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Technical note

Enzootic ataxia due to copper deficiency in captive red deer (*Cervus elaphus*) in Colima, Mexico

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Abstract:

The objective of the study was to describe a case of enzootic ataxia in a captive *Cervus elaphus* (red deer) associated with copper deficiency, in the state of Colima, Mexico. In July and October 2018, two female red deer aged 3 and 7 yr manifested incoordination with weakness of the hind limbs and an anatomopathological diagnosis of progressive ataxia was established. In September 2019, a 13-yr-old female showed nervous signs similar to the 2018 cases, so a blood sample was taken for serum copper measurement. The animal was euthanized for *post-mortem* examination and tissue samples were collected for histology, liver, kidney, forage and soil samples were also taken for copper and molybdenum measurement. The main lesions were found microscopically in spinal cord, which showed leukomalacia, demyelination, spheroid bodies and neuronal chromatolysis. The copper concentration was 2.7 in liver, 4.67 in kidney and 0.08 in serum (mg/kg DM or ppm). The Cu:Mo ratio for soil 1 was Cu 8.48; Mo 3.00; Cu:Mo 2.83:1, soil 2: Cu 9.10; Mo 3.00; Cu:Mo 3.03:1. Forage 1: Cu 6.59; Mo 7.35; Cu:Mo 0.90:1; forage 2: Cu 2.77; Mo 6.12 ± 0.61; Cu:Mo 0.45:1. Clinical signs, microscopic lesions, and low levels of Cu in serum, liver, and forage are consistent with enzootic ataxia due to primary copper deficiency. As far as known, this is the first report of enzootic ataxia in a captive red deer in Mexico.

Key words: *Cervus elaphus*, Colima, Copper, Enzootic ataxia, Red deer.

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Red deer (*Cervus elaphus*) is prone to metabolic diseases when bred in captivity. Copper (Cu) deficiency in this species has been associated with osteochondrosis, enzootic ataxia and poor growth in young deer[1-4]. These syndromes have been recognized in farmed deer, but not in wild populations. Enzootic ataxia is a metabolic disease of the deer that causes slow and progressive paralysis mainly affecting the hind limbs. Clinical signs are the result of leukomyelomalacia, that is, necrosis of the white matter of the spinal cord. Microscopically, it is characterized by a demyelination of the spinal cord axons associated with a copper deficiency[5,6,7]. In addition, there may be degenerative changes in neurons of the brain or cerebellum, which also exhibit lysis or nuclear rexis and only in some cases cerebrocortical necrosis occurs with acute cerebral edema[5,7]. The morbidity of this disease is low, less than 1 %, although in some cases it can be up to 13 %[8,9,10]. Enzootic ataxia in deer has been described in Europe and New Zealand, where it is considered a widespread health problem in red deer farms[2,11,12]. In America, it has been reported in red deer from Argentina[5].

In Mexico, there are 54 Management Units for wildlife conservation (UMAs, for its acronym in Spanish) of *Cervus elaphus* and they are of extensive type, distributed in 16 states of the Mexican Republic, and Colima has one of them[13]. In Mexico, there have been no reports of enzootic ataxia in the UMAs of captive red deer, so it is important to describe for the first time the presence of this type of disease. The objective of this work was to describe the clinical and pathological aspects of enzootic ataxia in a captive red deer (*Cervus elaphus*) and its relationship with copper deficiency, in the state of Colima, Mexico.

The "Rancho el Peregrino" UMA (SEMARNAT-UMA-IN0013-COL/2003) consists of 21 red deer (*Cervus elaphus*) (4 males, 9 females and 8 offspring), aged between 1 month and 15 years. It is located at 19° 15´ N and 103° 43´ W and at 490 m asl in the municipality of Colima, Mexico[14]. The purpose is the captive breeding of *C. elaphus* in an extensive system to produce meat and hard antlers. Eleven hectares are destined, divided into six paddocks, with *Cynodon nlemfuensis* grass, *Pithecellobium dulce* trees and *Acacia farneciana*; in addition, they are supplemented throughout the year with 300 g animal/d of ground corn, soybeans, wheat bran, coconut paste and alfalfa meal added with mineral salts (each 100 g contains: phosphorus, calcium, iron, magnesium, copper, zinc, manganese, cobalt, iodine, selenium and vitamin A). For reproductive management, mounts are carried out from October to December and calving occurs in June and July; as a preventive medicine, deer are dewormed and vaccinated against clostridiosis. Every 21 d, they are sprayed with tick during the spring and autumn.

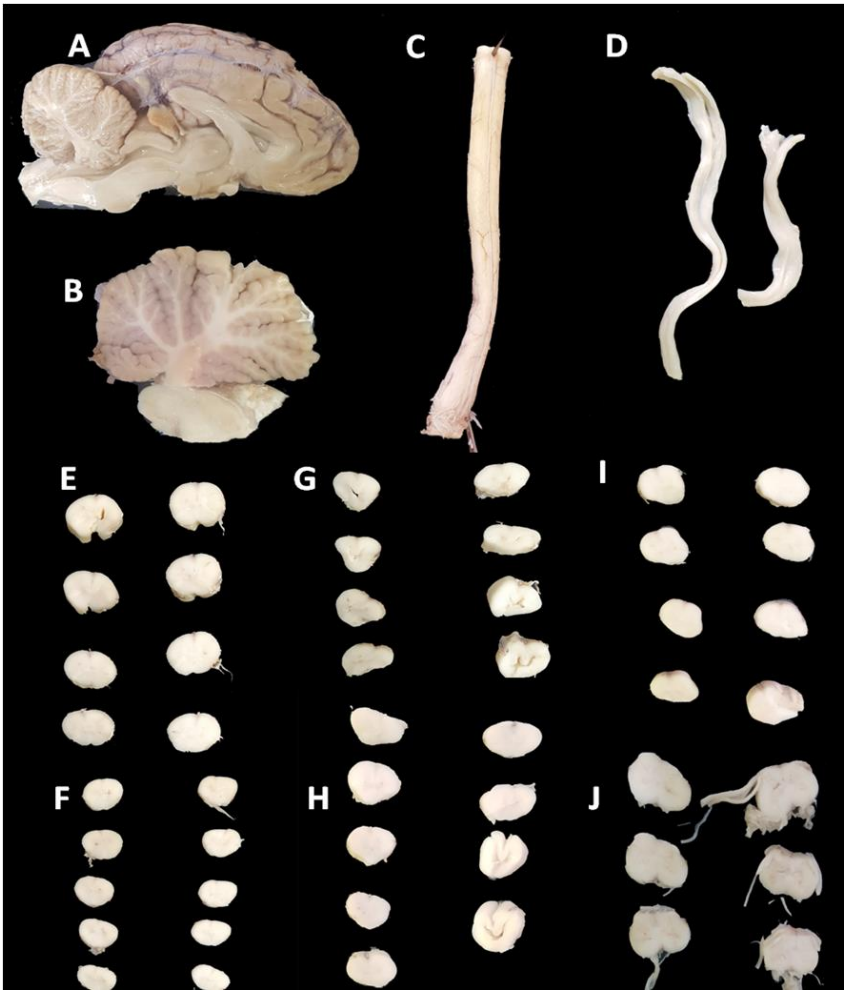
During 2018, there were two female red deer aged 3 and 7 yr (average weights of 60 and 80 kg respectively) with a clinical history of incoordination, weakness of the pelvic limbs, frequent falls and altered locomotion from 3 to 18 mo of evolution; there was also progressive weight loss and finally prostration. The first of these cases occurred in July and the second in October of the same year. In both animals, only the necropsy and histopathological study were performed, whose final diagnosis was severe bilateral leukoencephalomalacia, suggestive of progressive ataxia due to copper deficiency. In September 2019, another of the females of the UMA showed clinical signs similar to the cases observed in 2018. This female was 13 yr old and had an average weight of 95 kg. In the clinical examination, some lacerations in the skin were observed in different regions of the body, caused by prostration. A remote neurological examination was carried out, where mild and slowly progressive proprioceptive ataxia was observed. These signs were more evident when the animal walked in circles, or when pulling its tail to one side. It also showed weakness of the hind limbs, impaired ambulation (wobbly incoordination and crisscrossed pelvic limbs (scissor step), dysmetria due to hypermetria, circumduction movements (animal is circling), loss of conscious proprioception, stiff neck and difficulty standing. In the same way, it showed difficulty to lift the tarsi, which it dragged during the march; it also had weight loss, opisthotonos, tonic extension of limbs, paraparesis, exacerbation, prostration and depression. Considering that the clinical history and signs were similar to the cases of 2018, a blood

sample was taken to obtain serum and perform the serum copper measurement. Due to the severity of the clinical signs, euthanasia was performed by intravenous overdose of barbiturate (5 ml/1.0 kg body weight) (Pisabental® pentobarbital sodium 6.3 %, Pisa, SAGARPA, Q-7833-215, Guadalajara, Jalisco, Mexico).

The corpse was referred to the necropsy room of the Pathology laboratory of the Faculty of Veterinary Medicine and Zootechnics of the University of Colima, to carry out the *post-mortem* study. During the necropsy, no obvious macroscopic lesions were observed, so the complete spinal cord was obtained, and according to the anatomical location, it was divided into the following sections: cranial cervical, caudal cervical, cranial thoracic, caudal thoracic, cranial lumbar, caudal lumbar, cranial sacral, caudal sacral and coccygeal; likewise, samples were taken from the brain and sciatic nerves. The collected samples were fixed in 10 % formalin (pH 7.2), processed with the routine histological technique, included in paraffin and cut to 6 µm thick to be stained with hematoxylin and eosin (HE). Luxol Fast Blue staining was also used to evaluate spinal cord myelin[15]. On the other hand, liver, kidney, forage and soil samples were taken from two paddocks where the deer grazed, to determine the levels of copper and molybdenum using the atomic absorption spectrometry technique[16,17].

The most relevant macroscopic changes at the necropsy were: poor body condition (scale 2/5), hirsute hair, alopecic areas, skin lacerations and subcutaneous hematomas; caused by prostration. The liver showed multifocal discrete areas of capsular fibrosis. The other organs showed no obvious macroscopic changes (Figure 1). In the histological study, the most relevant lesions were found in the spinal cord, mainly in the ventral and lateral funiculi, with less evidence in the dorsal funiculi; these lesions were bilateral and symmetrical. In the white matter, extensive areas of demyelination were observed, characterized by the remarkable distension of the myelin sheaths, which inside showed few hypereosinophilic fragments of axons and some Gitter cells (digestion chambers); some axons were swollen and hypereosinophilic (spheroid bodies). By staining Luxol Fast Blue in each of the sections of the spinal cord, the loss of myelin (demyelination) in the lateral and ventral funiculi was evidenced, mainly in the areas adjacent to the ventral median fissure. The affected areas were characterized by the loss of tinctorial affinity. In the efferent nerves of the lumbar portion, foci of dystrophic mineralization were appreciated, as well as few spheroid bodies. Some neurons, mainly from the ventral horn of the gray matter were swollen, with loss of the Nissl substance or with the granules marginalized to the periphery (central chromatolysis).

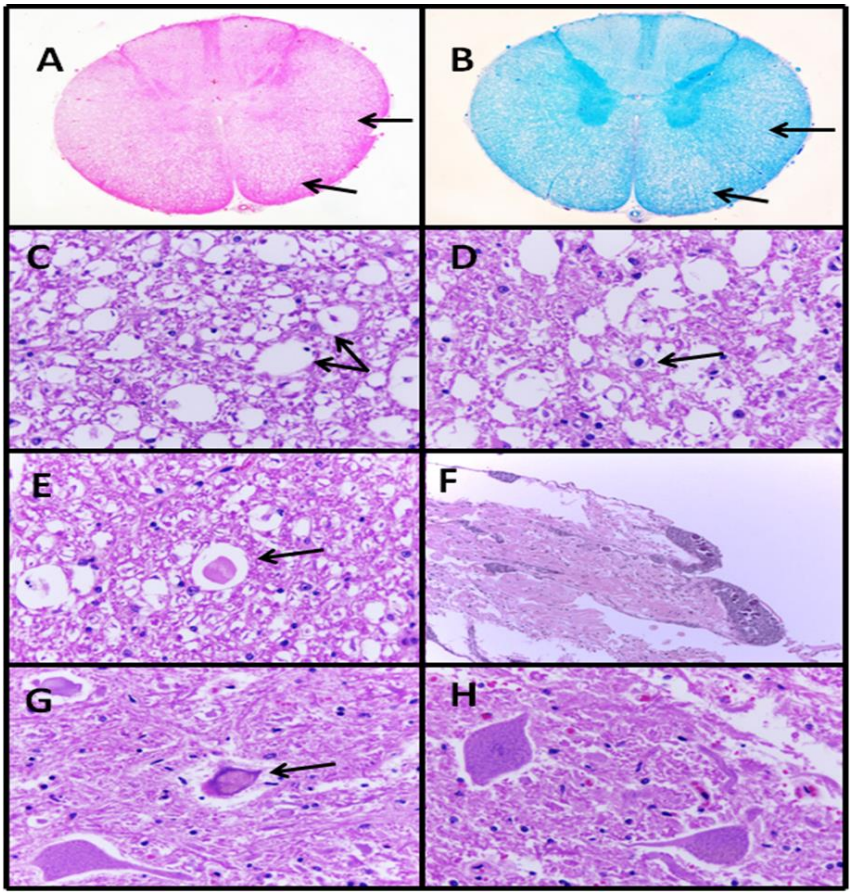
Figure 1: Nerve tissue samples from red deer (*C. elaphus*) for histopathological evaluation



A. Brain, **B.** Cerebellum, **C.** Spinal cord, **D.** Sciatic nerves, **E.** Cranial cervical, **F.** Caudal cervical, **G.** Cranial thoracic, **H.** Caudal thoracic, **I.** Lumbar, **J.** Sacral.

Additionally, for each section evaluated, there were a small number of neurons lacking a nucleus and their cytoplasm contained a moderate amount of ochre brown granules compatible with lipofuscin (Figure 2). No inflammatory reaction was observed in any of the spinal cord cuts. Histologically, no lesions were observed in the brain, sciatic nerve, spleen, rumen, reticulum, omasum, abomasum, kidneys, lungs, trachea, thyroid gland, pancreas and bladder. At the hepatic level, mild fibrosis located at the capsular level was microscopically corroborated and considered to be of no diagnostic relevance. In Table 1, the values of copper in tissues and blood serum are shown, while in Table 2, the values of the microminerals of Cu, Mo and the Cu:Mo ratio in forage and soils are shown.

Figure 2: Cross-section of the cervical portion of the spinal cord, stained with H-E



A. The ventral and lateral funiculi are observed with extensive bilateral symmetrical areas of demyelination (arrows). **B.** Cross-section of the cervical portion of the spinal cord stained with Luxol fast blue, areas of demyelination with loss of tinctorial affinity (arrows) become evident. **C.** Cranial cervical portion of the spinal cord, the myelin sheaths are dilated and inside there are fragments of axons and demyelination (arrows). **D.** Digestion chambers, inside there is a Gitter cell (arrow) and remains of an axon. **E.** Spheroid bodies (arrow). **F.** Mineralization areas in the efferent nerves of the dorsal horn of the dorsal portion of the spinal cord. **G.** Central chromatolysis in a neuron and loss of nuclei (arrow). **H.** Granular ochre brown pigment in a neuronal soma compatible with lipofuscin.

Table 1: Copper values (mg/kg DM or ppm) in tissues and blood serum of red deer (*Cervus-elaphus*) in captivity

Sample	Cu	Cu, Reference
Serum	0.08	0.5-1.5
Liver	2.70	6.4-29
Kidney	4.67	3.3-7.2

Reference values taken from:[7,12,18].

Table 2: Values of copper, molybdenum and the Cu:Mo ratio (mg/kg DM or ppm) in soil and forage of two meadows of the El Peregrino UMA

Sample	Cu (ppm)	Reference Cu	Mo (ppm)	Reference Mo	Cu:Mo	Reference Cu:Mo
Soil 1	8.48±0.45	5.1-10 (Ẋ= 7.5)	3.00±0.30	1-5 (Ẋ= 3.0)	2.83:1	2:1
Soil 2	9.10±0.48	5.1-10 (Ẋ= 7.5)	3.00±0.30	1-5 (Ẋ= 3.0)	3.03:1	2:1
Forage 1	6.59±0.35	8-11 (Ẋ= 9.5)	7.35±0.74	0.07-5.0 (Ẋ= 2.5)	0.90:1	2:1
Forage 2	2.77±0.15	8-11 (Ẋ= 9.5)	6.12±0.61	0.07-5.0 (Ẋ= 2.5)	0.45:1	2:1

References: [19,20,21].

Enzootic ataxia is a neurodegenerative disease caused by copper deficiency, whether primary or secondary. Primary or absolute deficiency occurs when forages or soil are poor in this element, and therefore there is insufficient intake, meanwhile the secondary or conditioned is caused by a reduction in its absorption at the intestinal level, generating low availability for tissues[6,7,12]. This disease mainly affects sheep, although it has also been described in goats, piglets and red deer[5]. Its diagnosis is carried out through clinical signs, microscopic lesions in the central nervous system and the determination of copper levels in the liver[12]. In some reports, it is reported that the morbidity of enzootic ataxia in red deer and red wapiti hybrids is around 1 %, although it can reach up to 13 %[5,10]; however, these proportions could vary depending on the geographical region, in addition, the adequate supply with Cu supplements to animals can effectively prevent the disease, while treatment of affected animals produces some remission of signs without eliminating the disease, so ataxia usually progresses to the death of animals[7,8]. In the "El Peregrino" UMA, morbidity was 9.8 % and mortality 0.1 %, remaining within the range established according to the literature[8,9,10]. The most common causes of morbidity and mortality in captive red deer in the "El Peregrino" UMA have been: 33.4 % babesiosis transmitted by the tick of the genus *Rhipicephalus*, 17.7 % secondary polyarthritis to an omphalophlebitis, 13.7 % suppurative bronchopneumonia, 9.8 % enzootic ataxia, 7.8 % fractures due to trauma, 3.9 % diarrhea and tympanites, 2.0 % of endoparasites *Fasciola hepatica* and *Oesophagostomum* spp[22].

The clinical signology of enzootic ataxia in deer presented with incoordination and weakening of the pelvic limbs, which was progressive and finally culminated in the prostration of the animal. These signs are perfectly consistent with those observed by other authors[10,11,12]. Spinal cord injuries in enzootic ataxia are purely microscopic, and this explains why no lesions were observed at necropsy. The distribution of leukomyelomalacia, mainly affecting the lateral and ventral funiculi adjacent to the ventral median fissure, was also consistent with other reports in the literature[5,7,12]. Microscopically, the observed

findings were bilateral and symmetrical in the white matter of the spinal cord with Wallerian degeneration, demyelination, scarce spheroid bodies, as well as Gitter cells; in neurons, central chromatolysis and neuronal necrosis are usually observed from the ventral horns[23,24,25]. The pathogenesis of this disease is not fully clarified, however, it is known that low levels of copper in the body interfere with the proper functioning of various enzymes, including superoxide dismutase, ceruloplasmin and cytochrome oxidase, which causes the suppression of mitochondrial respiration and therefore the decrease in the production of phospholipids and myelin[26,27]. To this is added the action of free radicals causing a demyelinating axonopathy. In other words, the lesions are typical of a neurodegenerative disease in deer[5,26,27].

The values of copper in blood serum and liver were below the values considered as normal limits (for blood serum of 0.5 to 1.5 mg/kg and 50 ppm in dry matter for liver). However, cases of enzootic ataxia have been described, where it manifests below 25 ppm and below 15 ppm[28,29,30]. In the present study, they were found well below these limits, although the body is very efficient in maintaining blood copper levels among optimal values, these characteristics have also been observed and recorded by other authors[28,29,31,32]. The liver has the ability to cause the redistribution of Cu and subsequently favor its accumulation in the kidney to then be excreted in the urine[26], this generates that the levels within the renal tissue, as in this case (4.67 mg/kg), are not affected and are found within the established range (3.3 to 7.2 mg/kg).

The analysis of copper and molybdenum performed on pastures and in the soil, as well as their Cu:Mo ratio suggests a low copper in the grass, which correlates with the low levels of copper found in liver and blood serum of deer, this being indicative of a primary deficiency. However, it should be considered that the disease occurs throughout the year, but can be seasonal due to the variation of the nutritional requirements of animals during the year and differences in the mineral composition of soil and pastures, depending on the season of the year; decreasing in winter-spring and increasing in summer-autumn as mentioned by other authors[19,20,21]. In farmed deer, Cu concentrations in the liver <4 µg/kg and serum concentrations <0.3 µg/ml indicate a deficiency in this element. It is considered as Cu deficiency in forages <10 ppm and <3 ppm molybdenum, losing the Cu:Mo ratio of 2:1[6], as in the present study 0.90:1 and 0.45:1. This suggests that molybdenum did not interfere with Cu metabolism in these deer. It is considered that for the liver, Cu concentrations >6.35 mg/kg are considered adequate and <3.81 mg/kg of tissue represents the range of deficient. For serum Cu concentrations, the ranges of <0.32 mg/L are deficient and >0.51 are adequate[2]. In severe copper deficiencies, bone and joint problems have been reported, such as osteochondrosis and spontaneous fractures[1,7,28], however, this was not evident in the deer of the UMA of Colima.

Clinical, necropsy and histopathology findings along with the analysis of Cu and molybdenum were used to make the final diagnosis of bilateral leukomyelomalacia or enzootic ataxia due to copper deficiency. This nutritional disease of the UMA in Colima was classified as a primary or absolute copper deficiency due to the low levels of Cu in the forage, which were insufficient to meet the nutritional requirements of the animals, reflecting low levels of Cu in liver, serum and favoring the development of progressive degenerative lesions in the central nervous system. Although Cu levels were not analyzed in the cases occurred in 2018, the signology and anatomopathological lesions were suggestive of enzootic ataxia; however, it is important that in the face of the manifestation of neurological signs in stabled deer, this disease is considered as a differential diagnosis, and that in addition to the clinical-pathological study, the measurement of Cu and Mo (serum, liver, forage and soil) is carried out to make a definitive diagnosis and to be able to establish prevention measures. Based on the literature search, this is the first report of enzootic ataxia in a captive red deer in Mexico.

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