

Examination of Some Endoparasites Prevalence in Romanov Sheep Imported from Ukraine

Adnan AYAN^{1*}, Turan YAMAN², Ömer Faruk KELEŞ², Hidayet TUTUN³

¹Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey.

²Department of Pathology, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey.

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey.

Geliş Tarihi: 11.09.2018

Kabul Tarihi: 27.05.2019

Abstract: The purpose of this study was to investigate some endoparasites spread in the Romanov sheep imported from Ukraine. The flotation, sedimentation and Baerman-Wetzel techniques were used to analyze the fecal samples collected from the sheep (n=156) and the samples were examined under the light microscope. Furthermore, from this herd, the internal organs of the sheep that had died were pathologically examined on macroscopic and microscopic level. Among fecal samples examined 69 (44.23%) were found parasitically positive, 66 of these (42.3%) were found positive for *Dicrocoelium dendriticum*, 3 samples (1.92%) were positive for *Nematodirus* spp. and *Eimeria* spp, while *Giardia* spp. was not detected. The pathological examination of the internal organs of eight of these sheep revealed adult forms of *D. dendriticum* only in the liver. The parasitological and pathological findings of this study indicated a high incidence of *D. dendriticum* that causes economic losses due to cases of death, in the Romanov sheep, which has been imported to country in large numbers in recent years.

Keywords: *Dicrocoelium dendriticum*, Helminth, Protozoan, Romanov sheep.

Ukrayna'dan İthal Edilen Romanov Koyunlarında Bazı Endoparazitlerin Yaygınlığının İncelenmesi

Özet: Bu çalışmada Ukrayna'dan ithal edilen Romanov kuzularında bazı endoparazitlerin yaygınlığı araştırılmıştır. Kuzulardan toplanan dışkılarına (n=156) parazitolojik muayene yöntemlerinden flotasyon, sedimentasyon ve Baerman-Wetzel yöntemleri uygulandı ve örnekler ışık mikroskopunda incelendi. Ayrıca bu sürülerden ölen kuzuların iç organları makroskobik ve mikroskobik olarak incelendi. Kuzuların 69'u (%44,23) paraziter açıdan pozitif olarak tespit edildi. Bunların 66'sı (%42,3) *Dicrocoelium dendriticum* yönünden, 3'ü (%1,92) *Nematodirus* spp yönünden pozitif bulundu. Protozoon etkenlerden ise *Eimeria* spp. ve *Giardia* spp. saptanmadı. Bu kuzuların iç organlarının patolojik incelemesinde karaciğerde yaygın olarak *D. dendriticum*'un erişkin formları tespit edildi. Sonuç olarak, son yıllarda ülkemize çok sayıda ithal edilen Romanov kuzularında yüksek *D. dendriticum* varlığından dolayı gerçekleşen ölümler ekonomik kayıplara neden olmaktadır.

Anahtar Kelimeler: *Dicrocoelium dendriticum*, Helminth, Protozoon, Romanov koyunu.

Introduction

It is widely acknowledged that parasitic infections of sheep result in large-scale economic losses for the livestock industry and agricultural communities due to death of infected animals, reduction in animal weight gain, and the affected organs being unusable after slaughter (Gıcık et al., 2002; Kara et al., 2009; Suarez and Buseti, 1995; Tsotetsi and Mbatı, 2003; Wang et al., 2006; Yılmaz et al., 2014). Some helminths found in sheep can directly or indirectly cause serious clinical diseases in humans, such as hydatidosis/echinococcosis and dicrocoeliasis (Cengiz et al., 2010; Karadag et al., 2005; Altintas 2008; Ing et al., 1998). According to data from the Turkish Statistical Institute, in Turkey in February 2018, the number of bovine animals was 16.1 million and the total number of small ruminants was 44.3 million comprising 33.6 million sheep and 10.6 million goats (Anonym, 2017). Although in recent years, the number of animals in Turkey has increased, it is not sufficient to meet the

growing demand for meat due to socio-economic development. Turkey fills the gap between supply and demand by importing live animals from abroad. However, since the presence of parasites in imported live animals can cause serious economic losses, it is crucial to perform a parasitic evaluation on these animals to increase their economic efficacy.

Dicrocoeliasis is caused by *Dicrocoelium dendriticum*, also known as the lancet liver fluke. This parasite which is seen all over the world lives in the gallbladder and bile ducts of the host animals and causes weight loss and decreased milk production. Dicrocoeliasis continues to spread among sheep populations due to the expansion of dry, scrub-type habitats and increased resistance to anthelmintics (Otranto and Traversa, 2003). Sheep, cattle, and other ruminants are the primary hosts of this parasite, and humans and other animals are alternative hosts (Albogami et al., 2015; Yener et al., 2016). Dicrocoeliasis usually occurs due to the

consumption of metacercariae-carrying ants by sheep, goats, and cattle, and sporadically by humans. In addition, pseudo-parasitism may develop in humans when raw or undercooked infected liver is consumed. When taken by the final host, young parasites in the metacercariae are released and pass through the intestinal wall into the portal system. Dicrocoeliasis has a worldwide prevalence, covering Europe, Asia, Africa, North and South America, and Australia. It is epidemic in pastures or mountain meadows that provide adequate conditions for the survival and development of terrestrial snails and ants. This parasite tends to be found in dry, calcareous and alkaline soils favored by intermediate hosts (Arbabi et al., 2011). In these areas, *D. dendriticum* eggs are resistant because they can survive hard winters and remain infectious for up to 20 months in grasslands. In Mediterranean countries, *D. dendriticum* egg excretion in sheep feces is seasonal and reaches its peak in winter (Manga-Gonzalez et al., 1991). In cases of dicrocoeliasis, pathological changes include pale or hardened liver, tension and inflammation of bile ducts, presence of parasites in bile ducts and gallbladder, whitish foci on the liver, scarring, fibrosis, and cirrhosis occur depending on the severity of the infection (Jithendran and Bhat, 1996; Yener et al., 2016). *D. dendriticum* is commonly seen in cattle and sheep in Ukraine (Savchuk, 1956).

Considering the economic losses arising from parasitic infections in imported live animals and due to the reduced quality of meat in Turkey, we aimed to investigate the prevalence of *D. dendriticum* among the Romanov sheep imported from Ukraine in the present study.

Material and Method

This study was conducted on 156 Romanov sheep imported from Ukraine to Van province of Turkey. According to the recommendation of a veterinary surgeon, the sheep were treated first with a commercial preparation containing 1% doramectin; one week later, with a preparation containing oxfendazole and oxclozanide; and a further week later, with a preparation containing rafoxanide and thiabendazole. One week after these applications, fecal specimens were collected from the rectum of the sheep and placed in containers. The specimens were transferred to the laboratory for examining macroscopically in terms of cestode rings and microscopically to identify nematode and cestode eggs and Eimeria oocysts using the Fulleborn saturated salt solution method and trematode eggs using the modified Benedek sedimentation method (Çelikkol, 1995). The Baerman-Wetzel method was employed for the

detection of lungworm larvae. For this purpose, 5 grams of fecal specimens was incubated in a Baermann apparatus for a day. Then, 2 mL of solution was obtained from the bottom of the centrifuge tube to examine the presence of lungworm larvae (Eysker, 1997). In addition, a direct examination (Native-Lugol) was performed to identify Giardia cysts (Özbel and Dağcı, 1997). The preparations were examined using x10 and x40 objective lenses. From the same herd, eight sheep died. Necropsy was performed on these sheep to macroscopically examine their livers in terms of the presence of *D. dendriticum*. For histopathologic examination, liver sections were fixed in a 10% formalin solution for 24 hours. Following a routine tissue follow-up procedure, 4 µm sections cut from the paraffin-embedded blocks were stained with Hematoxylin and eosin and Masson's trichrome connective tissue stain to be examined under light microscope (Luna, 1968).

Results

By examining the fecal samples 69 of the 156 Romanov sheep (44.23%) were found to be parasitically positive. Sixty-six (42.3%) of these sheep were positive for *D. dendriticum* and three (1.92%) for *Nematodirus* sp., Eggs of parasites including *Fasciola hepatica*, *Fasciola gigantica*, *Taenia ovis*, *Strongyloides papillosus*, *Moniezia* spp., *Paramphistomum* spp., *Oesopagostomum* spp., *Bunostomum* spp., *Cooperia* spp., *Haemonchus* spp., *Marshallagia* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Trichuris* spp. were not found in the feces. Furthermore, no lungworm larva belonging to *Dictyocaulus filaria*, *Cystocaulus ocreatus*, *Muellerius capillaris*, *Protostrongylus* spp. or *Neostrongylus linearis* was detected.

Finally, protozoa examination did not reveal any Eimeria sp., oocysts or Giardia cysts. Macroscopically, the infected livers were sclerotic in appearance and had hard and blunt edges; furthermore, diffuse gray-whitish branching masses were detected on both visceral and parietal surfaces (Figures 1A and 1B). On the cross-section of the liver, the bile ducts were marked and thickened (Figure 1C). When manual pressure was applied to the liver, a large number of adult *D. dendriticum* along with dark brown fluid from the bile ducts were observed. Histopathological examination showed diffuse capsular hepatic fibrosis and severe cholangiohepatitis (Figures 1D and 1E). Proliferation and dilation were present in the bile ducts with increased fibrosis tissue. Adult forms of parasites were also detected in the bile ducts (Figure 1F).

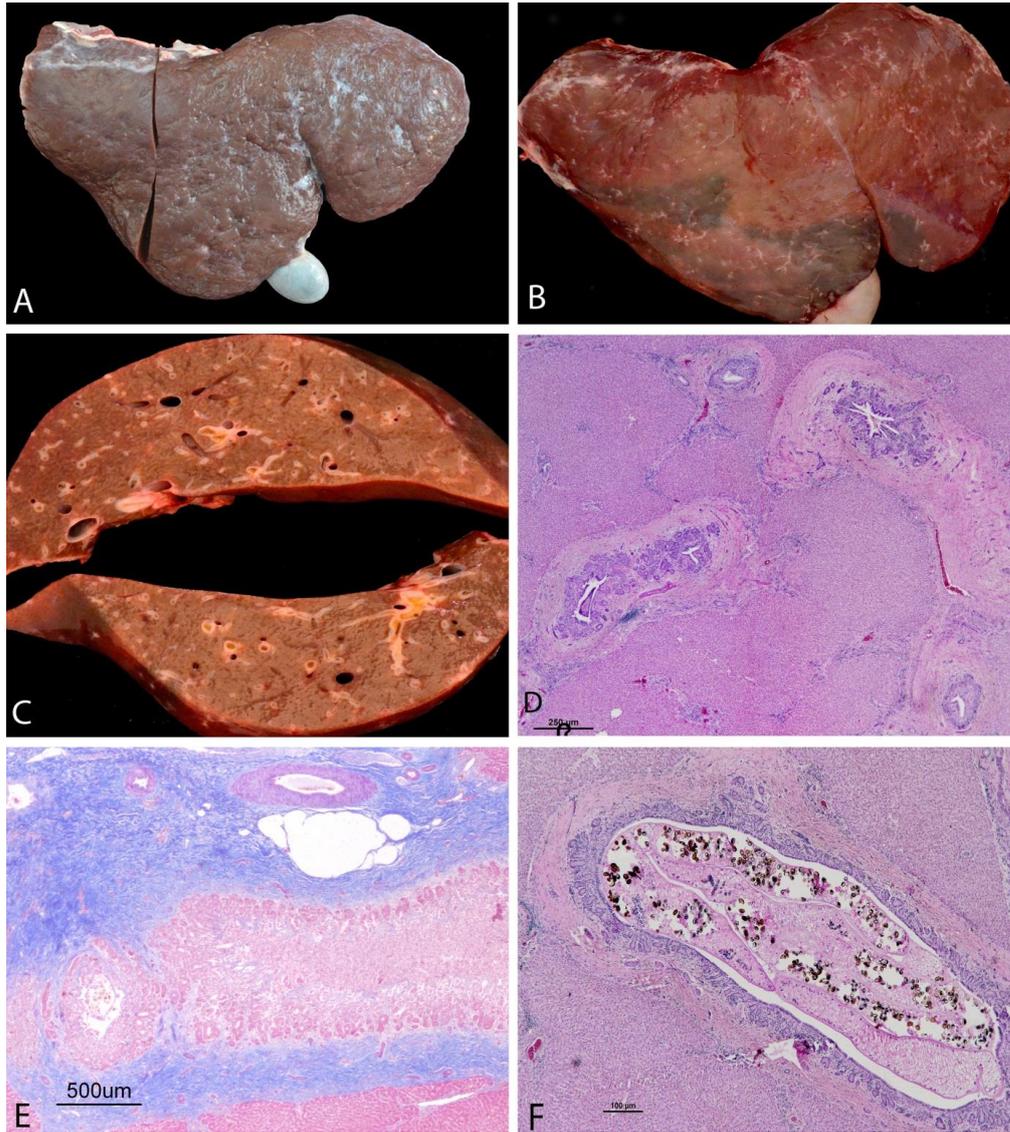


Figure 1. A) Liver sclerotic appearance, massive marginal edges, diffuse gray-whitish colored branched masses on parietal faces. B) Findings in another sheep, liver sclerotic appearance and multi fokal gray-whitish colored branched masses on parietal faces. C) On the cross section of the liver, the bile ducts were marked and thickened. D) In the liver, extensive capsular fibrosis and severe cholangiohepatitis were detected. Proliferation and dilatation were observed in the bile ducts with increased fibrosis tissue, H. E. X 10. E) In the liver, extensive capsular fibrosis and cholangiohepatitis were detected, M. T. C. X 20. F) Adult forms of parasites were detected in bile ducts, H. E. X 10.

Discussion

In the case of severe infections caused by *D. dendriticum*, there is clinical evidence of edema and anemia in the animals. The reduced milk and wool yield, as well as deaths in infected animals cause economic losses (Güralp, 1981). Infections of *Moniezia* sp. (Öncel, 2000; Kırçali Sevimli et al., 2006), lungworms (Öncel, 2000; Umur and Arslan, 1998), gastrointestinal worms (Celep et al., 1995; Kırçali Sevimli et al., 2006; Öncel, 2000; Umur, 1997), and *Trichuris* sp. (Kırçali Sevimli et al., 2006; Umur and Arslan, 1998.) have been reported in sheep from different regions of Turkey. In the

current study, the Romanov sheep imported from Ukraine showed positivity only for *D. dendriticum* and *Nematodirus* spp. The *D. dendriticum* infection may have developed because the areas in which the imported Romanov sheep are reared in Ukraine are favorable for the survival of the intermediate hosts of this trematode. *D. dendriticum* is common in cattle and sheep in Ukraine (Savchuk, 1956). Infection has also been detected in sheep in Turkey (Adanır and Cetin, 2016; Balkaya et al., 2009; Biçek and Değer, 2005; Değer et al., 2017; Gargılı et al., 1999; Gıcık et al., 2002; Kaplan et al., 2014; Kara et al., 2009; Kırçali Sevimli et al., 2006). In the current study, 66 (42.3%) of the 156 Romanov sheep were

positive for *D. dendriticum*. *Nematodirus abnormalis*, *N. spathiger* and *N. filicollis* are the causes of intestinal nematodes, and Turkey has high prevalence of these infections in small ruminants, whereas *N. lanceolatus* is rarely seen (Burgu et al., 1999; Cantoray et al., 1992; Umur and Yukarı, 2005). Similarly, in the current study, 1.92% of the sheep were found to have *Nematodirus* spp. The livers of the infected animals were hardened and had a pale color due to increased connective tissue. It was previously reported that *D. dendriticum* caused extensive cholangiohepatitis in the liver with fibrosis, and on the cross-section, the bile ducts were more marked and highly parasitic. Histopathologically, extensive hepatic fibrosis and cholangiohepatitis, inflammation of the bile ducts, and cirrhosis were noted (Güralp, 1981; Wolff et al., 1984; Camara et al., 1996; Yener et al., 2016). The macroscopic and microscopic findings obtained in this study are consistent with the above-mentioned reports in the literature. The macroscopic examination revealed hardened, sclerotic liver, thickened bile ducts and adult parasitic form on the cross-sectional image, and diffuse gray-whitish masses on the visceral and parietal surfaces. Microscopically, diffuse capsular hepatic fibrosis and severe cholangiohepatitis were present, and proliferation and dilatation of the bile ducts were observed.

The management of *D. dendriticum* is challenging due to the complexity of its biological life cycle and epidemiology, and the methods currently employed are not adequate. The management of and struggle against this parasite are mainly based on the control of infections in primary and secondary intermediate hosts and the antiparasitic treatment of infected animals. However, the control of intermediate hosts can only be performed in small areas due to the high cost of application in large areas and difficulties resulting from varying soil conditions (Otranto and Traversa, 2002). Anthelmintic for the treatment of *D. dendriticum* infections includes the derivatives of benzimidazole (albendazole, triclabendazole, fenbendazole, mebendazole, cambendazole, and thiabendazole) and probenzimidazole (thiophanate and netobimine) (Onar, 1990), as well as praziquantel (Akkaya et al., 2006). The oral use of these drugs decreases the *D. dendriticum* load by more than 90% (Akkaya et al., 2006; Onar, 1990; Otranto and Traversa, 2002). Benzimidazoles are frequently used against gastrointestinal nematodes (Köse et al., 2007). Some antiparasitic drugs used following the importation of animals are also effective against *D. dendriticum* and *Nematodirus* spp. in reducing the load of these parasites.

Conclusion

In conclusion, in recent years, economic losses have been observed due to death in Romanov sheep imported to Turkey, especially due to the presence of infections caused by *D. dendriticum* and *Nematodirus* spp, and the lack of preventive measures against these helminths. The results of this study show that the imported animals should be controlled by the authorized institutions in terms of parasitic diseases causing serious economic losses.

References

- Adanır R, Cetin H, 2016: Antalya Belediye Mezbahası'nda (An-Et) kesilen koyunlarda karaciğer trematodlarının yaygınlığı. *Mae Vet Fak Derg*, 1(1), 15-20.
- Akkaya H, Deniz A, Sezen A, 2006: Effect of praziquantel on *Dicrocoelium dendriticum* in naturally infected sheep. *Med Weter*, 62, 1381-1382.
- Albogami BM, Kelany AHM, Abu-Zinadah OA, 2015: Prevalence of *Dicrocoelium dendriticum* infection in sheep at Taif Province, West Saudi Arabia. *J Egypt Soc Parasitol*, 45, 435-442.
- Altıntaş N, 2008: Parasitic zoonotic diseases in Turkey. *Vet Ital*, 44, 633-646.
- Anonym, 2017: <http://www.tuik.gov.tr/PreHaberBultenleri.do?id=27704> Erişim tarihi; 25.06.2018
- Arbabi M, Dalimi A, Ghafarifar F, Froozandeh Moghadam M, 2011: Prevalence and intensity of *Dicrocoelium dendriticum* in sheep and goats of Iran. *Res J Parasitol*, 10(3923), 1-8.
- Balkaya İ, Terim Kapakin KA, Küçükkalem ÖF, 2009: *Dicrocoelium dendriticum* ile enfekte koyun karaciğerleri üzerinde parazitolojik ve patolojik incelemeler. *Atatürk Üniversitesi Vet. Bil. Derg*, 4(3): 169-175.
- Biçek K, Değer S, 2005: Tatvan belediye mezbahasında kesilen koyun ve keçilerde karaciğer trematodlarının yaygınlığı. *Yü Vet Fak Derg*, 16, 41-43.
- Burgu A, Gönenç B, Sarımehtemoğlu O, 1999: Tiftik keçilerinde Skrjabinema ve diğer helmint enfeksiyonlarının yayılışı. *Ankara Üniv Vet Fak Derg*, 46, 137-142.
- Camara L, Pfister K, Aeschlimann A, 1996: Histopathological analysis of bovine livers infected by *Dicrocoelium dendriticum*. *Vet Res*, 27(1), 87-92.
- Cantoray R, Aytakin H, Güçlü F, 1992: Konya yöresindeki keçilerde helmintolojik araştırmalar. *Veterinarium*, 3, 27-30.
- Celep A, Açıcı M, Çetindağ M, Gürbüz İ, 1995: Samsun yöresi koyunlarında parazitler epidemiyolojik çalışmaları. *Türkiye Parazitol Derg*, 19, 290-296.
- Cengiz ZT, Yılmaz H, Dülger AC, Çiçek M, 2010: Human infection with *Dicrocoelium dendriticum* in Turkey. *Ann Saudi Med*, 30, 159-161.
- Çelikkol G, 1995: Parazitolojide başlıca teknik ve tanı metotları. Yüksek Lisans Tezi, Yü Sağlık Bilimleri Enstitüsü, Van.

- Değer S, Biçek K, Karakuş A, 2017: Prevalence of *Dicrocoelium dendriticum* in sheep and goats slaughtered in Van region (Van municipality slaughterhouse). *Van Vet J*, 28(1), 21-24.
- Eysker M, 1997: The sensitivity of the Baermann method for the diagnosis of primary *Dictyocaulus viviparus* infections in calves. *Vet Parasitol*, 69, 89-93.
- Gargılı A, Tüzer E, Gülanber A, Toparlak M, Efil İ, Keleş V, Ulutaş M, 1999: Prevalence of liver fluke infections in slaughtered animals in Trakya (Thrace), Turkey. *Turk J Vet Anim Sci*, 23, 115-116.
- Gıcık Y, Arslan MÖ, Kara M, Akça A, 2002: Kars ilinde kesilen koyunlarda karaciğer kelebeklerinin yaygınlığı. *Kafkas Üniv Vet Fak Derg*, 8, 101-102.
- Güralp N, 1981: Helmintholoji, 2nd ed., Ankara Üniversitesi Veteriner Fakültesi Yayınları., Ankara.
- Ing MB, Schantz PM, Turner JA, 1998: Human coenurosis in North America: case reports and review. *Clin Infect Dis*, 27, 519-523.
- Jithendran KP, Bhat TK, 1996: Prevalence of *Dicrocoeliosis* in sheep and goats in Himachal Pradesh, India. *Vet Parasitol*, 61, 265-271.
- Kaplan K, Başpınar S, Özavcı H, 2014: 2008 – 2012 Yılları Arasında Elazığ'da Kesilen Hayvanlarda Karaciğer Trematodlarının Görülme Sıklığı. *Fü Sağ Bil Vet Derg*, 28(1), 41-43.
- Kara M, Gıcık Y, Sari B, Bulut H, Arslan MO, 2009: A slaughterhouse study on prevalence of some helminths of cattle and sheep in Malatya Province, Turkey. *J Anim Vet Adv*, 8, 2200-2205.
- Karadag B, Bilici A, Doventas A, Kantarci F, Selcuk D, Dincer N, Oner YA, Erdinler DS, 2005: An unusual case of biliary obstruction caused by *Dicrocoelium dendriticum*. *Scand. J Infect Dis*, 37, 385-388.
- Kırcalı Sevimli F, Kozan E, Köse M, Eser M, 2006: Dışkı muayenesine göre Afyonkarahisar İli koyunlarında bulunan helmintlerin yayılışı. *Ankara Üniv Vet Fak Derg*, 53, 137-140.
- Köse M, Kozan E, Sevimli Kırcalı F, Eser M, 2007: The resistance of nematode parasites in sheep against anthelmintic drugs widely used in Western Turkey. *Parasitol Res*, 101, 563-7.
- Luna LG, 1968: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed., The Blakiston Division., McGraw-Hill Book Company., USA.
- Manga-Gonzalez MY, González-Lanza C, Del-Pozo-Carnero P, 1991: Dynamics of the elimination of *Dicrocoelium dendriticum* (Trematoda, Digenea) eggs in the faeces of lambs and ewes in the Porma basin (León, NW Spain). *Ann Parasitol Hum Comp*, 66(2), 57-61.
- Onar E, 1990: Efficacy of thiophanate and albendazole against natural infections of *Dicrocoelium dendriticum*, *Fasciola hepatica*, and gastro intestinal nematodes and cestodes in sheep, and gastrointestinal nematodes and cestodes in sheep. *Vet Parasitol*, 35, 139-145.
- Otranto D, Traversa D, 2002: A review of *Dicrocoeliosis* of ruminants including recent advances in the diagnosis and treatment. *Vet Parasitol*, 107, 317-335.
- Otranto D, Traversa D, 2003: *Dicrocoeliosis* of ruminants: a little known fluke disease. *Trends Parasitol*, 19, 12-15.
- Öncel T, 2000: Güney Marmara bölgesindeki koyunlarda helmint türlerinin yayılışı. *Türkiye Parazitoloj Derg*, 24, 414-419.
- Özbel Y, Dağcı H, 1997: Giardiasisin laboratuvar tanısı. In "Giardiasis", Ed; Özcel MA and Üner A, Türkiye Parazitoloji Derneği Yay no:14; İzmir.
- Savchuk N, 1956: The distribution and control of helminthiasis of farm animals in the Odessa area. In: Problemi parazitologii. Transactions of the Scientific Conference of Parasitologists of the Ukrainian SSR, 2nd, pp. 183-184.
- Suarez VH, Buseti MR, 1995: The epidemiology of helminth infections of growing sheep in Argentina's Western Pampas. *Int J Parasitol*, 25, 489-494.
- Tsotetsi AM, Mbatı PA, 2003: Parasitic helminths of veterinary importance in cattle, sheep and goats on communal farms in the northeastern Free State, South Africa. *J S Afr Vet Assoc*, 74, 45-48.
- Umur Ş, 1997: Kars yöresi koyunlarının mide-bağırsak nematodları ve mevsimsel dağılımları. *Turk J Vet Anim Sci*, 21, 57-65.
- Umur Ş, Arslan MÖ, 1998: Kars yöresi sığır ve koyunlarında akciğer kılkuçları. *Türkiye Parazitoloj Derg*, 22, 88-92.
- Umur Ş, Yukari BA, 2005: Seasonal activity of gastrointestinal nematodes in goats in Burdur region, Turkey. *Turk J Vet Anim Sci*, 29, 441-448.
- Wang CR, Qiu JH, Zhu XQ, Han XH, Ni HB, Zhao JP, Zhou QM, Zhang HW, Lun ZR, 2006: Survey of helminths in adult sheep in Heilongjiang Province, People's Republic of China. *Vet Parasitol*, 140, 378-382.
- Wolff K, Hauser B, Wild P, 1984: *Dicrocoeliosis* in sheep: pathogenesis and liver regeneration after therapy. *Berl Munch Tierarzt Woch*, 97(10), 378-387.
- Yener Z, Uyar A, Yaman T, Keleş ÖF, 2016: Veteriner Özel Patoloji. Birinci Baskı, Matus Basım evi, Ankara.
- Yılmaz R, Özyıldız Z, Yumuşak N, 2014: Pathomorphological Findings of *Coenurus cerebralis* in Sheep. *Harran Üniv Vet Fak Derg*, 3(2), 73-77.

***Corresponding author:** Adnan AYAN

Van Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Genetik Anabilim Dalı, 65080, Zeve Kampüsü, Tuşba/Van
e-mail: adnanayan@yyu.edu.tr