasia. An increased MCH is an artefact as it is not possible that MCH is increased beyond the upper reference limit. The white blood cell count (WBC) is increased 12.40 G/L, (RI:5-10 G/L); with concurrent neutrophilia and lymphopenia which could be due to corticosteroid-associated causes such as stress in combination with an inflammatory process or infection.

Patient 2: The complete blood count (CBC) revealed a decreased mean haemoglobin cell concentration (MCHC) 34.8 g/dL, (RI: 36-40 g/dL); which remained the same during the second measurement. In other species it is associated with a regenerative response where reticulocytes are released in large numbers.

Patient 3: The complete blood count (CBC) revealed a decreased mean haemoglobin cell concentration (MCHC) 34.2, (RI: 36-40 g/dL).

Biochemistry interpretation:

Patient 1: The biochemistry results in this patient showed an increase in gamma-glutamyltransferase (GGT) 271.4 U/L, (RI: -30 U/L); and a slight increase in direct bilirubin: 6 µmol/L, (RI: 1.7-5.1 µmol/L), which is associated with cholestasis and possible biliary hyperplasia. Glucose 2.71 mmol/L, (RI: 3.05-5.27 mmol/L) and urea 2.16 mmol/L, (RI: 3.33-6.66 mmol/L) are decreased which supports the thesis of impaired liver function. Lactate-dehydrogenase (LDH) 587 U/L, (RI: -450 U/L); is increased what suggests liver damage although it is normally located in cardiac and skeletal muscle too. Magnesium (Mg) 0.5 mmol/L, (0.7-1.2 mmol/L); is decreased suggesting a gastrointestinal disturbance, the other two differential diagnosis endotoxaemia or sepsis are less probable since there is no evidence of a left shift or toxic changes in the neutrophils (15). Cholesterol (CHOL) 4.66 mmol/L, (RI: 0.5-3.1 mmol/L); could be elevated due to postprandial effects or due to liver damage with cholestasis.

Patient 2: The biochemistry results in this patient showed significantly increased liver enzymes alkaline-phosphatase (ALP) 1456 U/L, (RI: -350 U/L); aspartate-transferase (AST) 693 U/L, (RI: -500 U/L); glutamate dehydrogenase (GLDH) 57.5 U/L, (RI: -13 U/L); GGT: 435 U/L, (RI: -30 U/L); lactate dehydrogenase (LDH) 1629 U/L, (RI: -450 U/L) as well as indirect and direct bilirubin. This changes suggest cholestasis and possible biliary duct hyperplasia as in the previous patient. Urea 2.33 mmol/L, (RI: 3.33-6.66 mmol/L) is decreased suggesting liver impairment together with the previous data and the glucose concentration (GLU) 6.66 mmol/L, RI: (3.05-5.27 mmol/L); is above the upper reference limit which could be due to stress (glucocorticoids), pain (catecholamines) or postprandial. The increased level of triglycerides 9.27 mmol/L, (RI: 0.57 mmol/L) ; and cholesterol 4.66 mmol/L ,RI: (0.5-3.1 mmol/L) would support a postprandial effect but the high cholesterol level can also be due

to liver damage and cholestasis. The low magnesium (MG) 0.5 mmol/L, (RI: 0.7-1.25 mmol/L) suggests a gastrointestinal disturbance.

In this patient a control of the liver enzymes was conducted on day 10 and showed no significant changes except worsening of GGT 486 U/L, (RI: -30 U/L); AST 847 U/L,(RI: -500 U/L) and higher total protein levels 85.9 g/L, (RI: 55-75 g/L).

Patient 3: The biochemistry profile of the third patient showed elevated liver enzymes aspartat-transferase (AST) 549 U/L, (RI: -500 U/L); glutamate-dehydrogenase (GLDH) 31.3 U/L, (RI: -13 U/L); (GGT) 101 U/L, (RI: -30 U/L); laktat dehydrogenase (LDH) 741 U/L, (RI: -450 U/L); and elevated creatin-kinase (CK) 233 U/L, (RI: -200 U/L). The AST increase can be due to muscle injury (cardiac or skeletal), hepatocyte injury or both. Creatin-kinase is increased due to muscle injury as a majority of serum CK is of muscle origin.

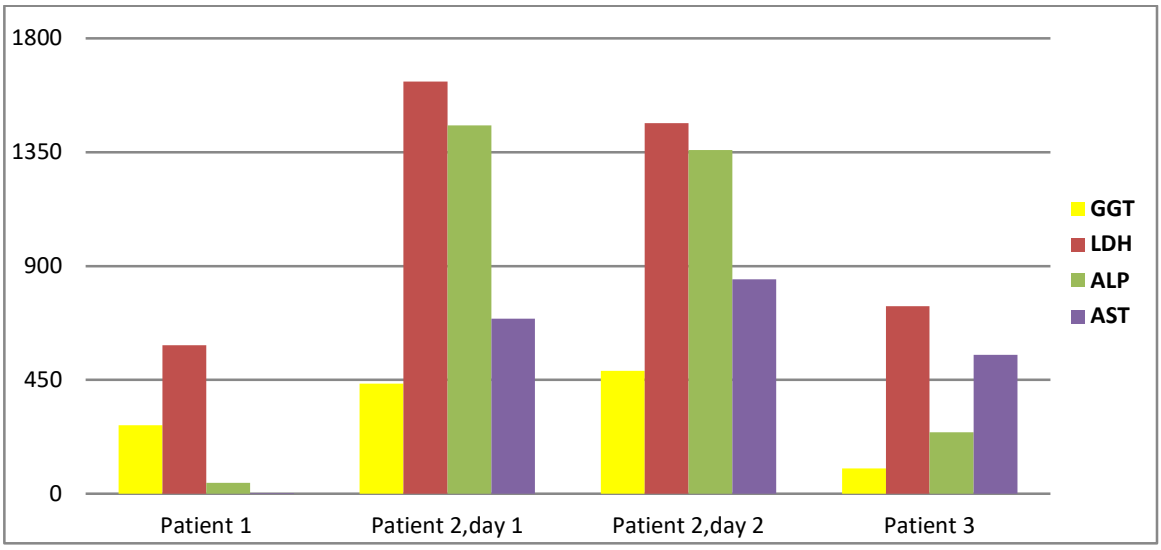

Fig. 1: Liver enzyme abnormalities

Discussion: The *Senecio* species (*Compositae* family) is composed of more than 1200 species distributed worldwide of which 30 have produced human and livestock poisoning such as " stomach staggers", "walking disease", "Pictou disease", etc. (1,2). These plants contain a series of pyrrolizidine alkaloids (PA). Common ragwort (lat. *Senecio jakobea*) typically contains six pyrrolizidine alkaloids (PA): seneciphylline, senecionine, jacozine, jacobine, jacoline and jaconine which are present as free bases and N-oxides (3). The PA are not directly toxic. They are apsorbed in the GI tract and then the bioactiviation occurs in the liver by mixing function oxidases to toxic dehydropyrrolizidine alkaloids (pyrroles) (4). The damage of the liver tissue occurs because the pyrroles are electrophiles that bind to DNA, proteins, amino-acids and glutathione with resultant cytotoxic and antimitotic damage (1).

Pyrrolizidine alkaloid toxicosis associated with consumption of common ragwort can cause large economic losses in livestock industry. The most resistant are goats and sheep, while pigs are less susceptible then horses (16). This plant is unpalatable but can usually be admixed with hay or silage (8). The clinical symptoms are a result of liver damage and cholestasis and include jaundice and CNS symptoms such as aimlessly walking and ptyalism due to hepatic encephalopathy. (5,7) Other clinical symptoms that can occur are deprived appetite, anorexia and depression (5,9). From the internal

organs the liver is most affected with typical increases in liver enzymes, most commonly GGT (5,7,8) which is also present in all three of our cases. There are a few reported cases with ragwort poisoning where an increased WBC count (7,8) is described as in patient 1 in our report who had also confirmed poisoning with common ragwort. The typical pathohistologic changes in the liver include focal hepatocyte necrosis (piecemeal necrosis), minimal peri-biliary fibrosis and mild duct proliferation and the damaged hepatocytes can develop into large megalocytes (4,5). Hepatic megalocytosis has been cited as a hallmark of pyrrolizidine alkaloid poisoning (6). A case report including 110 horses for over 4 years on a farm in Costa Rica died after showing neurological symptoms, pathology reports of liver changes from two sick horses that were introduced to the Veterinary school matched those present in the literature. The liver appeared to be severely damaged with interlobular fibrosis, hepatocellular anisomorphia characterized by megalocytosis, retention of bile pigments, vacuolar and fatty degeneration and proliferation of the bile ducts (9).

The diagnosis of poisoning is based on typical pathohistological changes (4,5) as well as detection of ragwort alkaloids in hay or silage using liquid chromatography/time of flight mass spectrometry (3). This was done for hay in patient 1.

Humans that have ingested foods such as herbal teas, milk, honey and contaminated grains in addition to herbal remedies containing pyrrolizidine alkaloids developed a hepatic sinusoidal obstruction syndrome (HSOS) with symptoms such as hepatomegaly, bilirubinaemia and ascites (10,11). The mechanism of development is primary vascular in nature which is supported by a study in which rats ingested PA and the sinusoidal endothelial cells where more affected than the hepatocytes (12).

Common ragwort is widely spread in many regions in Austria. The most toxic parts are young leaves and flowers (16). While horses avoid larger plants on pastures, plants or plant parts in hay or silage are eaten. In the other two patients a ragwort poisoning was suspected because of the common appearance of the plants on those regions and previous poisonings there.

The two main differential diagnosis in horses could include aflatoxicosis and Tyzzers disease (Clostridium piliformae) due to the similar clinical presentation, biochemical and pathohistological changes (13,14). The mycotoxins such as aflatoxin from fungal species *Apergilus flavus* causes an increase in liver enzymes GGT and ALP and pathohistological changes which include megalocytosis und biliary hyperplasia. (13)

Horses with Tyzzer´s disease which were investigated in a retrospective study from 1969-2000 showed clinical-pathological findings which included increased liver enzymes (AST, LDH, ALP) and elevated bilirubin concentrations (14).

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