Enzootic ataxia associated with copper deficiency in a farmed red deer: a case report

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ABSTRACT: The occurrence of enzootic ataxia in a farmed red deer (*Cervus elaphus*) is reported. A nine-year-old male presented with progressive ataxia, hind limb weakness, sudden falling down while running and temporary inability to rise. Gross and histopathological examinations of organs, haematology, serum biochemistry and measurement of concentrations of copper and molybdenum in the liver were done. Examination of fresh liver tissue and blood serum revealed very low copper concentrations of 15.7 mmol/kg and 1 mmol/l, respectively. Histopathology revealed typical severe bilaterally symmetrical vacuolisation of white matter of pons cerebelli and spinal cord, and hepatic haemosiderosis. According to the clinical signs and laboratory results, a diagnosis of enzootic ataxia due to copper deficiency was made. To the best of our knowledge, these cooper values in blood serum and liver tissue are the lowest ever reported in deer with clinically expressed enzootic ataxia.

Keywords: *Cervus elaphus*; enzootic ataxia; copper; diagnosis

Copper (Cu) is an essential microelement that plays a variety of biochemical and metabolic roles in animals (Grace 1983). Cu deficiency causes various syndromes in domestic ruminants (MacPherson 1989) that have also been described in deer (Audige et al. 1995; Wilson and Grace 2001). In farmed deer, Cu deficiency is commonly associated with enzootic ataxia (Wilson et al. 1979; Peet and Hepworth 1993; Audige et al. 1995; Geisel et al. 1997; Soler and Cseh 2007), osteochondrosis and skeletal abnormalities (Thompson et al. 1994; Audige et al. 1995). Cu deficiency may be primary, associated with low Cu intake, or secondary, when Cu absorption or metabolism is adversely affected due to high levels of molybdenum (Mo), iron (Fe), sulphur (S) or zinc (Zn) in the diet (Grace 1983). According to Suttle (1991) and Smith et al. (2006) a dietary excess of Mo is one of the most common reasons for secondary Cu deficiency. This paper reports a case of enzootic ataxia in a farmed red deer (*Cervus elaphus*) associated with low concentrations of serum and liver Cu.

Case description

A nine year old male red deer showed ataxia, hind limb weakness, sudden falling down while running

and temporary inability to rise. The flight response was slow and the animal was in poor condition. The outbreak occurred on a 9 ha enclosure established 15 years ago in the region of the Slovenske Gorice. The farm is divided into three equal paddocks, on which approximately 25 red deer, 30 fallow deer and 15 mouflons are kept. Animals graze perennial ryegrass in the summer, while they are fed with baled grass silage during the winter. Hay and salt lick blocks are available *ad libitum* all year round. Food supplements (e.g. trace elements) have not been administered for several years. The affected deer developed hind limb weakness two weeks before the farmer euthanised it by shooting. The farmer stated that similar clinical signs that lasted several weeks were observed in two hinds and one red deer stag in previous years; however, the veterinary examination and treatment of these animals was not performed.

The animal was shot and a gross examination of organs was conducted on the field. Blood samples for haematological and biochemical examinations were collected from the heart within a few minutes after the animal's death. Samples from the cerebellum, cervical spinal cord and liver were taken for histopathological examination, fixed in 10% neutral-buffered formalin and routinely paraffin-
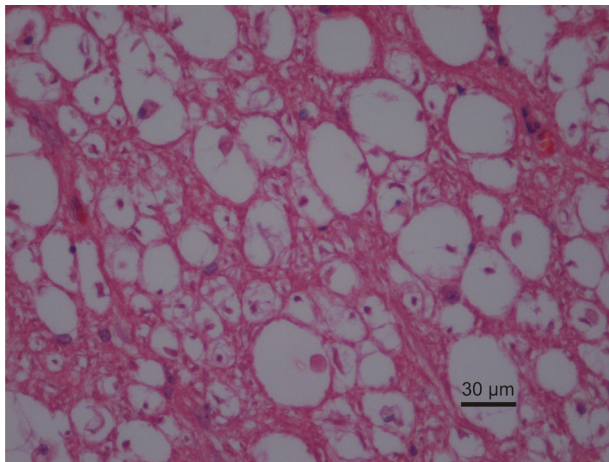
Figure 1. Severe vacuolation with loss of the axons in the white matter of the spinal cord; HE staining, bar = 30 μm
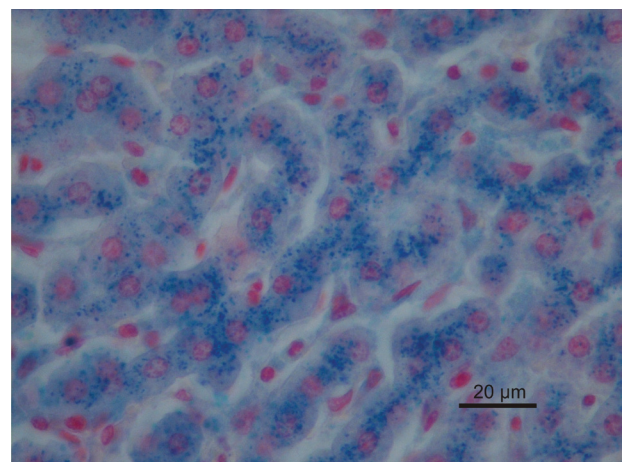

Figure 2. Severe haemosiderosis of hepatocytes; Prussian blue staining, bar = 20 μm

embedded. Four-μm thick sections were stained with haematoxylin and eosin and examined under a light microscope. Fresh liver samples were collected for the trace element analysis.

Pathological findings. The animal was in poor condition, but antlers were apparently in good shape. Its coat was dull and dry, and exhibited hair loss in the right hip region. Small multifocal haemorrhages were found in several skeletal muscles while extensive haemorrhages were present in the right triceps brachii muscle. No significant gross pathological changes were detected in the other examined organs. Histopathology revealed severe bilaterally symmetrical vacuolation of the white matter of spinal cord and pons cerebelli. Myelin sheets were severely dilated and with small, eosinophilic remnants of the axons and infiltration

of single Gitter cells (Figure 1). Small groups of very small, shrunken neurons with picnotic nuclei were also noted in the pons cerebelli, and small multifocal haemorrhages were found in the pons cerebelli and in meninges. Hepatocytes and Kupffer cells contained abundant haemosiderin deposits, positive for Prussian blue (Figure 2).

Haematological and biochemical findings. The haematological findings (using an automated haematology analyser (SCIL Vet ABC Plus)) and the majority of biochemical values (using the RX Daytona biochemical analyser (Randox Laboratories Ltd., Ireland), with the enzyme assay performed at 37 °C) were in the normal range for red deer. The results of the measurement of various blood constituents are given in Tables 1 and Table 2. Regarding the biochemical constituents,

Table 1. Results of serum biochemistry from the affected deer

Parameter	Value	Parameter	Value
AST (IU/l)	114	Glucose (mmol/l)	21.3
ALT (IU/l)	46	Fe (μmol/l)	13
LDH (IU/l)	479	Na (mmol/l)	140
GGT (IU/l)	56	K (mmol/l)	10.7
Urea (mmol/l)	7.8	Cl (mmol/l)	101
Creatinine (μmol/l)	133	Ca (mmol/l)	2.55
CK (IU/l)	266	Mg (mmol/l)	0.91
TSP (g/l)	76.3	iP (mmol/l)	2.17
Albumin (g/l)	32.1	Se (μg/l)	75.6
Triglycerides (mmol/l)	0.53	Cu (μmol/l)	1

AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; GGT = gamma glutamyltransferase; CK = *creatine* kinase; TSP = total serum protein; Fe = iron; Na = sodium; K = potassium; Cl = chloride; Ca = calcium; iP = inorganic phosphorus; Mg = magnesium; Se = selenium; Cu = copper

Table 2. Results of haematology from the affected deer

Parameter	Value	Parameter	Value
RBC (× 10^{12}/l)	7.68	Ne (%)	46
Ht (%)	33.2	Eo (%)	4
Hb (g/dl)	10.5	Ba (%)	1
MCV (fl)	43	Ly (%)	43
MCH (pg)	13.7	Mon (%)	5
MCHC (g/dl)	31.7	PLT (× 10^9/l)	183
WBC (× 10^9/l)	5.3		

RBC = erythrocytes; Ht = haematocrit; Hb = haemoglobin concentration; MCV = mean cell volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; WBC = leucocytes, Ne = neutrophil granulocytes; Eo = eosinophil granulocytes; Ba = basophil granulocytes; Ly = lymphocytes; Mon = monocytes; PLT = platelets

the serum activity of creatine kinase (CK) (normal 198 IU/l (Wilson and Pauli 1983)) and gamma glutamyltransferase (GGT) (normal 19.5 IU/l (Wilson and Pauli 1983)) and the concentration of glucose were elevated (normal 6.9 IU/l (Wilson and Pauli 1983)), while concentrations of iron (Fe) (normal 30.8 mmol/l (Woodbury 2002)) and Cu (normal 8 mmol/l (Mackintosh et al. 1986)) in serum were considered below the normal range for red deer. Inductively coupled plasma mass spectrometry (ICP-MS) was used for detection of Cu and Mo concentration in liver tissue and selenium (Se) in blood serum. Examination of fresh liver tissue revealed a strikingly low Cu concentration of 15.7 mmol/kg (normal 100 μmol/kg fresh tissue (Mackintosh et al. 1986; Wilson and Grace 2001) and a normal molybdenum (Mo) concentration of 19.4 mmol/kg (up to 40 mmol/kg in a wide range of animal species (Underwood 1977)).

DISCUSSION AND CONCLUSIONS

The clinical condition presented here was similar to cases described in the literature for enzootic ataxia in red deer (Wilson et al. 1979; Peet and Hepworth 1993; Geisel et al. 1997; Handeland and Flaoyen 2000; Soler and Cseh 2007). According to Millar et al. (2003), culling is the only option for severely affected animals as the demyelination characteristic of enzootic ataxia is irreversible. The diagnosis of enzootic ataxia in our case was based on clinical findings, the presence of low Cu level in blood and liver tissue and typical microscopic lesions in the brain and spinal cord of the affected animal. The liver is the central storage organ for Cu and a reliable index of Cu reserves in the body

(O'Cuill et al. 1970; Grace 1983). The level of Cu in serum is controlled by homeostatic mechanisms and can be normal also when liver Cu levels are low (Clark and Hepburn 1986). However, if the Cu levels in the liver become depleted, as a result of a Cu-deficient diet, the level of Cu in serum will decline (Grace 1983; Mackintosh et al. 1986). Nevertheless, while the serum Cu concentration may be used to confirm adequacy or inadequacy at the time of sample collection, it cannot be used to predict liver Cu storage (Wilson and Grace 2001). Enzootic ataxia in red deer occurs when the liver Cu concentration is below 60 μmol/kg fresh tissue and serum Cu concentration is below 3–4 μmol/l (Wilson and Grace 2001). In the present study analysis of fresh liver tissue and blood serum revealed Cu concentrations of 15.7 mmol/kg and 1 mmol/l, respectively. These concentrations were significantly lower than any reference values published for the stated species. Some authors even suggested that liver Cu concentrations above 100 μmol/kg fresh tissue and serum Cu concentrations above 8 mmol/l in deer reflect an adequate Cu status (Mackintosh et al. 1986; Wilson and Grace 2001). Enzootic ataxia was also confirmed by histological examination of brain and spinal cord. Histopathological examination revealed vacuolisation of cerebellum white substance and spinal cord with no inflammatory response and neuronal degeneration of the base of the cerebellum. Similar histopathological lesions were found in several other outbreaks of enzootic ataxia in farmed red deer (Wilson et al. 1979; Peet and Hepworth 1993; Handeland and Flaoyen 2000; Millar et al. 2003; Soler and Cseh 2007). Marked haemosiderin deposition in hepatocytes and Kupffer cells was found in the liver. Haemosiderin deposition in Kupffer cells was also reported by

Peet and Hepworth (1993) in an ataxic red deer. Diffuse haemosiderosis is a common finding in emaciated animals (Kelly 1993; Josefsen et al. 2007), and an increased amount of liver iron in winter has been reported in Svalbard reindeer (Borch-Iohnsen and Nilssen 1987); therefore, liver siderosis can be a result of catabolism of blood and lean tissue during a period of sub-maintenance food intake. On the other hand, according to Watts (1989) copper deficiency impairs Fe absorption, reduces haem synthesis, and increases Fe accumulation in storage tissues. This can influence serum Fe concentration, which usually decreases as also occurred in our case. Cu deficiency can also result in haemosiderosis, due to impaired reutilisation of haemoglobin Fe (Watts 1989) or in haemolytic anaemia (Harvey 2008). The accumulation of large amounts of Fe in hepatocytes results in hepatocellular injury (Harvey 2008). Hepatic haemosiderosis was presumably the reason for serum GGT elevation as GGT is one of the most sensitive indicators known for monitoring and diagnosing hepatobiliary disease (Turk and Casteel 2008). Increased serum concentrations were also observed for CK and glucose. Enzyme CK is found primarily in the heart and skeletal muscles and is the most sensitive indicator of muscular disorders (Chapple et al. 1991). Considerable injury to any of these tissues will lead to a measurable increase in CK concentrations (Ishak 2004). Similarly a gunshot wound might cause increased enzyme activity; however, in shot red deer only a non-significant increase in CK activity was reported (Kent et al. 1980). The elevated CK in our case could be the result of muscle injuries due to repeated falling down. High glucose levels in deer may be associated with stress, which activates the sympathetic nervous system and therefore increases the secretion of adrenaline. Within a few seconds adrenaline accelerates the conversion of glycogen to glucose, which is released from the liver into the blood stream. It seems that stress levels have a major influence on blood glucose values (Kolb et al. 1995).

The mineral composition of the foodstuff can have major effects on Cu availability (Suttle 1986). Elevations of dietary Mo and Fe can interfere with proper Cu utilisation (Grace 1983; Audige et al. 1995). Considering that the hepatic concentration of Mo was within normal ranges and serum concentration of Fe was under the normal range, we suggest that the low Cu concentration was not related to high Mo or Fe ingestion, although high levels of Mo in the liver rapidly return to normal when

the administration of extra Mo ceases, which may limit its value for diagnosis (Underwood 1977). In our case the diagnosis of enzootic ataxia was based on the low concentrations of serum and liver Cu and typical histopathological lesions. Nevertheless, further studies with complete mineral profiles of pasture, soil and feed, as well as monitoring liver Cu concentrations in all seasons are required to better understand the complex mineral interactions occurring in the enclosure and the grazing animals. We advised the owner to start immediately with the feeding of supplements rich in trace elements and vitamins which are proven to be effective in preventing Cu deficiency in farmed deer (Haigh and Hudson 1993). However, the farmer refused further cooperation with the veterinary service.

Acknowledgement

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