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The Pathological Investigations on Nutritional Myopathy Causing Lamb Deaths in Neonatal Period

Orhan YAVUZ

Aksaray University Faculty of Veterinary Medicine Department of Pathology, Aksaray / TURKEY
orhanyavuz@aksaray.edu.tr

Abstract

In this study; between October 2014 and March 2016, 39 lambs evaluated grossly and histopathologically, which dead from nutritional myopathy (NM) symptoms showing such as inability to stand up and limping. Heart, intercostal muscles, diaphragm and musculus gracilis were evaluated in terms of paleness and calcification for gross pathology. After the routine tissue processing, samples were stained with H&E and examined under light microscope. Grossly, paleness in 39 cases and calcification in 22 cases were detected in the hearts. It was found that the most affected skeletal muscle was m.gracilis with paleness in 23 cases and calcification in 9 cases. In intercostal muscles, paleness in 11 cases and calcification in 2 cases were observed. Although, paleness in 8 cases were observed, there was no calcification found in diaphragm. Microscopically, hyaline degeneration and Zenker's necrosis found in heart in 35 cases, in m.gracilis in 18 cases, in diaphragm and intercostal muscles in 7 cases.

According to the findings, cardiac form is more effective to be found in Cental Anatolia region in Turkey. Moreover, NM was an important disease that threats the herd health in lambs.

Keywords: Lamb, nutritional myopathy, pathology, white muscle disease

Neonatal Dönemde Nutrisyonel Miyopati Kaynaklı Kuzu Ölümleri Üzerine Patolojik İncelemeler

Öz

Bu çalışmada 2014 Ekim - 2016 Mart tarihleri arasında Aksaray Üniversitesi Veteriner Fakültesi'nde getirilen; klinik olarak ayağa kalkamama, sallantılı yürüyüş, dik ve kısa adımlar halinde yürüme gibi nutrisyonel miyopati bulguları göstererek ölen 39 kuzunun makroskobik ve histopatolojik bulguları değerlendirildi. Patolojik incelemeler için kalp, interkostal kaslar, diyafram ve musculus gracilis kasları makroskobik olarak solgunluk ve kireçlenme yönünden değerlendirildikten sonra, rutin doku takibinin ardından H&E ile boyanarak ışık mikroskobunda incelendi. İncelenen olgular içerisinde kalpte 39 olguda solgunluk ve 22 olguda kalsifikasyon gözlenirken, iskelet kaslarından ise en çok m.gracilis'te 23 olguda solgunluk ve 9 olguda kalsifikasyon gözlendi. Bunları 11 olgu solgunluk ve 2 olgu kalsifikasyon ile interkostal kaslar izlerken, diyaframda 8 olguda solgunluk gözlendi ve kalsifikasyon tespit edilmedi. Mikroskobik olarak kalpte 35, m.gracilis'te 18, interkostal kaslar ve diyaframda da 7 şer olguda hiyalin dejenerasyonu ve Zenker nekrozu tespit edildi.

Elde edilen bulgular ışığında Aksaray bölgesindeki kuzularda daha çok kardiyak formun etkili olduğu ve hastalığın sürü sağlığını tehdit eden önemli bir metabolik bozukluk olduğu değerlendirilmiştir.

Anahtar Kelimeler: Beyaz kas hastalığı, kuzu, nutrisyonel miyopati, patoloji

Introduction

Nutritional myopathies are the degeneration in skeletal and cardiac muscles of lambs and kids as a result of Vitamin E (Vit E) and Selenium (Se) deficiency. This disease is also known as *White Muscle Disease* (Milli and Hazıroğlu, 2000; Yüksek and Basbuğan, 2015). Although this disease is mostly observed in lambs, kids, calves and camel cubs, it is also observed in sheep and goats (Baran, 1966; Hebert and Cowan, 1971; White and Rewel, 2007; Özdemir, et al., 2016). Reported first in Germany in 1925, this disease is now common worldwide (Cooper and Valentine, 2016). Although the incidence of this disease is reported around 1% in many countries, the incidence in Turkey and New Zealand has been indicated within the range of 20-30% (McDowell et al., 2002). In Turkey, the disease is mostly seen in the Central Anatolia, Eastern Anatolia and Southeastern Anatolia regions (Özcan, 1967; Beytut et al., 2001).

Selenium deficiency is more frequently seen in animals that graze on pasture and Vit E deficiency is more frequently seen in animals that are fed with animal feeds. It is reported that hyaline degeneration of muscles occurs as a result of formation of free radical associated with Vit E and Se deficiencies (Başoğlu and Sevinç, 2004). Selenium is normally found in glutathione peroxidase (GSH-Px) enzyme which reduces hydrogen peroxide, super-oxide radicals and lipid peroxides to water (Hefnawy and Perez, 2010). Se is mostly found in kidneys and liver and then in skeletal and cardiac muscles. Therefore skeletal and cardiac muscles need high amounts of Se. Vit E plays a role to block excessive peroxide formation and prevents hyaline degeneration (Fidancı, 1986; Cooper and Valentine, 2016).

The disease is clinically observed in two different forms; acute (cardiac) and subacute. Acute form is characterized with myocardial degeneration and sudden death especially in young animals such as lambs and kids. Subacute form is characterized mostly by skeletal muscle degeneration. In this form clinical findings include inability to stand, difficulty walking, stiff-legged walk, shortness of breath and difficulty holding up the head (Dabak et al., 2002). In another classification, the form seen in newborn lambs are called the *congenital form*, and the form that seen in 3-4 month old lambs are called the *acquired form*. (Sugen and Güneş, 2008).

Hyaline degeneration which is characterized as pale areas in the skeletal muscles and cardiac muscles, Zenker's necrosis and calcification areas are observed in macroscopic examination during necropsy. Muscles with hyaline degeneration have predominantly a pale appearance and such muscles have a look similar look to fish or chicken meat. Muscles that are normally brittle, later start to show linear or widespread white areas. Degeneration of cardiac muscles is mostly localized in subendocardial and subepicardial regions and does not tend to progress deeper. Heart ventricles wall, interventricular septum and papillary muscles are affected by degeneration. In cases with Zenker's necrosis, due to formation of dystrophic calcification crackling sensation (crepitation) is detected when such areas are incised (Özdemir and Ortatatlı, 2016).

Histopathologically; degenerative muscle fibers are pink- homogenous colored and swollen. It has been reported that such muscles lose their striation. In more advance cases, Zenker's necrosis of muscle fibers is observed and integrity of muscle fibers is compromised and they become dark pink in color. Calcification may form in necrotic muscle fibers in further stages of the disease. Increased connective tissue with mononuclear cell filtration can be observed in the necrotic region (Özdemir and Ortatatlı, 2016).

Enzyme activities including GSH-Px, creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LD) can be measured in laboratory tests of the disease. Red blood cell GSH-Px activity in healthy lambs is 30-60 mU/mg hemoglobin and any value lower than 30 indicates an insufficiency. However the enzyme GSH-Px is affected by some factors and therefore does not always give the correct result (Beytut et al., 2001; Başoğlu and Sevinç, 2004).

The objective of this study is to evaluate pathological findings observed in lambs died as a result of nutritional myopathy in Central Anatolia region in Turkey and to determine in which organs lesions are observed the most.

Material and Method

The material of this study conducted in Aksaray University Faculty of Veterinary Medicine Department of Pathology between the dates of October 2014 - May 2016 consists of 39 lambs which died showing clinical signs of nutritional myopathy such as inability to stand, wobbly walking, and stiff gait. Twenty five lambs were 1-3 weeks old and 14 lambs were 4-8 week old. While 26 of 39 lambs were *Akkaraman* race, 13 lambs were *Merinos* race. The regions where the lambs were brought are shown on the map (Figure 1).

Macroscopic examination of cardiac, intercostal, diaphragm and gracilis muscles of lambs were performed during necropsy. Then samples were taken from these tissues and fixed in 10% formaldehyde solution. Then, samples were embedded into paraffin blocks after the routine pathological process and sections 5µ thickness were cut from the blocks using a microtome and stained with hemotoxylin-eosin and examined under a light microscope. Hyaline degeneration, Zenker's necrosis, calcifications and inflammatory reactions were investigated in histopathological examinations.

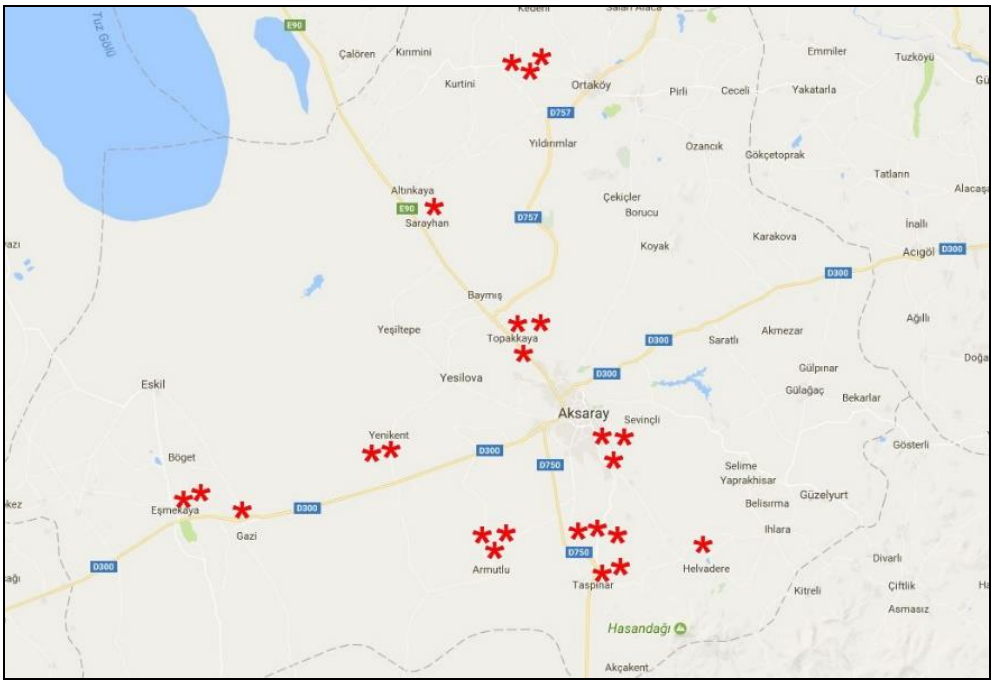

Figure 1. The villages where lambs were brought for the necropsy (Red asterisks).

Results

Macroscopic Findings

Macroscopic and histopathological findings are shown in Table 1. The villages where the lambs were brought are given in Table 2. Based on the necropsy, the organ where lesions are observed the most was found to be the heart. Paleness in heart was observed in all of 39 cases (100%) and calcification was observed in 22 cases (56.4%). Paleness was observed in both ventricles in the epicardium and endocardium of the heart (Figure 2A). Additionally, paleness and calcifications in papillary muscles were observed. The calcified areas especially in the endocardium were typical (Figure 2B). Among the skeletal muscles examined, the muscle that was affected the most was gracilis muscle on the inside of the thigh. These are followed by intercostal muscles and diaphragm muscle. The affected muscles were pale and had a look similar to chicken meat (Figure 2C). In cases with severe NM, white linear areas were prominent (Figure 2D). Paleness was observed in 23 cases (58.9%) in gracilis muscle and in 11 cases (28.2%) in intercostal muscles and in 8 cases (20.5%) in diaphragm. Calcification were found in 9 cases (23%) in gracilis muscle and only 2 cases (5.1%) in intercostal muscles, but no calcification was found in diaphragm.

Table 1: Counts and percentages of grossly and microscopically observed cases (N:39).

N:39	Gross Findings		Histopathological Findings		
	Paleness	Calsification	Inflammation	Zenker's Necrosis	Calsification
Heart	39 (100 %)	22 (56.4 %)	21 (53.8 %)	35 (89.7 %)	26 (66.6 %)
M. Gracilis	23 (58.9 %)	9 (23 %)	14 (35.8 %)	18 (46.1 %)	11 (28.2 %)
Intercostal	11 (28.2 %)	2 (5.1 %)	6 (15.3 %)	7 (17.9 %)	2 (5.1 %)
Diaphragm	8 (20.5 %)	-	3 (7.6 %)	7 (17.9 %)	-

Table 2: The villages, counts of farms and cases

Villages	Farms	Cases
Aksaray Merkez	3	4
Armutlu	3	5
Eşmekaya	2	3
Helvadere	1	2
Gazi	1	2
Sarayhan	1	2
Taşpınar	5	8
Topakkaya	3	6
Yenice	3	5
Yenikent	2	2
TOTAL	24	39

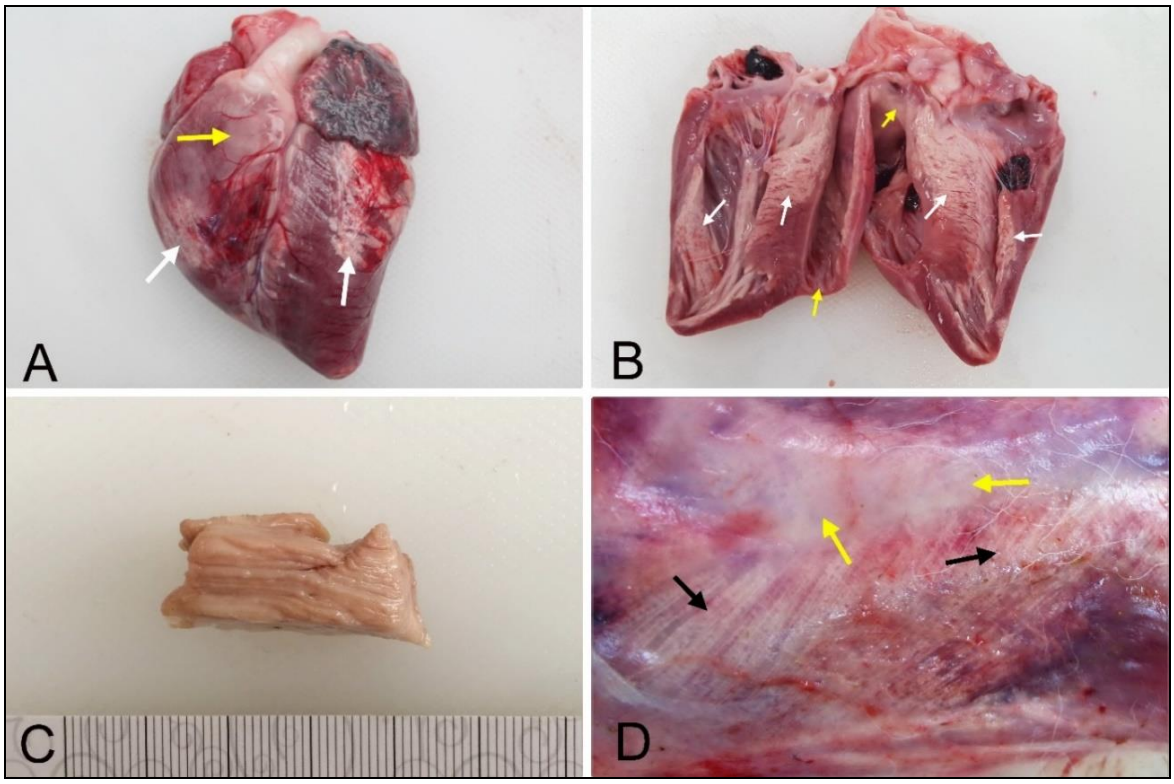

Figure 2. A. Pale (yellow arrow) and calcification areas (white arrows) in the epicardium of the heart. **B.** Widespread areas of dystrophic calcification (white arrows) and pale areas (yellow arrows) in the endocardium. **C.** Paleness appearance in the musculus gracilis. **D.** Pale areas (yellow arrows) and linear calcification areas (black arrows) in the diaphragm.

Histopathological Findings

The number of lesions based on microscopic examinations is given in Table 1. Histopathologically, inflammation, degeneration, necrosis and calcification were observed the most in heart and then in gracilis muscle, intercostal muscles and diaphragm muscle. It was noted that degenerative muscle fibers were swollen and had a pink homogenous color (Figure 3A). Zenker's necrosis was detected in more advanced cases. Muscle fibers in Zenker's necrosis had a dark pink color and clumps of pyknotic nuclei (Figure 3B). Degeneration and necrosis were observed in heart in 35 cases (89.7%), in gracilis muscle in 18 cases (46.1%) and in diaphragm and intercostal muscles in 7 cases each (17.9%). During calcification which is the next stage of necrosis, muscle fibers were found to be deep purple in color and had irregular shapes (Figure 3C). Calcification was mostly found in heart with 26 cases (66.6%), in gracilis muscle in 11 cases (28.2%), in only 2 cases in intercostal muscles (5.1%) and in diaphragm was not found of any cases. In these stages inflammatory cells caused mostly by macrophages together with mononuclear cell infiltrations around lesions were seen (Figure 3D).

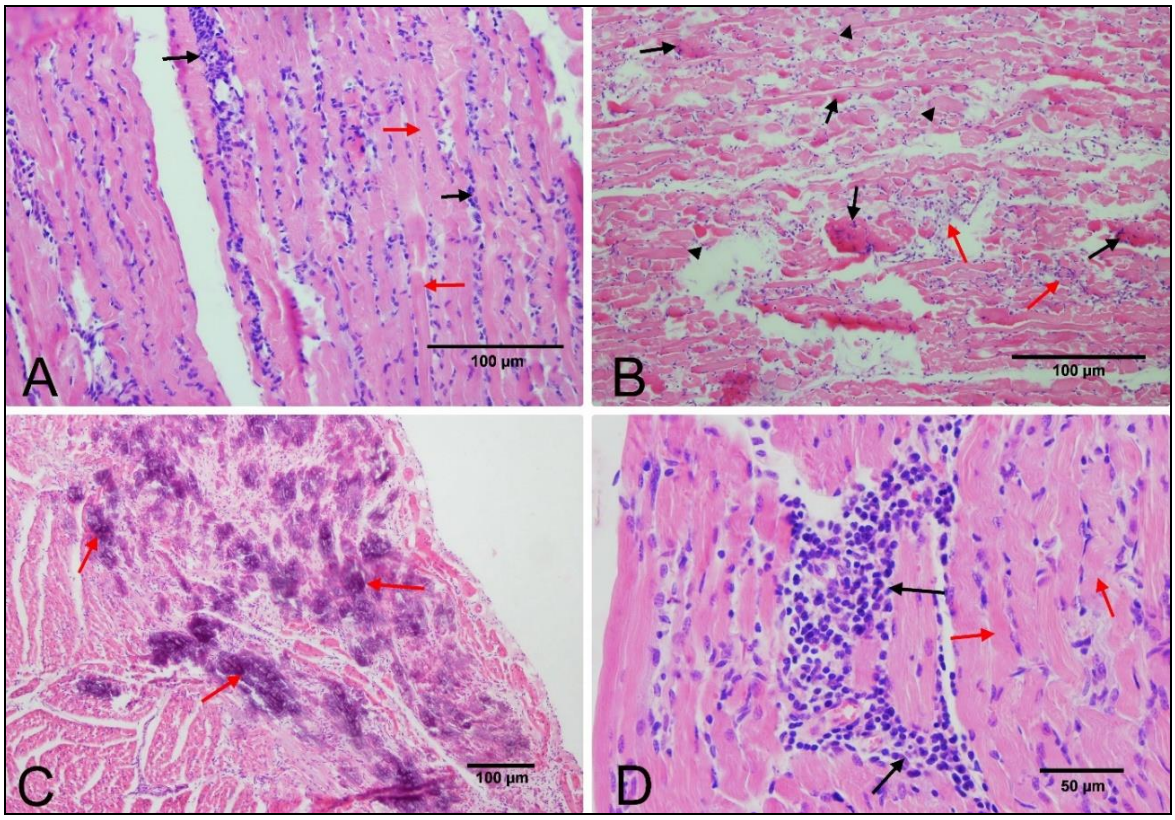

Figure 3. A. Muscle fibers suffered from homogenous pink hyaline degeneration (red arrows) and mononuclear cell infiltrations (black arrows) between muscular fibers. M. gracilis. HE. **B.** Degenerative muscle fibers (arrow heads), Zenker's necrosis in muscle fibers (black arrows) and mononuclear cell infiltrations (red arrows) between these fibers. Diaphragm. HE. **C.** Muscle bundles (arrows) undergoing calcification. Heart. HE. **D.** Degenerative muscle fibers (red arrows) in the gracilis muscle and cellular infiltrates (black arrows) formed by macrophages and mononuclear cells in the interstitial areas. M. gracilis. HE.

Discussion and Conclusion

Nutritional myopathy is a metabolic disease associated with Vitamin E and SE deficiency in lambs (Başoğlu and Sevinç, 2004; Yüksek and Basbuğan, 2015). In live animals, enzyme values such as GSH-Px, CK, AST and (LDH) in blood and Vit E and Se concentrations were measured in laboratory tests to make a diagnosis (Nizamlıoğlu et al., 1991; Voyvoda et al., 1996; Keleş et al., 2000; Beytut et al., 2001). However due to the fact that measurement of such biochemical parameters are difficult in the field conditions, the enzyme GSH-Px is affected by some factors, and that it is difficult to detect Se and Vit E in body fluids and that they are sometimes within the normal limits this reportedly cannot always give correct results (Başoğlu and Sevinç, 2004). Therefore diagnosis based on necropsy findings is also preferred (Ferrer et al., 2002).

In this study, twenty five lambs were 1-4 weeks old and 14 lambs were 4-8 weeks old. The typical form of the disease has been reported to be observed mostly in lambs that are 1 day to 2 months old and this study also determined that the same age groups were affected by the disease in a similar way (Cooper and Valentine, 2016). The fact that older lambs are not affected much by this disease can be explained by the recovery of the animals from subacute form with an effective Se and Vit E treatment. The lambs in the study received treatment after the disease symptoms were seen but no improvement could be achieved. This is explained by the fact that treatment of cases with acute cardiac failure is not possible (Başoğlu and Sevinç, 2004).

It were reported in the literatures that the disease affects mostly cardiac muscles and then leg muscles and other muscles and similarly this study also found that the cardiac and then to a lesser degree gracilis and intercostal muscles and diaphragm muscle were affected the most (Baran, 1966; Köküuslu et al., 1986; Cooper and Valentine, 2016). Paleness and calcification areas in endocardium and epicardium of the heart were similar to the findings reported by other researchers (Ferrer et al., 2002; Milli and Hazıroğlu, 2000). Among skeletal muscles, gracilis muscle on the inside of the thigh and intercostal and diaphragm muscles were found to be affected the most by the disease and similar to the literature (Köküuslu et al., 1986).

Histopathologically, swollen and pink homogenous muscle fibers and irregular shaped, dark pink-purple color dystrophic calcification areas and inflammatory cell infiltration mostly accompanied with macrophages seen in both cardiac and skeletal muscles in severe cases were similar to the findings reported in previous studies (Özdemir et al., 2016; Özdemir and Ortatatlı, 2016). Paleness in gracilis muscle was in 23 cases in macroscopic analysis and the number of degeneration and necrosis was 18 cases in microscopic examinations. Similarly, paleness in intercostal muscles was observed in 11 cases and histopathologically degeneration and necrosis was observed only in 8 cases. Cooper and Valentine (2016) emphasized that skeletal muscles of lambs look normally whitish and gross findings alone are not sufficient for diagnosis and that diagnosis should be supported by techniques such as histopathology.

In conclusion, it was shown that both macroscopic and microscopic examination of first cardiac muscles and then gracilis muscle, intercostal and diaphragm muscles is necessary for pathological diagnosis of nutritional myopathies. It was determined that this metabolic disease which has been prevalent in the Central Anatolian region since 1950s and is still common and threatens herd's health.

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