Outbreak of enzootic ataxia in lambs in the semi-arid state of Pernambuco, Brazil. Surto de ataxia enzoótica em cordeiros no semiárido do estado de Pernambuco, Brasil.

Emanuel Felipe de Oliveira Filho1*, Pierre Castro Soares2, Marta Miranda3, José Augusto Bastos Afonso4, Carla Lopes Mendonça4, Rodolfo José Cavalcanti Souto4, Daniel Nunes de Araújo Gonçalves5, Antônio Flávio Medeiros Dantas6, Taciana Holanda Kunst7, Ana Paula Silveira Paim7, Marta López-Alonso8

1 Programa de Pós-Graduação em Medicina Veterinária/UFRPE. Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife, PE, CEP: 52171-900. E-mail: felipe130188@gmail.com
2 Departamento de Medicina Veterinária/UFRPE. Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife, PE, CEP: 52171-900. E-mail: pcastro.pe@gmail.com
3 Departamento de Anatomía, Producción Animal e Ciencias Clínicas Veterinarias, Universidade de Santiago de Compostela, Facultad de Veterinaria, 27002, Lugo, Spain. E-mail: marta.miranda@usc.es
4 Clínica de Bovinos de Garanhuns/UFRPE - Campus Garanhuns. Av. Bom Pastor - Boa Vista, Garanhuns - PE, 55292-270, Brasil. E-mail: afonsojab@oi.com.br, carlalopes.mendonca@gmail.com, rodolfo.souto@hotmail.com
5 Programa de Pós-Graduação em Ciência Animal Tropical/UFRPE. Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife, PE, CEP: 52171-900. E-mail: daniel.nagon@gmail.com
6 Departamento de Medicina Veterinária/UFCG - Campus Patos. Avenida Universitária, s/n - Santa Cecília, Patos - PB, Brasil. E-mail: dantas.af@uol.com.br
7 Departamento de Química Fundamental/UFPF. Av. Jornalista Aníbal Fernandes, s/nº Cidade Universitária - Recife, PE, CEP 50.740-560. E-mail: taciana.kunst@ufpe.br, anaspaim@ufpe.br
8 Departamento de Patoloxía Animal, Universidade de Santiago de Compostela, Facultad de Veterinaria, Lugo, Spain. E-mail: marta.lopez.alonso@usc.es
*Corresponding autor E-mail: felipe130188@gmail.com

Abstract

An outbreak of delayed enzootic ataxia in lambs in southern in the southern semi-arid state of Pernambuco. Affected lambs (n=18) showed symptoms of ataxia of the hind limbs, as well as difficulty in getting up and inability to walk, with a “dog sitting” posture. Histopathological examination of the spinal cord revealed vacuolization, axonal spheroids and myelin remains phagocytosed by macrophages and demyelination of the white matter. Low serum concentration of copper (6.88 μmol/L) was observed. Serum iron, zinc and molybdenum concentrations were at normal levels for the species. Clinical and laboratory data allowed concluding diagnosis of primary enzootic ataxia.

Keywords: Copper deficiency, diagnostics, enzootic ataxia, sheep
Resumo

Descreve-se surto de ataxia enzoótica em cordeiros no semiárido do estado de Pernambuco. Os cordeiros afetados (n=18) apresentaram sintomas de ataxia dos membros posteriores, além de dificuldade de se levantar e incapacidade de andar, com postura de "cão sentado". O exame histopatológico da medula espinhal revelou vacuolização, esferoides axonais e restos de mielina fagocitados por macrófagos e desmielinização da substância branca. Baixa concentração sérica de cobre (6,88 μmol/L) foi observada. Concentrações séricas de ferro, zinco e molibdênio estavam em níveis normais para a espécie. Os dados clínicos e laboratoriais permitiram concluir diagnóstico de ataxia enzoótica primária.

**Palavras-chave:** Deficiência de cobre, diagnóstico, ataxia enzoótica, ovinos

Introduction

Copper (Cu) is one of the most important essential microelements for animals and plants. It is an essential constituent of numerous enzymes such as ceruloplasmin, cytochrome C oxidase, superoxide dismutase, lysyl oxidase, galactosyl transferase ceramide and dopamine beta hydroxylase with essential roles in haematopoiesis, connective tissue metabolism, myelin and bone formation, pigmentation and wool and hair formation (CAVALHEIRO; TRINDADE 1992; RADOSTITS et al., 2007; SUTTLE, 2010).

Among domestic animals, ruminants (and particularly sheep) are particularly susceptible to Cu deficiency, and hypocuprosis is a common problem worldwide (SUTTLE, 2010). Paradoxically, sheep are also extremely susceptible to Cu toxicity. As Cu concentrations in forage crops are generally low, and sheep have a poor ability to absorb Cu in the intestine, this species has evolved a system of low biliary excretion in order to retain Cu, which leads to excessive hepatic Cu accumulation on exposure to high levels of Cu in the diet. Although Cu toxicity can occur under natural conditions, it is often associated with excessive Cu supplementation to treat Cu deficiency.

Moreover, Cu metabolism is very complex in ruminants as important interactions with other trace elements can occur in the rumen. The most important of these is the non-competitive interaction with sulphur (S) and molybdenum (Mo), leading to the formation of insoluble tetra-thiomolybdates in the rumen; competitive interactions with high levels of dietary zinc (Zn) and iron (Fe) can also occur (SUTTLE, 2010). Thus, Cu requirements in ruminants not only depend on the Cu dietary concentrations, but also on their antagonists. This is particularly true for grazing animals, in which ingestion of soil may represent an important source of elements (SUTTLE, 2010). Consequently, Cu deficiency is classified as primary (related to low concentrations of Cu in the diet) or secondary (when dietary Cu concentrations are adequate, but the presence of Cu antagonists inhibits Cu absorption).

Within Cu deficiency disorders in ruminants, enzootic ataxia (EA) or swayback causes the most important problems in lambs and goats worldwide (SUTTLE, 2010). EA affects animals up to 180 days old and is characterized by demyelination of the central nervous system, causing symptoms such as loss of function of the hind limbs and (to a lesser extent) anterior limbs, flaccid or spastic paralysis, total incapacity to walk and, finally, death. Two types of EA are described on the basis of the location of the lesion and the clinical evolution of the condition. The congenital form is more severe, affects neonates in the first days of life and is characterized by destruction of the cerebral white matter, whereas the late or delayed form occurs after the third week of life and is characterized by injuries in the brainstem and motor tracts of the spinal cord (SANTOS et al., 2006).
In Brazil, EA was first described by Tokarnia et al. (1966), in sheep in the state of Piauí, in the northeast region of the country. The disease has also been detected in goats and sheep in the semi-arid region of Paraíba state (PB) (SILVA et al., 2014) and in the municipality of Mossoro (Rio Grande do Norte, RN). Within the state of Pernambuco, it has also been diagnosed in goats and sheep in the Pernambuco wilderness (SOUZA et al., 2009; MARQUES et al., 2011), and an outbreak of EA has been described in goats and sheep in the central semi-arid area of the municipality of Surubim (SANTOS et al., 2006). The aforementioned researchers suggest that Cu deficiency seems to be secondary to elevated concentrations of Fe in the diet, and in fact some soils in Pernambuco are characterized by medium to high Fe availability (OLIVEIRA; NASCIMENTO, 2006).

As far as we are aware, this is the first report of an outbreak of EA in lambs in the southern region of the semi-arid state of Pernambuco (PE). This is important as this region is located in the main dairy producing area of the state with high numbers of goats and sheep. The findings will allow the scientific community to identify other episodes of Cu deficiency and to enable measures of control through technical guidance.

Material and Methods

An outbreak of EA occurred in a property located in the municipality of Garanhuns, in the southern region of the semi-arid state of Pernambuco. The sheep were raised in an extensive system, with no regular mineral supplementation, and were fed on Brachiaria spp. and pangola grass (Digitaria decumbens) and provided water ad libitum. In May 2014, five crossbred Santa Inês sheep (from a flock of 70) showed difficulties in locomotion, with uncoordinated movements and "sitting dog" posture; however, at that time the farmer did not ask for veterinary advice. In April 2015, 18 animals were newly affected and were attended at the Garanhuns Cattle Clinic (CBG-UFRPE) for clinical examination following the protocol described by Radostits et al. (2007). The lambs were less than five months old and clinical signs first appeared at age one month. During the course of the outbreak, 5 of the 18 lambs died within a 2 week-period, and the remaining lambs were maintained in the hospital for 4 weeks until clinical recovery (some with clinical consequences). The farm owner reported that new cases of disease had appeared, with sheep showing the same clinical symptoms, and that some (number not specified) had died.

Although EA was suspected on the basis of the clinical examination, necroscopy (histological examination) was carried out to confirm the diagnosis and differentiate it from that of other possible neurological diseases (abscess or congenital abnormalities of the spinal cord, listeriosis or the nervous form of caprine arthritis encephalitis virus: Santos et al., 2006). Necropsies were carried out in 5 animals. Macroscopic examination did not reveal any significant findings in the central nervous system (CNS). Samples obtained from the CNS were placed in 10% neutral buffered formalin and processed by routine histopathological methods and stained with Hematoxylin-Eosin (HE). Spinal cord samples were processed by Luxol Fast Blue staining, with freezing cutting, to enable characterization of the myelination. This procedure was performed in the Animal Pathology Laboratory of UAMV/UFCG-Patos - PB.

Blood samples were collected by jugular venipuncture, into vacutainer tubes without anticoagulant, to obtain serum for trace element determination (Cu, Fe, Zn and Mo). Samples were maintained at room temperature for clot retraction and serum collection. Subsequently, all samples were sent to the Clinical Laboratory of CBG-UFRPE where they were centrifuged (15 minutes at 500 g), aliquoted and stored in an ultra-freezer at -80 until analysis.
Serum samples (2 mL) were digested in 5 mL of concentrated nitric acid in a microwave-assisted digestion system (Mars Xpress-CEM Technology Inside®). The digested samples were transferred to polypropylene sample tubes and diluted to 10 mL in ultrapure water. Trace element determination was carried out by inductively coupled plasma atomic absorption spectrometry (ICP-OES) (ICP model OES Optima 7000 DV, PerkinElmer, USA) in the Analytical Central of the Department of Chemistry Fundamental of the Federal University of Pernambuco. Calibration solutions were prepared immediately before analysis. An analytical quality control was applied throughout the study. Blanks were processed with the samples, and the readings obtained were subtracted from the sample readings to calculate the final values (NOMURA et al., 2005). The element concentrations were expressed in μmol/L.

The trace element concentrations in serum were analyzed using the SAS software suite (SAS, 2009). Descriptive statistical analysis of the variables (average and standard deviation, median and range) and Pearson correlation analysis (to verify the degree of relationship between the variables) were performed. Differences in concentrations were considered statistically significant at a probability level of 5%.

Results

Description of the outbreak

At the beginning of the outbreak, the affected animals showed symptoms including loss of visual acuity (amaurosis was observed in three animals), apathy, inappetence, anorexia, frequent falls and difficulty in standing, with uncoordinated movement of the pelvic limbs (Figure 1). As the disease progressed, the animals showed symptoms such as anaemia, weight loss, changes in wool colour, decreased sensory and motor responses with a "sitting dog" posture and diarrhea. Most of the lambs affected were ≤ 60 days old. The clinical evolution of the disease ranged from one week to 15 days.

Figure 1. Animal showing uncoordinated movements and hind limbs ataxia, difficulty in standing and incapable of walking.
Histopathological study

The results of the histological study are presented in Figure 2. In the HE staining of the spinal cord fragments (white matter, mainly of the ventral funiculi) there was slight vacuolization, scarce axonal spheroids, and myelinic remains phagocytosed by macrophages with foamy cytoplasm (Gitter cell) (Figure 2A). In the luxol fast-blue histochemical staining (Figure 2B), areas of discrete demyelination were observed in the white matter. The changes observed in the spinal cord showed a symmetrical pattern affecting the nerve fibres.

**Figure 2 A** - HE staining, showing slight vacuolization (+), scarce axonal spheroids (arrow) and myelinic debris being phagocytosed by macrophages with foamy cytoplasm (Gitter cell) (*).

**Figure 2 B** – Luxol fast blue histochemical staining, revealing areas with discrete demyelination (arrows).

Trace element analysis

The results of the trace element analysis of the serum of the clinically affected animals (n=18) are shown in Table 1. Mean Cu concentrations in serum (6.88±4.31 µmol/L) were found. As for the Fe (44.02±28.51 µmol/L) and Zn (16.13±7.9 µmol/L) and there was no significant relationship between Cu or either of these elements in the serum (Figure3). The Mo concentrations in serum were very low was higher than the limit of quantification (0.05 µmol/L) in only one animal.

| Table 1. Cu, Fe and Zn concentration in serum (expressed in µmol/L) in lambs (n=18) affected by enzootic ataxia (EA) in the southern region of the Semiarid state of Pernambuco. Mo concentrations were found below the limit of quantification (<0.050 µmol/L) in 17 of the 18 samples. |
|-----------------|-----------------|-----------------|
|                  | Cu              | Fe              | Zn              |
| Mean±SD          | 6.88±4.31       | 44.02±28.51     | 16.13±7.90      |
| Median           | 7.46            | 36.4            | 13.9            |
| Range            | (0.97-16.02)    | (12.50-106.10)  | (6.40-38.70)    |
| Adequate range¹ | 9.0-15.00       | 18.2-54.40      | 12.3-18.50      |

¹Suttle (2010)
Figure 3. Scatterplot showing the relationship between Cu and its antagonists Fe and Zn in serum. The horizontal line denotes the lower limit of adequate Cu concentrations in serum.

Discussion

Description of the outbreak

In their respective studies, Santos et al. (2006) and Souza et al. (2009) stated that during progress of the clinical episode, the animals may show symptoms such as weight loss, decubitus lesions, frequent falls and progressive apathy. The disease can cause death of the animals between 7 and 20 days after the initial diagnosis, and locomotion and growth may be negatively affected in surviving animals.

Amaurosis was observed in three animals, one of which was blind, even after receiving specific mineral supplementation. No studies published in the north-eastern region of Brazil have reported the presence of amaurosis in cases of late AE. In addition, the following symptoms were not observed in any of the animals examined by Santos et al. (2006): lack of awareness, amaurosis, nystagmus, cortical deafness, pupillary reflex decrease and anaemia. New research is needed to better define the occurrence of amaurosis in lambs with EA. This clinical condition probably occurs as a result of a higher level of neuronal degeneration in the brain, which may cause severe damage to optic nerves or central nerves and even lead to animal blindness, as seen in this outbreak.

Histopathological study

All of these morphological lesions are suggestive of Cu deficiency and have previously been described in outbreaks of the late form of EA (BANTON et al., 1990; SANTOS et al., 2006; GUEDES et al., 2007; OZKUL et al., 2012; SILVA et al., 2014; OHFUJI, 2015 p. 625–628). As in the present case, the most significant findings were the degeneration of axons and myelin in the cerebellum and motor tracts of the spinal cord (RADOSTITS et al., 2007). Animals affected by AE demonstrate deficient myelination, as well as alteration of the white matter in the brain, neurons and necrosis (ZATTA; FRANK, 2007). The critical period in the development of the disease occurs during late gestation, when myelination occurs most rapidly. Cu deficiency depresses cytochrome oxidase activity, leading to inhibition of aerobic metabolism and phospholipid synthesis (ZATTA; FRANK, 2007).
Trace element analysis

Once the diagnosis of EA is confirmed on the basis of the combination of the clinical signs and the results of the histological examination, tests should be carried out to determine whether the episode of Cu deficiency had a primary (related to low dietary Cu concentrations) or secondary (related to high levels of Cu antagonists, namely Fe, Zn or Mo) origin. Measurement of trace element concentrations in soils and diets can provide valuable information; however, the results are not always conclusive as Cu requirements in sheep do not depend directly on the Cu concentration in the feedstuffs, and they are closely related to their availability in plants, and most importantly, to the interactions with the Cu antagonists (SUTTLE, 2010). Alternatively, determination of trace element concentrations in serum may provide a clear indication of the possible presence of high concentrations of the Cu antagonists, as well as the magnitude of the Cu deficiency.

The results of the trace element analysis of the serum of the clinically affected animals (n=18) are shown in Table 1. Mean Cu concentrations in serum (6.88±4.31 µmol/L) were below the adequate range for sheep (9-15 µmol/L; SUTTLE, 2010), but within the range of a marginal deficiency (3-9 µmol/L; SUTTLE, 2010). This result is consistent with delayed presentation of EA. While the congenital form occurs in pregnant females with extreme dietary deficiency of Cu, the late or delayed form, affecting older neonates, is associated with less severe Cu deprivation (ZATTA; FRANK, 2007). Notably, in 7 of the 18 affected lambs, the Cu concentrations were within the adequate range (see Figure 3). Marques et al. (2011) reported that the mean serum and hepatic contents of Cu were lower than the limits considered normal for this species, indicating the need for Cu supplementation. Nevertheless, Santos et al. (2006) described serum Cu concentrations within the adequate range in both lambs and kids (11.3±2.35 μmol/L) clinically affected by the disease and not differing from those in the unaffected animals. Although most Cu in the serum occurs within ceruloplasmin (which represents the Cu exported from the liver to the tissues needed for their normal metabolism), the concentrations may be affected by several physiological and pathological conditions. Ceruloplasmin is an acute phase protein and its concentrations increase greatly under inflammatory conditions. Moreover, the clinical neurological signs of EA occur as a consequence of the lack of Cu during foetal development and are more closely related to the Cu status of the sheep/mother during the late stages of gestation than to the Cu status of the lamb at the time of the diagnosis.

By contrast, the Mo concentrations in serum were very low, and the Mo concentration was higher than the limit of quantification are similar to the results have been reported by Silva Junior et al. (2015) for sheep in Sertao do vale do Rio Sao Francisco (Pernambuco), where Mo concentrations were unquantifiable in most samples (71.2%) and very low in the remaining samples (0.10-0.20µmol/L). Molybdenum requirements in animals are very low and signs of Mo deficiency are not observed in practice and are only reported when animals are fed purified diets under experimental conditions (NRC, 2005). However, very low Mo concentrations in the diet of ruminants can lead to excessive hepatic Cu accumulation (SPEARS, 2003). Nonetheless, this does not seem the case here, as the sheep were suffering from Cu deficiency. Taking into consideration all of these results, it seems that the episode of EA observed was associated with primary Cu deficiency.
Conclusion

The combination of the histopathological finding sand determination of the trace element concentrations in serum enabled us to confirm the presumptive (based on the clinical manifestations) diagnosis of delayed EA in lambs, as well as to suggest primary Cu deficiency as the most probable aetiology. As the affected animals suffer from irreversible lesions and can only be supported with palliative treatment, preventive measures ensuring adequate Cu in the diets of pregnant females, especially in the second half of gestation, are essential to avoid new outbreaks of the disease. In this case, we recommended the inclusion of a mineral supplement in the diet, and no other signs of Cu deficiency were subsequently observed in the herd. However, Cu supplementation must be carefully administered in sheep and only after prescription by a veterinarian or animal-nutrition specialist on the basis of analytical data. Sheep are very sensitive to hepatic Cu accumulation, and episodes of Cu toxicity can occur when Cu supplementation is given at levels in excess of requirements.

References


