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Araştırma Makalesi

HISTOPATHOLOGIC EXAMINATION OF THE BRAIN TISSUE IN LAMBS WITH NEUROLOGICAL SYMPTOMS: ENZOOTIC ATAXIA

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ABSTRACT

The aim of this study was to retrospectively evaluate the pathomorphological findings detected in the brains of lambs which died and were reported to have shown neurological symptoms in clinical anamnesis. Macroscopically, gelatinous softening and cavities were observed in the cortex. Brain tissue sections were stained with hematoxylin and eosin (HE). Multifocal gliosis was observed in six cases, calcification of neurons in four, of which one case was severe, and demyelination in four, of which three had gitter cells. The lambs showed neurological symptoms, such as brain lesions of different characteristics and severity, on histopathological examination before death and were diagnosed with enzootic ataxia.

Key Words: Brain, enzootic ataxia, histopathology

SİNİRSEL SEMPTOMLAR GÖSTEREN KUZULARDA BEYİN DOKUSUNUN HİSTOPATOLOJİK İNCELENMESİ: ENZOOTİK ATAKSİ

ÖZ

Bu çalışmada klinik anamnezinde sinirsel semptomlar göstererek ölen kuzuların beyninde rastlanan patomorfolojik bulguların retrospektif değerlendirilmesi amaçlandı. Makroskobik olarak kortekste jelatinöz yumuşamalar ve kavitasyonlar gözlendi. Beyin dokusu kesitlerine rutin hematoksilen eozin (HE) boya yöntemi uygulandı. 6 olguda multifokal odaklar halinde gliozis dikkat çekti. Birisinde şiddetli olmak üzere 4 olguda nöronlarda kalsifikasyon, ayrıca 4 olguda demiyelinizasyon ile bunların 3'ünde gitter hücreleri mevcuttu. Farklı karakter ve şiddetteki beyin lezyonları histopatolojik olarak tespit edilerek; sinirsel semptomlar göstererek ölen kuzulara enzootik ataksi tanısı konuldu.

Anahtar kelimeler: Beyin, enzootik ataksi, histopatoloji

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INTRODUCTION

Primary or secondary copper deficiency in lambs causes enzootic ataxia, characterized by degeneration of the central nervous system and developmental disorders (1, 2, 3). In our country, it is reported that the disease is observed in the Black Sea region, some parts of Central Anatolia, and the province of Denizli, with an incidence rate of 3%–80% (1, 4, 5).

Primary copper deficiency occurs due to soil being deficient in copper, whereas secondary copper deficiency is caused by the presence of some elements (molybdenum, calcium, and sulfate), which negatively affect the utilization of copper despite being adequately present in the feed (2, 6). The enzymes copper cytochrome oxidase, amine oxidase cytochrome c, and superoxide dismutase play an important role in the functioning of the protein ceruloplasmin. These enzymes are involved in various processes, such as oxidative phosphorylation, iron transport, antioxidation, neutralization, and neurotransmitter synthesis (2, 3, 5, 7).

Copper deficiency is observed in two different forms in newborn lambs and goats: congenital (Swayback) and delayed (enzootic ataxia) (2, 3). In the congenital form of the disease, animals occasionally become blind and are unable to stand. Bilateral symmetric gelatinous softening and cavities occur in the white matter; however, the histological pathogenesis of these phenomena has not been established (3, 6, 8). It has been suggested that these are caused due to hypo- and demyelination. In the delayed form, the disease is characterized by neurological findings. Lying down and ataxia resulting in death are important implications and are responsible for the name of the disease (3, 7). Neurological symptoms, such as coordination defects, weakness, uncoordinated walking, staggering and falling, and sitting on the back legs as dogs while trying to stand, are characteristic findings of the disease (2, 6, 8). All these symptoms have been stated to be pathologically consistent with the degeneration in the white matter of the brain (9). Microscopically, demyelination, hypomyelination of the white matter and central chromatolysis, degeneration resulting in necrosis, and calcification are observed in the neurons (7, 10). Although few, gitter cells are found in the demyelinated areas, but inflammatory reaction does not occur (8). Despite extensive studies, the role of copper in the development of the nervous system remains unclear (3). In this study, the objective was to determine and grade the histopathological findings in the brains of lambs showing late clinical symptoms of enzootic ataxia.

MATERIALS AND METHOD

The study material comprised brain tissues obtained from nine 1–2-day-old Akkaraman crossbreed lambs (7 female, 2 male) that were reported to show neurological symptoms in anamnesis and died later. Brain tissues were fixed in 10% buffered formalin. Next, 5-µm thick sections were cut from paraffin-embedded blocks after series of alcohol and xylol treatments, deparaffinized in xylol, and stained with Hematoxylin and Eosin (11) after being passed through a series of 100%, 96%, 80%, and 70% alcohol treatments. Microphotographs (Olympus DP12) were obtained after examination under a light microscope (Olympus CX31).

RESULTS

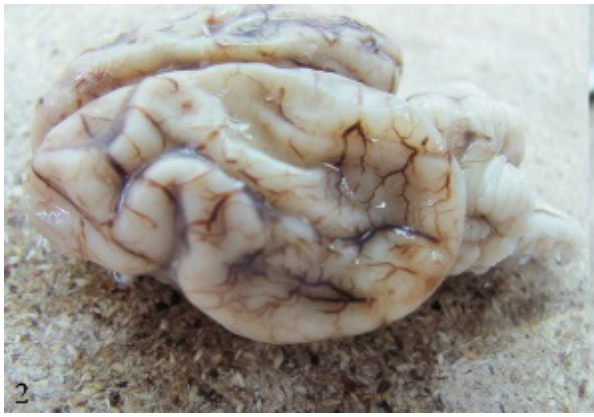
In animals which died within 1–2 days following birth, symptoms such as head being bent forward,

difficulty in standing, blindness, inability to stand, sitting on back legs (dog sitting position), and ataxia were observed (Figure 1). Macroscopically, there were cavities filled with clear cerebrospinal fluid in the cross-sections of apparently collapsed regions in the cerebrum, accompanied by flattening of the cerebral cortical gyri (Figures 2-3). There was remarkable cerebral hypoplasia, and cross section of normal cerebral hemispheres showed subcortical bilateral gelatinous softening (Figures 4-5). In the histopathological evaluation of the brain tissue sections, there were varying degrees of hyperemia (from mild to severe) and changes ranging from degeneration to necrosis in all lambs, except one (8/9). There was multifocal gliosis (6/9), satellitosis (6/9) in almost all lambs, and four (4/9) had flaky neuronal calcification of moderate (1/9) and mild (3/9) intensities (Figures 6-7). Moreover, there was demyelination in four lambs (4/9), of which three showed the presence of gitter cells (3/9) (Figure 8). Two lambs had meningitis. Additionally, parenchymal perivascular mononuclear cell infiltration was observed in three (3/9) lambs (Figure 9). Histopathological findings were graded as mild, moderate, and severe and are summarized in Table 1.

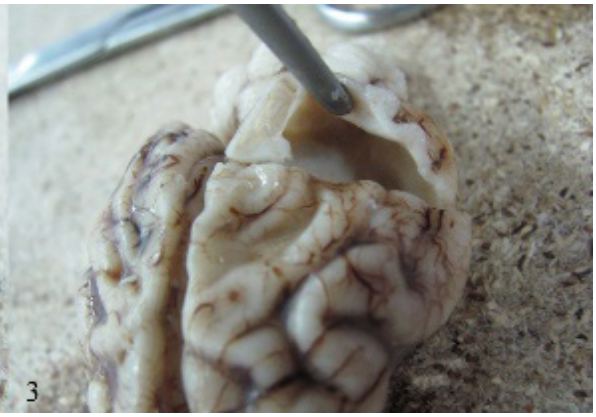
Şekil 1. Kuzu, klinik bulgu; baş öne eğik, ayakta durmada güçlük.
Fig 1. Lamb, clinical finding; head being bent forward, difficulty in standing.

DISCUSSION

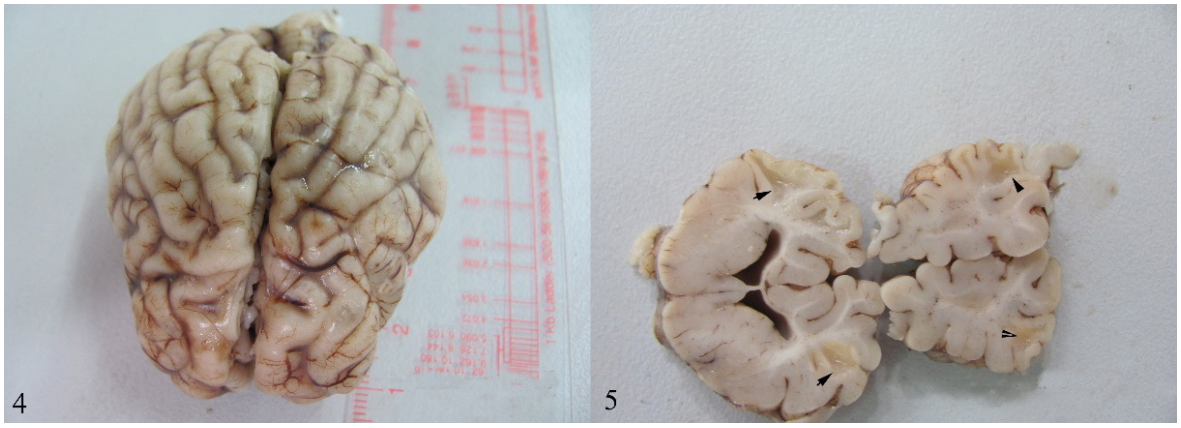
Copper is an essential trace element and is found in at least 10 enzymes which catalyze oxidation reactions in plants and animals. It is one of the most important elements necessary for the development of the embryo and fetus. Its deficiency in pregnant animals results in deficiency in the fetus as well. Thus, enzootic ataxia occurs in babies born from sheep fed on copper-deficient pastures. As a result, there is embryonic death or developmental defects and pathological defects in the skeletal and central nervous systems of the newborns. In lambs, demyelination in the central nervous system



Şekil 2. Serebrumda çökük görünümlü alanlar.
Fig 2. Apparently collapsed regions in the cerebrum.

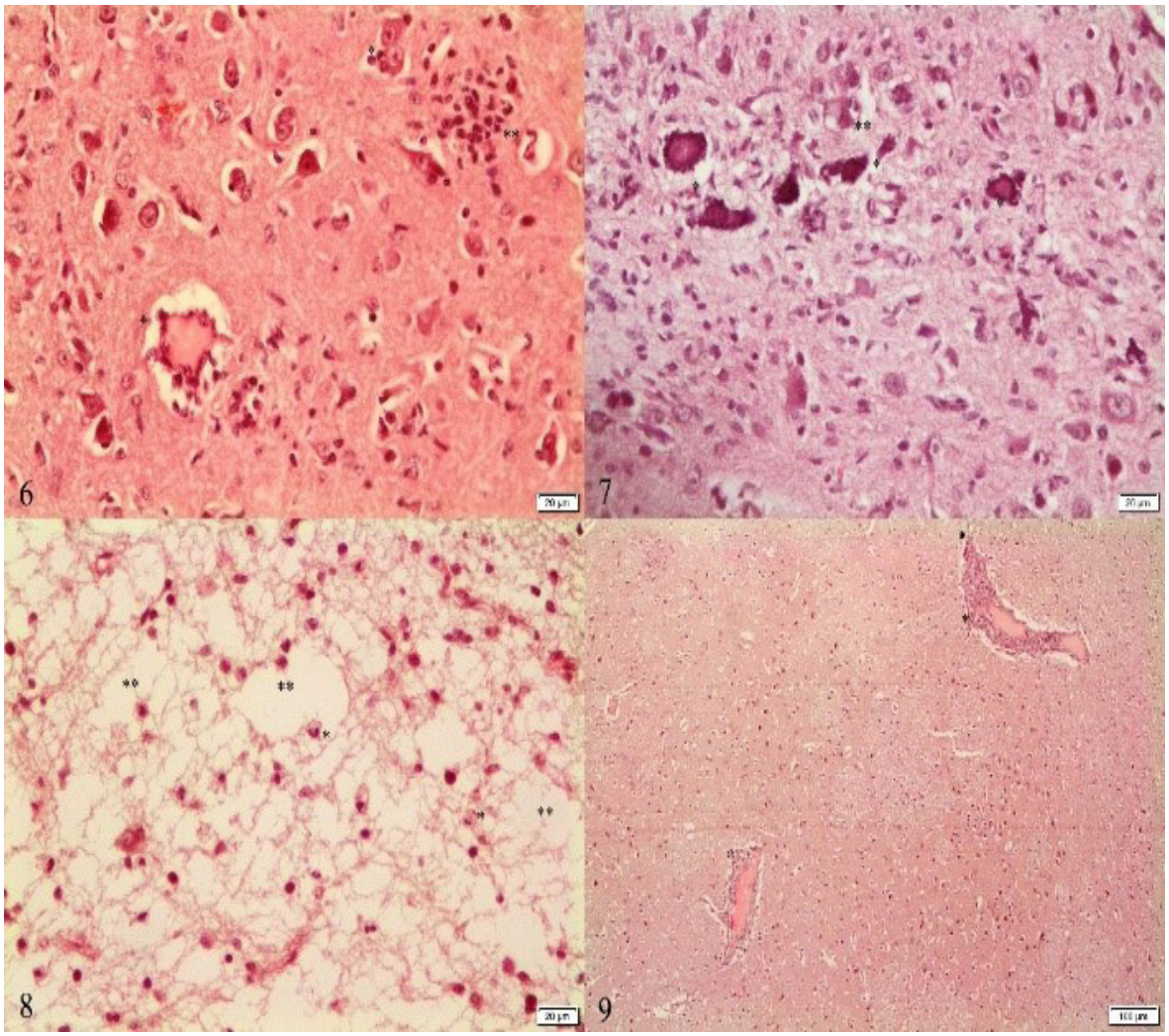


Şekil 3. İçleri şeffaf serebrospinal sıvı ile dolu kavitasyonlar.
Fig 3. The cavities were filled with clear cerebrospinal fluid.



Şekil 4. Normal görünüşte beyin.
Fig 4. Normal appearance of brain.

Şekil 5. Serebral hemisferde subkortikal bilateral jelatinöz yumuşamalar.
Fig 5. Subcortical bilateral gelatinous softening in the cerebral hemisphere.



Şekil 6. Serebrumda satelitoz (*) ve gliozis (**), H&Ex20 µm.
Fig 6. Satellitosis (*) and gliosis (**) in cerebrum, H&Ex20 µm.
Şekil 7. Nöronlarda kalsifikasyon (*) ve satelitoz (**), H&Ex20 µm.
Fig 7. Neuronal calcification (*) and satellitosis (**), H&Ex20 µm.
Şekil 8. Demiyelinizasyon alanları (*) ve gitter hücreleri (**), H&Ex20 µm.
Fig 8. Demyelination areas and (*) ve gitter cells (**), H&Ex20 µm.
Şekil 9. Perivasküler yerleşimli mononüklear hücre infiltrasyonları (*),H&Ex20 µm.
Fig 9. Perivascular mononuclear cell infiltration (*), H&Ex20 µm.

Tablo 1. Sinirsel semptomlar gözlenen kuzuların beyinlerinde rastlanan histopatolojik bulgular
Table 1. Histopathological findings detected in the brains of lambs which shown neurological symptoms

Animal no and sex	Histopathological findings								
	Demyelination	Neuronal degeneration and necrosis	Satellitosis	Hyperemia	Gliosis	Calcification	Gitter cells	Meningitis	Perivascular mononuclear cell infiltration
1. Male	-	++	+	+++	+	++	++	-	-
2. Female	+++	+	+	++	++	-	+++	-	-
3. Female	+++	+	+	++	+	+	++	+	-
4. Female	-	+		++	-	+	-	-	-
5. Male	-	+++		+++	-	+	-	+	+
6. Female	-	+	+	++	+	-	-	-	+
7. Famele	+	-		-	+	-	+	-	++
8. Female	++	+		+++	-	-	-	-	-
9. Female	-	+	+	+	+	-	-	-	-

during embryonic development and after birth can be caused by the inadequacy of the cytochrome oxidase enzyme system. As in previous studies, findings compatible and incompatible with ataxia, similar neurological symptoms, demyelination, and similar morphological changes were observed (12, 13, 14). The swayback posture in lambs is clinically classified into congenital and delayed forms (2, 3).

Many researchers (15, 16, 17, 18, 19) have reported hardening and straightening of wool and color changes in black wool in lambs due to depigmentation; however, because the lambs in our study were Akkaraman crossbreed, no depigmentation and changes in wool were observed. Although the number of females was higher than that of males, the role of sex in the occurrence of enzootic ataxia has not been proven (19, 20). Previous studies have reported that neuronal degenerative changes were observed in lambs in the absence of clinical symptoms, and that minimal lesions can be detected in lambs showing clinical symptoms (3). In this study, symptoms such as head being bent forward, ataxia, and difficulty in standing were supported by histopathological findings, including demyelination, neuronal degeneration, and necrosis, which was consistent with previous studies. The observation of these lesions in the nervous system immediately after birth supports the notion that the lesions are formed in the intrauterine period. Similar to the present study, some previous studies have reported blindness in lambs with enzootic ataxia (21), whereas others reported no such finding (10). Bilateral gelatinous cavities macroscopically observed in the brain tissues of lambs were similar to those reported previously (5).

In conclusion, the disease in our study was the congenital form of enzootic ataxia because the lambs died within 1–2 days following birth, showed severe neurological symptoms, and visible lesions in the central nervous system, which were supported by histopathological findings.

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