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Research Article

The Prevalence of Liver Trematodes in Slaughtered Ruminants in Aydın Province

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ABSTRACT

Fasciola hepatica and *Dicrocoelium dendriticum* are trematodes widely found worldwide including in Turkey. Liver trematode infections cause serious losses in livestock production. Also, it adversely affects the development and live weight gain of animals and damage the national economy by causing the loss of economical significant organs such as the liver. This study was performed to investigate the reveal the prevalence of *Fasciola* and *Dicrocoelium* parasites at the slaughterhouse in Aydın province and to investigate the resulting economic losses. This study was carried out from the samples collected in the Efeler slaughterhouse in Aydın province between May 2015 and June 2016. According to this, liver and bile ducts of slaughtered sheep-goat and cattle have been examined in terms of *Fasciola* spp. and *Dicrocoelium* spp. in a year. During the study a total of 3193 cattle and 3659 small ruminant (sheep and goat) livers were examined. Two species of liver fluke, *F. hepatica* and *D. dendriticum*, were encountered in the study.

As a result *F. hepatica* was detected in six of 3193 cattle (0,18%) and in one of 3659 (0,02%) small ruminants. In addition, *D. dendriticum* was detected in 13 of 3659 (0,3%) small ruminants. *Fasciola gigantica* and *D. dendriticum* were not found in cattle livers that were examined. The data in this study give valuable information concerning the prevalence of *F. hepatica* and *D. dendriticum* in slaughtered cattle and sheep in Aydın. To the best of our knowledge, this study is the first study about the examination of liver flukes in an abattoir in Aydın. Eventhough the fluke infection rates of slaughtered animals in Aydın are pretend to be remissible at present however the impact of economic losses arising due to liver destruction on the country should not be neglected.

Keywords: Dicrocoelium dendriticum, Fasciola hepatica, prevalence, ruminants, Aydın,

Aydın Mezhabalarında Kesilen Ruminantlarda Karaciğer Trematodlarının Yaygınlığı

ÖZET

Fasciola hepatica ve *Dicrocoelium dendriticum*, Türkiye dahil olmak üzere tüm dünyada yaygın olarak bulunmaktadır. Karaciğer trematod enfeksiyonları, hayvancılık üretiminde ciddi kayıplara neden olmaktadır. Ayrıca bu hastalıklar hayvanların gelişimini, canlı ağırlık artışlarını olumsuz etkiler ve karaciğer gibi ekonomik açıdan önemli organların kaybına neden olarak ülke ekonomisine zarar verir. Bu çalışma, Aydın ilinde bulunan mezhabada kesilen sığır ve koyunlardaki *Fasciola* spp. ve *Dicrocoelium* spp. parazitlerinin prevalansını ortaya çıkarmak ve meydana gelen ekonomik kaybı tespit etmek için yapılmıştır. Çalışma Aydın'da bulunan Efeler mezbahasında Mayıs 2015- Nisan 2016 tarihleri arasında bir yıl süre ile yapılmıştır. Buna göre kesimi yapılan koyun ve sığırların karaciğer ve safra kanalları karaciğer kelebekleri yönünden incelenmiştir. Çalışma sırasında toplam 3193 adet sığır ve 3659 adet koyun kesilmiştir. 3193 sığırın altısında (%0,18) ve 3659 koyunun bir tanesinde (%0,02*) F. hepatica* erişkin paraziti tespit edilmiştir. Ayrıca 3659 koyunun 13 tanesinde (%0,3) *D. dendriticum* erişkin parazitine rastlanılmıştır. Sığır karaciğerlerinde *Fasciola gigantica* ve *D. dendriticum* parazitlerine rastlanılmamıştır. Çalışmadaki veriler, Aydın'da kesilen sığır ve koyunlarda *F. hepatica* ve *D. dendriticum*'un yaygınlığı hakkında değerli bilgiler vermektedir. Bilindiği kadarıyla, yapılan bu çalışma Aydın mezbahalarında karaciğer parazitleri ile ilgili yapılan ilk incelemedir. Aydın'da kesilen hayvanların karaciğer kelebek enfeksiyon oranları şu an için önemsiz gibi görünse de, karaciğer tahribatından kaynaklanan ekonomik kayıpların ülke üzerindeki etkisi ihmal edilmemelidir.

Anahtar kelimeler: Dicrocoelium dendriticum, Fasciola hepatica, prevalans, ruminant, Aydın

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Introduction

Fasciola hepatica and *Dicrocoelium dendriticum* are trematodes widely found worldwide including in Turkey (Kara et al., 2009). Fasciolosis is a zoonotic trematode disease that causes pathological lesions in the liver and bile ducts in humans as well as animals such as cattle, sheep, goats, camels and causes economic losses (Saltan and Taşçı, 2020). Dicrocoeliasis is an another disease caused by *Dicrocoelium* spp. trematodes in the bile ducts and pancreas of carnivores, pigs, rabbits and humans as well as domestic and wild ruminant animals (Kaufmann, 1996; Toparlak and Tüzer, 2002). Liver trematode infections cause serious losses in livestock production. Also it adversely might affects the development and live weight gain of animals and damage the national economy by causing the loss of economic significant organs such as the liver (Acıöz, 2019). Kaplan et al. (2009) have stated that the total economic value of the liver that was destroyed in Elazığ between 1998-2000 due to fasciolosis was 17,143 United States Dollars (USD). In another study showing the economic loss due to liver destruction, Balkaya and Şimşek (2010) were calculated this amount as 17.560 Turkish Liras (TL). Furthermore, liver flukes also negatively affect human health besides threatening animal health. According to the data of the World Health Organization (WHO), it has been reported that because of foodborne trematode infections two-hundred thousand people became ill and, 7000 of them died around the world (WHO, 2015). There are many studies in Turkey about presence of liver flukes according to stool or slaughterhouse examinations by various researchers. The studies conducted in Turkey report that the frequency of *F. hepatica* infections in cattle, sheep, and goats varies between 1.5-21%, 1.6-2.11%, and 0.8-41.21%, respectively. According to previously studies about *D. dendriticum* prevalence in different regions of Turkey; rate of infected sheep liver 24,6% in Antalya, 3,99% in Thrace, 11% in Afyon, 5% in Şanlıurfa were detected (Gargılı et al., 1999; Balkaya and Şimsek, 2005; Sevimli et al., 2006; Yıldırım et al., 2007; Kara et al., 2009; Adanır and Çetin, 2016; Acıöz, 2019 Saltan and Taşçı, 2020).

In order to make more efficient livestock production, it is necessary to raise knowledge of the producers about the extent of economic losses, to reveal the existing species responsible for diseases and also to develop strategic-effective treatment and control methods against them. This study was performed to investigate the prevalence of *Fasciola* spp. and *Dicrocoelium* spp. at a slaughterhouse in Aydın province and to investigate the resulting economic losses.

Material and Method

This study was carried out from the samples collected in the Efeler slaughterhouse in Aydın province between May 2015 and June 2016. In the first phase of the study, Efeler slaughterhouse in Aydin was visited to determine the prevalence of *Fasciola* spp. and *Dicrocoelium* species. For this aim, liver and bile ducts of slaughtered sheep-goat and cattle have been examined for the presence of *Fasciola* spp. and *Dicrocoelium* spp. in a year. During the study, a total of 3193 cattle and 3659 small ruminants (sheep and goats) livers were examined.

The liver was examined according to the method described by Ogamba-Ongoma (1972) and the parasites were identified by the morphological peculiarities according the literatures (Soulsby, 1982; Reinecke, 1983).

Results

Two species of liver fluke, *Fasciola hepatica* and *Dicrocoelium dendriticum*, were found in this study. As a result *F. hepatica* was detected in six of 3193 cattle (0,18%) and in one of 3659 (0,02%) small ruminants. *Fasciola* infection rate was higher in male (four) cattle than female (two) cattle. In addition, *D. dendriticum* was detected in 13 of 3659 (0,3%) small ruminants. *Fasciola gigantica* and *D. dendriticum* were not found in non

Table 1. The prevalence of liver fluke infections in slaughtered animals

	Sheep					Cattle				
	Fasciola hepatica			*Dicrocoelium dendriticum*		*Fasciola hepatica*			*Dicrocoelium dendriticum*	
Date	Positive	%	*Num of PE	Positive	%	Positive	%	*Num of PE	Positive	%
May 15	-	-	447	3	0.67	2	0.54	369	-	-
June 15	-	-	405	-	-	1	0.3	329	-	-
July 15	-	-	405	3	0.74	-	-	323	-	-
Aug 15	1	0.2	489	7	1.43	-	-	298	-	-
Sep 15	-	-	395	-	-	-	-	243	-	-
Oct 15	-	-	272	-	-	2	1.08	184	-	-
Nov 15	-	-	124	-	-	1	0.49	202	-	-
Dec 15	-	-	124	-	-	-	-	233	-	-
Jan 16	-	-	167	-	-	-	-	262	-	-
Feb 16	-	-	285	-	-	-	-	291	-	-
Marc 16	-	-	268	-	-	-	-	214	-	-
Apr 16	-	-	278	-	-	-		245	-	-

* Number of post-mortem examinatios

of cattle livers that were examined. *Dicrocoelium* infected liver rates were found to be similar in male (seven) and female (six) individuals. One of the sheep was detected as mixed infected for *D. dendriticum* and *F. hepatica*. The number of examined and infected animals, and the prevalence rates were given in **Table1**.

Infected 19 livers (6 cattle, 13 small ruminants) were destroyed in accordance with the medical waste-disposal regulations. In the calculation that made to determine the economic losses, the average cattle liver was calculated as 7 kg and sheep liver as 2 kg. Besides that, the selling price per kilogram of the liver has been determined as 40 TL. Accordingly, this shown that the economic losses caused by a total of 19 livers destroyed during a year was calculated as 2720 TL.

Discussion

The data in this study give valuable information concerning the prevalence of *Fasciola hepatica* and *Dicrocoelium dendriticum* in slaughtered cattle and sheep in Aydın. To the best of our knowledge, this study is the first study about the examination of liver flukes in an abattoir in Aydın. On the prevalence of liver fluke in Turkey, there are many studies carried out based on post-mortem examination and coprological test (Gargılı et al., 1999; Gıcık et al., 2002; Kara et al., 2009; Caya, 2012; Adanır and Çetin, 2016; Çelik and Çelik, 2018; Acıöz, 2019). The prevalence of fasciolosis in different regions varies depending on the snail population, livestock breeding conditions, the environmental and climatic conditions in the region. Different prevalence rates reported in previous studies may be associated with these factors (Yıldırım et al., 2007). The prevalence of *Fasciola hepatica* in sheep (0,02%) and cattle (0,18%) in the present study is lower than the previous studies in some provinces of Turkey (Gargılı et al., 1999; Gıcık et al., 2002; Kara et al., 2009; Caya, 2012; Adanır and Çetin, 2016). This situation can be explained by farm management differences. The fact that *F. hepatica* is more common in traditional farms when compared to dairy farms supports the herd management thesis (Keyyu et al., 2005; 2006). Yıldırım et al. (2007) was attributed the high prevalence in these farms to contaminated pastures and inadequate infrastructure systems. Studies on the evaluation of fasciolosis prevalence in cattle by gender have yielded conflicting results, and no significant difference was reported between genders in these studies (Maqbool et al., 2002; Opara, 2005). Although the number of infected males were found to be higher in our study, the numbers of males and females were found to be considered close to each other.

Although many studies were carried out about dicrocoeliasis in sheep and fascioliasis infection in Turkey, the number of studies on the spread of the parasite in cattle are very limited (Saltan and Taşçı, 2020). The rate of sheep that infected with *Dicrocoelium dendriticum* (0,3%) reported in the current study was pretty low compared to the rates reported previously in Turkey (Gargılı et al., 1999; Kırcalı et al, 2005; Kara et al., 2009). This result can be explained by the lack of a suitable environment for the intermediate host. Also, it has been considered that the reason for the low number of infected animals may be due to the regular fight against parasites in animals in Aydın.

As a result in this study, liver trematodes were detected at a relatively low rate compared to studies conducted in other regions. It is thought that this situation may be due to intensive animal husbandry and limited use of pasture in the region.

Eventhough the fluke infection rates of slaughtered animals in Aydın are pretend to be remissible at present however the impact of economic losses arising due to liver destruction on the country should not be neglected. We are agree with Yıldırım et al. (2007) as they suggest there is a need more practical approaches to reduce the economic losses and to control these diseases.

More examination studies about liver flukes including different types of diagnostic tests (coprological, serological, molecular) need to be performed in the study area to understand more about liver flukes prevalence in the region.

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Conflict of interest

The authors declare that they have no competing interests

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Research Article

Prevalence of *Dirofilaria immitis* infection in dogs from Aydin and Izmir Provinces, Turkey

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A B S T R A C T

Mosquito-borne nematodes, *Dirofilaria immitis* and *D. repens* are among the most important filarial parasites of dogs. *D. immitis* is the causal agent of heartworm that primarily infects domestic and wild canids. Rarely, humans become accidental hosts to this parasite. *D. repens*, which is the main agent of human dirofilariosis, usually causes a non-pathogenic, subcutaneous infection in dogs. The aim of the present study was to investigate the prevalence of *Dirofilaria* spp. in the owned dogs in Aydin and Izmir regions using four different diagnostic techniques. To this end, blood samples were collected from 150 dogs in Aydin (n: 122) and Izmir (n: 28) provinces. The presence of the microfilariae of the parasite in the blood samples were examined by wet blood film method, modified Knott's test, membrane filtration-acid phosphatase staining techniques and polymerase chain reaction (PCR). A commercial antigen test kit (HESKA Solo Step CH Batch Test Strips) was also used to detect adult *D. immitis* antigens in serum samples. The overall prevalence of *D. immitis* was 10,0% as determined by both PCR and the antigen test kit. While the presence of the parasite determined by the modified Knott's and membrane filtration tests was 4,67%, only four animals were determined to be positive by direct microscopic examination (2,67%). All positive samples were detected in Aydin, majority in the Germencik district. The prevalence of *D. immitis* was higher in male dogs (11,93%) when compared to female dogs (4,87%). In conclusion, the present study demonstrates that canine dirofilariosis is prevalent in Aydın province (10,0%) of Turkey, even though the positivity is confined to a small area. Reliable epidemiological data is crucial to implement preventive control measures in order to decrease the incidence of canine dirofilariosis, which should be considered under the "One Health" concept in endemic areas.
Keywords: Dirofilaria immitis, prevalence, dog, diagnosis, epidemiology

Türkiye de Aydın ve izmir bölgelerindeki köpeklerde Dirofilaria immitis enfeksiyonunun prevalansı

ÖZET

Sivrisinek kaynaklı nematodlar, *Dirofilaria immitis* ve *D. repens* köpeklerin en önemli filarial parazitleri arasındadır. *D. immitis*, başlıca evcil ve yabani köpekleri enfekte eden kalp kurdunun nedensel ajanıdır. Nadiren, insanlar da bu parazite tesadüfi konaklık yapmaktadır. İnsan dirofilaryozunun asıl etkeni olan *D. repens*, köpeklerde genellikle patojenik olmayan, deri altı enfeksiyonunlara neden olur. Bu çalışmanın amacı, Aydın ve İzmir illerinde bulunan sahipli köpeklerde *Dirofilaria* spp.'nin prevalansını dört farklı tanı tekniği kullanarak araştırmaktır. Bu amaçla Aydın (n: 122) ve İzmir (n: 28) illerinde 150 köpekten kan örnekleri alınmıştır. Kan örneklerinde parazitin mikrofilaryalarının varlığı direkt kan muayene yöntemi, modifiye Knott testi, membran filtrasyon-asit fosfataz boyama tekniği ve polimeraz zincir reaksiyonu (PCR) ile incelenmiştir. Serum örneklerinde yetişkin D. immitis antijenlerini saptamak için ticari bir antijen test kiti (HESKA Solo Step CH Batch Test Strips) kullanılmıştır. Serum örneklerinde yetişkin D. immitis antijenlerini saptamak için ticari bir antijen test kiti (HESKA Solo Step CH Batch Test Strips) kullanılmıştır. Toplamda, *D. immitis'*in prevalansı hem PCR hem de antijen test kiti ile % 10,0 olarak belirlenmiştir. Modifiye Knott's ve membran filtrasyon testleri ile belirlenen parazit varlığı %4,67 iken, direkt mikroskobik inceleme ile sadece dört hayvan pozitif olduğu belirlenmiştir (% 2,67). Tüm pozitif örnekler Aydın'da, çoğunluğu Germencik ilçesinde tespit edilmiştir. Erkek köpeklerde (% 11,93) dişi köpeklere (% 4,87) göre *D. immitis* prevalansı daha yüksek olarak tespit edilmiştir. Sonuç olarak, bu çalışma, pozitifliğin küçük bir alanla sınırlı olmasına rağmen, Türkiye'nin Aydın ilinde (% 10,0) köpek dirofilaryozunun yaygın olduğunu göstermektedir. Bu tür çalışmalar, endemik bölgelerde "Tek Sağlık" kavramı altında ele alınması gereken köpek dirofilariozis insidansını azaltmak için önleyici kontrol tedbirlerinin uygulanmasında güvenilir epidemiyolojik verilerin ne kadar önemli olduğunu ortaya koymaktadır.
Anahtar Kelimeler: Dirofilaria immitis, prevalans, köpek, tanı, epidemiyoloji

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Introduction

Dirofilaria immitis and *Dirofilaria repens* are among the most important filarial nematodes of dogs, transmitted by mosquito bites. *D. immitis* is the causative agent of the heartworm disease in dogs as well as in other carnivores. Adult *D. immitis* occupies mainly the right ventricle and pulmonary arteries of in carnivores, resulting in severe consequences like heart failure, pulmoner odeamo *etc*. In contrast, *D. repens* is usually non-pathogenic, and causes subcutaneous infection in dogs. Microfilariae of these parasites circulating in blood are ingested by several species of competent mosquito vectors during their blood-feeding (Otranto et al., 2015). While *D. immitis* rarely infects humans, *D. repens* is the main agent of dirofilariosis in humans, considered to be a public health concern (Pampiglione and Rivasi, 2000).

Dirofilaria immitis occurs mainly in tropical, sub-tropical as well as temperate zones (Martin and Collins, 1985; Cringoli et al., 2001). However, *D. repens* is present only in Old World (Pampiglione and Rivasi, 2000). Nevertheless, it was indicated that *D. repens* has been spreading to northern Europe from the endemic areas of southern Europe more rapidly than *D. immitis* probably due to the climate change (Capelli et al., 2018).

It is established that *D. immitis* and *D. repens* are present in different parts of Turkey, where *D. immitis* is more common than *D. repens* (Doğanay, 1983; Tasan, 1984; Ağaoglu et al., 2000; Oge et al., 2003; Yildirim et al., 2007). Early reports were based primarily on the detection of adult worm during postmortem examination (Doğanay, 1983; Tasan, 1984) or microfilariae detection by whole blood examination. There has been a steady increase in the number of epidemiological studies during the last two decades using various molecular and serological diagnostic tests, as well as conventional tests such as whole blood wet smear and modified Knott's tests (Yildirim et al., 2007; Yaman et al., 2009; Güven et al., 2017) . It should be pointed out that each diagnostic test has its own advantages and disadvantages. For example, the diagnostic test based on detection of microfilariae could give false negative results for amicrofilaremic dogs with occult infection of dirofilariosis.

We aimed in the present study to determine the prevalence of *Dirofilaria* spp. in dogs in Aydin and Izmir provinces located in the West Aegean Region of Turkey using conventional, immunological, and molecular diagnostic techniques at the same time. The diagnostic sensitivity of these tests was also compared.

Materials and Methods

Study areas and sample collection

The present study was conducted within provinces located in Aydin and Izmir, both located in the West Aegean region of Turkey. Randomly selected 150 dogs of different breeds, ages and sexes were examined in Aydin and Izmir provinces (Table 1). Whole blood samples (EDTA-treated) and serum samples were collected from owned and stray dogs, as well as from dogs admitted to the veterinary clinics in Aydin between August 2007-2009. The presence of *Dirofilaria immitis* was examined using various conventional, immunological, and molecular diagnostic techniques.

Parasitological investigation

Two conventional tests, namely wet blood film method and modified Knott's concentration technique, were used to detect the presence of microfilarial of the parasite as described by (Hendrix, 1998), subsequently followed by microfilaria identification by (Soulsby, 1982). The circulating microfilariae in the whole blood was also examined by the polycarbonate membrane filtration (Millipore, TMTP 02500) technique (Acevedo et al., 1981; Yildirim et al., 2007), combined with acid phosphates staining methods for the identification of microfilariae using a commercially available kit (Leucognost-SP, Merck, Germany) as described by the manufacturer.

In order to detect adult *D. immitis*, the presence of antigen in the serum samples were examined immunologically using an antigen kit (HESKA Solo Step CH Batch Test Strips, Colorado, USA) as described by the manufacturer. *D. immitis*-specific PCR was also used in the present study to determine the presence of the microfilariae in the blood samples as described below.

DNA extraction and PCR assays

DNA was isolated from EDTA-treated blood (300 ml) using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, USA) as described by the manufacturer. DNA samples were stored at −20°C until used in PCR.

A primer set targeting a 302 bp fragment of the ITS2 (internal transcribed spacer region 2) gene of the ribosomal DNA of *D. immitis*. Following primers described by (Rishniw et al., 2006) were used: D.imm-F1 (5'-CATCAGGTGATGATGTGATGAT-3') and D.imm-R1a (5'-TTGATTGGATTTTAACGTATCATTT-3').

For the detection of *Dirofilaria repens* by PCR, primers targeting a 348 bp fragment of the 5S SSU rRNA of D. repens (D.rep-F1 (5'-TGTTTCGGCCTAGTGTTTCGACCA-3') and D.rep-R1 (5'-ACGAGATGTCGTGCTTTCAACGTG-3') were used as described in (Favia et al., 2000) .

The PCR assays for *D. immitis* and *D. repens* were performed

Table 1. Overall heartworm prevalence in studied areas

Research area	Diagnostic techniques used						
	Sample No	Wet smear	Modified Knott's test	Membran Filtration	PCR	Serological Kit	Total
Aydın/Merkez	56	-	1	-	1	1	1
Aydın/Germencik	19	4	6	6	12[a]	12	12
Aydın/Karpuzlu	29	-	-	-	-	-	-
Aydın/ Clinics	18	-	-	-	2	2	2
Izmir	28	-	-	-	-	-	-
Total	150	4[b]	7[b]	6[b]	15[a]	15[a]	15
				χ^2	74,890		
				P	0,000		

χ^2Pearson's chi-square test.
[a,b] The different letters within the same column indicate significant difference among groups.

Table 2. The prevalence of *D. immitis* correlated with age and sex

	Examined dogs No	Infected dogs No	%	χ^2	*P*
Sex					
Female	41	2[a]	4,87	1,645	0,200
Male	109	13[a]	11,93		
Age (year)					
0,5-3	81	8[a]	9,88	0,043	0,979
4-6	47	5[a]	10,64		
≥ 7	22	2[a]	9,09		

χ^2Pearson's chi-square test.
[a,b] The different letters within the same column indicate significant difference among groups.

in a final volume of 25 µl consisting of 1× buffer (Promega, Madison, WI, USA), 2 mM $MgCl_2$ (Promega, Madison, WI, USA), 200 µm of each deoxynucleotide triphosphate (Promega, Madison, WI, USA), 25 pmol of each primer, 1.25 U of hot start polymerase (hot-start *Taq* polymerase (ThermoFisher Scientific, USA) and 2.0 µl template DNA. The reactions were performed in an automated DNA thermal cycler (Techne™ TC-512 Gradient Thermal Cycler, UK). Reactions consisted of an initial 5 min denaturation step at 95°C, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Final extension was performed at 72°C for 7 min, followed by a hold step at 4°C. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel, pre-stained with ethidium-bromide (10 µl/ml), in TAE buffer at 100 V and bands were visualized under UV light.

Statistical analysis

Pearson's chi-square (χ^2) test was used to compare the prevalence of *D. immitis* among district, sex, age. The sensitivity the diagnostic tests were also compared. Statistical analyses were performed using the statistical package SPSS (Version 21.0). A value of $p < 0.05$ was considered significantly different.

Ethical statement

This study was approved by the Animal Ethics Committee of Adnan Menderes University, Aydin, Turkey (Protocol number: B.30.2.ADÜ.0.06.00.00/124-HEK/2007/022).

Results

Several different diagnostic tests were used to determine the prevalence of the infected dogs in Aydin and İzmir regions (Table 1). As expected, different diagnostic techniques produced different results. The highest prevalence of the parasite was % 10,0 (15 /150 samples) as detected by both PCR and the antigen test kit (Table 1). The presence of the parasite determined by the modified Knott's test and membrane filtration test was 4,67% (7/150) and 4,0% (6/150), respectively. The lowest number of positive animals was detected by wet blood film method (2,67%; 4/150). The Figure 1 shows microfilariae detected either by wet blood, the filtration and/or the modified Knott's test and acid phosphatase staining. The staining of the microfilariae by acid phosphatase indicated that all the microfilariae were *D. immitis*, as evidenced by red staining of both excretory pore and anal pore. These results were confirmed by PCR. All samples, determined to be positive by wet blood film method and the modified Knott's test were also determined to be positive for *D. immitis* by PCR. None of the samples were positive for *D. repens.*

None of the samples obtained from İzmir were positive. All the positive dogs were detected in Aydın region. The number of positive dogs differed significantly among the districts in Aydın, the majority of positive samples (12/15 positive samples) originating from Germencik district (Table 1). There was also a marked difference in positivity between the genders of dogs, The prevalence of *D. immitis* was higher in males (11,93%) compared to females (4,87%) (Table 2). However, there was no differences among the age groups of animals.

Discussion

Vector-borne parasitic nematodes, *D. immitis* and *D. repens*, are prevalent worldwide especially in temperate, tropical, and subtropical climatic regions. The intensity and the distribution of dirofilariasis appear to expand to countries that were once considered to be free of the infection due mainly to climatic and ecological changes (Simon et al., 2012; Baneth et al., 2016). Therefore, these parasites pose a growing public health concern considering their effect on dogs and man.

The epidemiological studies conducted in different parts of Turkey have indicated a wide-range of prevalence rate, *viz.* 0-46,2% (Simsek et al., 2011; Tascı and Kilic, 2012; Guven et al., 2017). Several factors other than ecological and geographical differences of the study region on the base of vector population, such as the detection method used, survey periods, sample size, breed of dog population and the type of of infection (patent or occult) might have contributed to the wide-range discrepancies in prevalence rates. The overall prevalence of the parasite was 10% in the present study, which is similar to previous studies conducted in different parts of Turkey (Yildirim et al., 2007; Guven et al., 2017). However, it should be noted that positive samples were obtained only from Aydin, and mainly in the Germencik district, indicating the presence of factors at a local scale.

The prevalence of infection determined by PCR and antigen detection kit was markedly higher than that revealed by microscopic examination, modified Knott's technique as well as filtration methods. This finding is in accordance with previous observations demonstrating that the reliability and reproducibility of the PCR assay is higher than that of microscopic examination (Watts et al., 1999). It should also be noted that identification of microfilariae by microscopic examination is prone to error and could potentially lead to miss-diagnosis of three species of canine filarial parasites, namely *D. immitis, D. repens,* and *Acanthocheilonema reconditum* (Soulsby, 1982). Beside the relatively low sensitivity in comparison to PCR, the acid-phosphatase staining technique is an easy and reliable

method for the identification of microfilaria (Peribanez et al., 2001).

It is well known that PCR might give false negative results especially in cases of occult infection. Nevertheless, we did not come across any false negative results in the present study as all serologically positive samples were also PCR positive indicating the presence of microfilaria in the peripheral blood.

The results obtained in the present study indicated that the proportion of male dogs infected with *D. immitis* was higher than female dogs. However, the difference was not statistically significant. This finding is in agreement with several studies indicating that the prevalence of the parasites is not different between the males and females (Oge et al., 2003; Hou et al., 2011; Wang et al., 2016). Biological basis underlying the higher prevalence in males, as detected in the present study, is not known. However, it is suggested that male dogs encounter the intermediate host, mosquitoes, more often than females, since they are kept outdoors more often for defense (Song et al., 2003). According to Johnson and Harrel (1986), the outdoors provide more favorable environmental conditions for mosquitos. It was also postulated that preferential attraction to the mosquitoes might also play a role leading to higher infection rates in male dogs (Montoya et al., 1998).

There is evidence indicating that the chance of getting infected with *D. immitis* increases as the dog gets older (Montoya et al., 1998). This is most likely due the fact that the longer the life span of a dog higher the likelihood of exposure to mosquitoes infected with *D. immitis* (Rhee et al., 1998). Nevertheless, no significant differences were determined in the present study among the age groups. This finding is in agreement with the hypothesis that the infection risk remains the same throughout life span of the dog (Rhee et al., 1998).

Conclusion

In conclusion, the present study demonstrates that canine dirofilariosis is prevalent in Aydın province (10.0%) of Turkey, even though the positivity is confined to a small area. In the face of global climate change that leads to warmer temperatures supportive of extrinsic development of *D. immitis,* it is important to determine areas of risk and be aware of the spread of the vector into new environments. The availability of sensitive and specific diagnostic tests is the most important prerequisite for epidemiological studies. In this context, the present study highlights the diagnostic value of molecular and serological tests in determining the prevalence of *D. immitis*. Reliable epidemiological data is crucial to implement preventive control measures in order to minimize the incidence of canine dirofilariosis, which should be considered under the "One Health" concept in endemic areas.

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Conflict of interest

The authors declare that they have no competing interests.

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Research Article

The Effect of Starvation on Acute Phase Proteins and Adiponectin Levels in Rats

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ABSTRACT

In this study, it was aimed to investigate the effect of starvation constituted in rats, on C-reactive protein (CRP), haptoglobin (Hp), albumin, ceruloplasmin and cytokine (adiponectin) synthesized from the adipose tissue.

Twenty adults, 250-300 g of weight, non-pregnant, Wistar type of female rats were used in this study. The rats were randomly divided into two groups, as control and experimental groups. The rats in the experimental group were given only water, while the rats in the control group were fed regularly according to their routine feeding habits for 48 hours. Blood samples were collected intracardiac under the anaesthesia. Serum CRP, Hp, albumin, ceruloplasmin, adiponectin, cholesterol, triglyceride, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), glucose and urea levels were studied. Albumin, total protein, triglyceride, glucose and ALT levels were significantly lower, whereas urea and AST levels were higher in the starving/fasting group compared to the control group. Regarding the acute phase proteins (APP), there was a statistically insignificant decrease in the CRP and Hp levels, and increase in ceruloplasmin levels in the starving/fasting group. Adiponectin and cholesterol levels were higher; GGT level was a lover in the starving group compared to the control group and both of the differences were statistically insignificant. Statistically significant increases in urea and AST levels were observed.

As a result, the levels of positive APP's didn't change significantly with acute starving whereas a negative APP, albumin, decreased significantly. The tendency in the increase of adiponectin levels is another important data that we obtained in this study. Starving of human beings may occur as a result of various psychological, social and organic reasons, and we speculate that the physiological and biochemical effects of starving stated in our study could be a reference for further studies related to this subject.

Key Words: Rat, starvation, acute phase proteins, adiponectin

Ratlarda Açlığın Akut Faz Proteinleri ve Adiponektin Düzeyleri Üzerine Etkileri

ÖZET

Bu çalışmada ratlarda açlığın C-reaktif protein (CRP), haptoglobin (Hp), albümin, seruloplazmin ve yağ dokusundan sentezlenen sitokin (adiponektin) üzerindeki etkisinin araştırılması amaçlanmıştır.

Bu çalışmada 250-300 g ağırlığında , gebe olmayan, 20 yetişkin Wistar tipi dişi rat kullanıldı. Ratlar, rastgele, kontrol ve deney grubu olarak iki gruba ayrıldı. Deney grubundaki ratlara sadece su verilirken, kontrol grubundaki ratlar 48 saat rutin beslenme programlarına göre beslendi. Kan örnekleri anestezi altında intrakardiyak olarak toplandı. Serum CRP, Hp, albümin, seruloplazmin, adiponektin, kolesterol, trigliserit, total protein, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), gama glutamiltransferaz (GGT), glukoz ve üre analizleri yapıldı. Kontrol grubuna göre açlık grubunda albümin, total protein, trigliserit, glukoz ve ALT düzeyleri anlamlı olarak düşük, üre ve AST düzeyleri yüksek olarak belirlendi. Açlık grubunda CRP ve Hp seviyelerinde istatistiksel olarak önemsiz bir azalma, seruloplazmin seviyelerinde artış görüldü. Açlık grubunda kontrol grubuna göre adiponektin ve kolesterol seviyeleri yüksek; GGT düzeyi ise düşük bulundu. Her iki fark istatistiksel olarak anlamsızdı. Üre ve AST düzeylerinde istatistiksel olarak önemli artışlar belirlendi.

Sonuç olarak, pozitif akut faz proteinleri (AFP) seviyeleri akut açlıkla değişmezken, negatif AFP olan albümin önemli ölçüde azaldı. Çalışmadan elde edilen diğer önemli bir veri adiponektin düzetindeki artma eğilimiydi. Bu çalışmanın sonuçlarının, insanlarda çeşitli psikolojik, sosyal ve organik nedenlerle ortaya çıkabilen açlığın fizyolojik ve biyokimyasal etkilerinin inceleneceği çalışmalarda referans olabileceği kanısına varıldı.

Anahtar Kelimeler: Rat, açlık, akut faz proteinleri, adiponektin

Introduction

Starvation is a metabolic process that is a result of inappropriate balance or absence of all or a portion of the basic constituents required for the survival of an organism (Lee et al., 2015a). Insufficient nutrition and starvation lead to the deficiency of essential nutrients which results in the catastrophic destruction of the body and even death (Pointer et al., 2013). Short and long term starvation causes some changes in the carbohydrate, fat and protein metabolisms (Lee et al., 2015a). The findings related to these metabolic changes caused by starvation are not clearly stated.

Acute phase reaction (APR) is a local or systemic nonspecific immune reaction, that may develop as a result of trauma, infection, neoplastic growth, tissue damage, inflammation or immunologic defects of a metabolism (Eckersall and Bell, 2010). The aim of the development of APR is the removal of infectious agents, harmful molecules and wastes that cause damage to the organism; so that the tissue or organ can be protected from further damage and the repair process can be initiated so that physiological homeostasis can be provided. APP's are proteins synthesized by the liver that are generally a reaction to inflammation and serum concentrations of APP's change during APR (Gruys et al., 2006). The levels of APP's are correlated with the magnitude and severity of the tissue damage. Therefore the determination of APP levels provides diagnostic and prognostic information (Eckersall and Bell, 2010). Adiponectin is a glycoprotein adipocytokine synthesized from the fat tissue (Frankenberg et al., 2017). Studies related to the physiologic importance and efficiency of adiponectin have increased in recent years. The most important effect of adiponectin besides its antiatherogenic and anti-inflammatory effects is its regulatory effect on insulin hormone activity (Sun et al., 2009).

There are not many studies related to the effect of starving on APP's and adiponectin in the literature. We consider that the results of this study regarding the effect of starvation on adiponectin, albumin, APP's like Hp, CRP, ceruloplasmin will be beneficial for further studies.

Material and Methods

Animals: The experimental protocols were approved by the ethics committee of Adnan Menderes University Animal Experiments Local Ethics Committee (64583101/2016/155).

Adult, healthy, female Wistar rats weighing approximately 250-300 g were used. During the study, the rats were kept in 425 x 265 x 180 mm sized transparent polycarbonate cages, covered with stainless steel in the controlled rooms of Adnan Menderes University, Veterinary Faculty, Experimental Animals Unit; which were regulated to have an optimal temperature (22°C), 40-60% relative humidity, 12 hours light and 12 hours dark media. The animals were put into the experiment rooms 1 week before the experiment for their adaptation to the environment. Food and water were given *ad libitum*. The groups were randomly selected as two groups; each consisting of 10 animals. Two rats were put into each cage, making a total of 10 cages.

Before the onset of the study, each rat was weighed and the result was recorded. The rats were put into two groups as experimental and control groups, each consisting of 10 rats. As the study started, the study group was given only water for two days, while the control group was fed as their routine feeding habit. At the end of the second day, each rat was weighed and the results were recorded. After the weighing procedure, intracardiac blood samples were taken, after being anaesthetized by ether. Blood samples were centrifuged and divided into three parts; and kept at -20°C temperature until the analysis was performed.

Biochemical Analysis: Serum albumin, total protein, cholesterol, triglyceride, urea, glucose, ALT, AST and GGT levels were measured with commercial test kits (Archem Diagnostic, Turkey) used in the biochemistry autoanalyzer (Rayto Chemray 120). CRP levels were measured with a quantitative competitive immunoassay technique kit, on the ELISA device (AssayPro, Assay Max Rat CRP, USA). Serum haptoglobin levels were measured with commercial test kits (ELISA commercial kit Tridelta LTD, Ireland) on the ELISA device. Serum ceruloplasmin levels were determined with the spectrophotometric technique that Sunderman and Numato (1970) presented (Schimatzu UV 1601, Japan). Rat adiponectin ELISA Kit (Boster Biological Technology, USA) based on standard enzyme-linked immunosorbent experiment technology was used for the measurement of serum adiponectin levels.

Statistical Analysis: The statistical analysis of the data obtained at the end of the study was performed using SPSS (for Windows Release Standart Version 15) program. Independent sample test (Mann-Whitney U) was used for the identification of the difference between the groups. The results were given as mean ± standard deviation. The interpretation of statistical results was determined as NS: Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Results

Albumin levels were 4,04±0,14 g/dL in the control group, and

Table1. Control and starvation groups mean serum albumin (ALB), Total Protein (TP), cholesterol, triglyceride, urea and glucose results
*****:p<0,001 **:p<0,01 *:p<0,05**

	ALB (g/dl) X±SD	TP (g/dl) X±SD	Cholesterol (mg/dl) X±SD	Trygliceride (mg/dl) X±SD	Urea (mg/dl) X±SD	Glucose (mg/dl) X±SD
Control (n=10)	4,04±0,14	6,57±0,25	75,33±17,25	64,15±11,41	38,70±5,28	130,05±24,81
Starvation (n=10)	3,97±0,63	6,12±0,38	90,00±26,64	50,90±11,03	47,48±6,94	106,20±26,12
t	0,346	3,107	-0,254	2,639	-3,185	2,093
p	0,043 *	0,007 **	0,529	0,023 *	0,011 *	0,019 *

Table 2. Control and starvation groups mean serum AST, ALT and GGT results
*****:p<0,001 **:p<0,01 *:p<0,05**

	ALT (U/L) X±SD	AST (U/L) X±SD	GGT (U/L) X±SD
Control (n=10)	55,05±10,93	135,64±26,73	2,63±0,67
Starvation (n=10)	38,25±6,30	214,88±103,20	2,44±0,83
t	4,211	-3,017	0,573
p	0,001 **	0,004 **	0,247

3,97±0,63 g/dL in the study group. Albumin levels were lower in the study group compared to the control group, statistically p<0,05. Total protein levels were 6,57±0,25 g/dL in the control group, whereas 6,12±0,38 g/dL in the study group. Regarding the serum total protein levels, the statistical difference between the two groups is p<0,01. Cholesterol level was 75,33±17,25 mg/dL in the control group, and 90,00±26,64 mg/dL in the study group. The increase in serum cholesterol levels in the starving group was statistically insignificant. Triglyceride levels were 64,15±11,41 mg/dL in the control group and 50,90±11,03 mg/dL in the study group. The decrease in the study group was statistically significant, p<0,05. Urea levels were 38,70±5,28 mg/dL in the control group, and 47,48±6,94 mg/dL in the study group; the increase was statistically significant p<0,05. Blood glucose levels were 130,05±24,81 mg/dL in the control group, and 106,20±26,12 mg/dL in the study group; the decrease in the study group was statistically significant p<0,05 (Table 1).

ALT levels were 55,05±10,93 U/L in the control group and 38,25±6,30 U/L in the study group. The relative decrease of serum ALT levels in the study group was statistically significant, p<0,01. AST levels were 135,64±26,73 U/L in the control group and 214,88±103,20 U/L in the study group. The relative increase of serum AST levels in the study group was statistically significant, p<0,01. The difference between the GGT levels between the control and study groups was statistically insignificant, 2,63±0,67 U/L and 2,44±0,83 U/L respectively (Table 2).

CRP levels were measured as 27,46±13,77 µg/mL in the control group and 17,11±12,65 µg/mL in the study group. The decrease in the study group was statistically insignificant. The difference of serum Hp levels were also statistically insignificant, 0,96±0,84 g/L in the control group and 0,69±0,33 g/L in the study group. Ceruloplasmin levels were 50,08±5,36 mg/dL in the control group and 55,17±12,40 mg/dL in the study group; the difference was also statistically insignificant. The difference between adiponectin levels of the control and study groups was statistically insignificant; 5,85±1,54 ng/mL and 6,41±1,52 ng/mL respectively (Table 3).

The mean weight of the rats in the control group was 323,7±18,80 g in the beginning of the study and increased to 326,8±22,84 g at the end; whereas the weight of rats decreased from 325,4±39,00 g to 292,1±36,34 g. The 48 hours starvation led to a 10% decrease in the body weights of the rats (Table 4).

Discussion

Starvation is a biological process in which the access of an organism to nutritional sources is restricted as a result of various causes. It is known that the restriction or cessation of food intake initiates a process that can result in death in all of the living organisms. The restriction of food intake causes in some changes of systemic activities of the organism (Karataş, 2014). We aimed to study the effect of starvation on blood levels of biochemical parameters, APP's and adiponectin levels in rats.

In our study, the total protein and albumin levels in starved rats were lower than the control group; and the difference was p<0,01 for total protein and p<0,05 for albumin, both of which were statistically significant. Similarly, Lee et al. (2015b) reported a decrease in total protein levels in the rats that were starving. The decrease in total protein was interpreted to be a reflection of inadequate food intake or a sign of the deterioration of the liver function in starvation. In our study, the blood AST levels were significantly higher in the study group compared to the control group; which was concluded to be the result of the effect of starvation on the liver function impairment. Albumin is the most important negative APP (Cray et al., 2009). The most important factors affecting albumin levels are nutrition and inflammation. Protein loss of the body causes a decrease in albumin levels. A decrease in protein intake results in reduced albumin synthesis and finally albumin concentration decreases (Burl et al., 2004). Factors like liver function impairment, starvation affect serum albumin levels (Hirvonen and Pyörälä, 1998; Petersen et al., 2004). Lee et al. (2015b) stated that long-term starvation leads to a severe decrease in serum albumin levels. Albumin synthesis of the organism is partly related to its nutrition, and especially to the protein intake. But studies have shown that inadequate nutrition itself -excluding severe starvation statuses- does not cause a significant decrease in albumin levels (Kaysen and Don, 2003).

As soon as the body faces malnutrition or starvation, it begins to provide the supplementation of some nutrients, like lipids, from organs and tissues like the muscles and the liver. This causes a decrease in blood and tissue triglyceride levels. In our study, serum triglyceride levels were significantly lower in the starvation group, which is compatible with the literature. Similarly, Lee et al. (2005) reported a decrease in serum triglyceride levels in long-term starved rat models. Zauner et al. (2017) stated a significant decrease in serum triglyceride levels in the second day of their study, in which 1, 2, 3, 4 days of starvation was performed on rats. The results of our study are compatible with the results of other experimental starvation animal model studies and we concluded the decrease in the triglyceride levels was a result of the usage of lipids by the metabolism to fulfil the energy need. In our study, blood cholesterol levels were not statistically different between the study and control groups. We concluded that 48 hours of starvation didn't have any effect on total cholesterol levels. Lee et al. (2015b) performed a prolonged (two weeks) starvation study on rats and stated that cholesterol and HDL levels don't change, but LDL cholesterol levels significantly increase with starvation. In another study, Zauner et al. (2017) reported that short term starvation didn't

Table 3. Control and starvation groups mean serum CRP, Hp, ceruloplasmin and adiponectin results
***:p<0,001 **:p<0,01 *:p<0,05

	CRP (µg/ml)	Haptoglobin (g/L)	Ceruloplasmin (mg/dl)	Adiponectin (ng/ml)
Control (n=10)	27,46±13,77	0,96±0,84	50,08±5,36	5,85±1,54
Starvation (n=10)	17,11±12,65	0,69±0,33	55,17±12,40	6,41±1,52
t	1,751	0,956	-0,454	0,814
p	0,089	0,796	0,143	0,353

cause a significant change in total cholesterol levels.

The decrease in blood glucose levels is directly related to the reduction of nutritional intake. In this study, blood glucose levels were significantly lower in the starved rats compared to the control group. The decrease in blood glucose levels in both short- and long-term starvation is reported in various studies (Karataş, 2014; Ryu et al., 2005; Zauner et al., 2017). The drop in the glucose with hunger is related to the decreased intake of glucose which is the major energy source for the metabolism and the consumption of hepatic glycogen.

The body develops various adaptation mechanisms during starvation, like decreasing the weight to decrease the energy need. This adaptation is correlated with the obesity status of the organism before starvation. The energy is obtained from the stored lipid in the individuals who have high-fat tissue; whereas it is obtained from the catabolism of proteins in individuals who have not. The usage of proteins as an energy source may lead to an increase in urea levels. Lee et al. (2015b) established two weeks starved rat model study, and reported a significant increase in the blood urea nitrogen levels and concluded this finding to be a result of the renal dysfunction and failure. In our study, the blood urea levels of starvation constituted rats were significantly higher compared with the control group, which we considered to be the related with the catabolism of proteins in starvation states of the organism. Similarly, Zauner et al. (2017) reported an increase in the blood urea nitrogen levels in all periods of short-term starvation, where the rats were kept at 1, 2, 3 and 4 days of starved periods. In our study, there was a decrease in the serum ALT levels. It is considered to be the result of decreased functional parenchymal tissue loss during starvation. The GGT levels didn't vary among the two groups.

Non-statistically significant decrease was observed in the levels of CRP, haptoglobin and ceruloplasmin in the fasting group compared to the control group in this study. It was thought that this decrease might be related to the decrease in protein synthesis in the liver and the bodyweight lost as a result of 48 hours starvation. Positive APPs are important parameters in monitoring and evaluating the disease in inflammatory processes. However, positive APPs may not give sufficient and correct answers in nutritional disorders and chronic infections. In such cases, looking at negative APPs helps to make a more accurate diagnosis (Gruys et al., 2005).

Recent studies have shown that adipose tissue is not only an energy store, but also an organ that has effects on energy metabolism and insulin sensitivity, and secretes hormones and various metabolites. The most important of these are adiponectin and leptin. Plasma adiponectin levels decrease in obesity and type 2 diabetes, unlike other adipocytokines. This condition is associated with insulin resistance and sensitivity. In a screening study conducted in India, it was observed that the rate of type 2 diabetes was low in individuals with high blood adiponectin levels and high in those with low blood adiponectin levels (Lindsay et al., 2002). Koca et al. (2005) reported that adiponectin levels decreased in their study on obese individuals. Arita et al. (1999) reported that there is a negative correlation between the decrease in adiponectin level and body mass index in obese patients. They stated that it could be associated with increased insulin resistance and TNF-α level with arteriosclerotic lesion and / or increase in adipose tissue. Pannaacciulli et al. (2003) reported in a study on 11 women with anorexia nervosa that blood adiponectin levels were higher than healthy samples in the control group. Besides, in studies conducted both in obese rats (Milan et al., 2002) and in humans (Yang et al., 2001), the decrease in body weight caused an increase in adiponectin level despite the decrease in adipose tissue, which is the only organ that synthesizes itself. Similarly, in our study, adiponectin levels in rats with starvation were found to be higher than the control group, but no statistical significance could be determined between the groups. It was concluded that the reason for this was related to the small number of samples in the experimental and control groups and the duration of fasting. Adiponectin is an adipocytokine that has an anti-inflammatory effect and suppresses TNF-α and IL-1 levels (Yalnız et al., 2012). APPs are proteins released by the stimulation of these cytokines in inflammatory reactions. Therefore, decreased adipose tissue and increased adipokine level due to starvation decreased the synthesis of proinflammatory cytokines. APP levels do not increase as a result of decreased protein synthesis from the liver with decreased protein intake; it is obviously clear that APP's levels have a decreasing trend. Expected decreases in total protein, albumin, triglyceride and glucose levels and increases in urea and AST levels were observed in this study. Impairment of liver function due to starvation resulted in a decrease in the levels of proteins synthesized from the liver. It is thought that the results obtained from this study will provide information about APPs occurring in starvation, some biochemical values and adiponectin concentrations and maybe a reference for future studies.

Conflict of interest

The authors declare that they have no competing interests.

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Research Article

AdipoR1 and AdipoR2 Receptor Gene Expression in Rats with Metabolic Syndrome Induced by Fructose Diet

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ABSTRACT

In this study, it was aimed to investigate serum adiponectin levels and AdipoR1 and AdipoR2 gene expressions in rats with metabolic syndrome with fructose diet. Twenty-four male Sprague Dawley rats were used for this purpose. The rats in the control group were fed only with water and rat food while the rats in the experimental group were fed with water containing 20% D-fructose and rat food for 16 weeks with *ad libitum*. In the control and metabolic syndrome groups, weight differences between the beginning and end of the experiment and the abdominal circumference measured at the end of the experiment were found statistically significant at the level of $p < 0.001$. Adiponectin levels were measured by ELISA in serum samples at the end of the experiment. Fructose administration caused a statistically significant decrease in serum adiponectin levels compared to the control group ($p < 0.001$). At the end of the period of fructose application time in liver tissue, quantitative change of AdipoR1 gene was observed as 1.97 fold decrease compared to control according to RT-PCR results, while a decrease of 3.11 fold was observed in the quantitative change of AdipoR2 gene.

As a result, high fructose consumption decreases serum adiponectin levels and significantly deteriorates adiponectin receptor expression in the liver.

Key Words: Adiponectin, AdipoR1, AdipoR2, Fructose, Metabolic syndrome

Fruktoz Diyetiyle Metabolik Sendrom Oluşturulan Ratlarda AdipoR1 ve AdipoR2 Reseptör gen Ekspresyonu

ÖZET

Bu çalışmada fruktoz diyetiyle metabolik sendrom oluşturulan ratlarda serum adiponektin düzeyleri ve AdipoR1 ve AdipoR2 gen ekspresyonlarının araştırılması amaçlandı. Bu amaçla 24 adet 8 haftalık erkek Sprague Dawley rat kullanıldı. Kontrol grubundaki ratlar sadece çeşme suyu ve rat yemi ile beslenirken deney grubundaki ratlar % 20'lik D-fruktoz içeren çeşme suyu ve rat yemi ile 16 hafta *ad libitum* beslendi. Kontrol ve metabolik sendrom grubundaki ratların deneme başlangıcı ve sonu arasındaki kilo farkları ve deneme sonunda *ölçülen* karın *çevreleri* gruplar arasında $p<0,001$ düzeyinde istatistiksel olarak anlamlı bulundu. Deneme sonunda serum örneklerinde adiponektin düzeyleri ELISA yöntemi ile ölçüldü. Fruktoz uygulaması kontrol grubu ile karşılaştırıldığında serum adiponektin düzeyinde istatistiksel olarak anlamlı azalışa neden oldu ($p<0,001$). Karaciğer dokusunda fruktoz uygulama süresi sonunda RT-PCR sonuçlarına göre AdipoR1 geninin kantitatif değişiminde kontrole göre 1,97 kat bir azalış gözlenirken, AdipoR2 geninin kantitatif değişiminde ise 3,11 katlık bir azalma belirlendi. Sonuç olarak yüksek fruktoz tüketimi, serum adiponektin düzeyini azaltırken karaciğerde adiponektin reseptörü ekspresyonunu belirgin şekilde bozduğu görüldü.

Anahtar kelimeler: Adiponektin, AdipoR1, AdipoR2, Fruktoz, Metabolik sendrom

Introduction

Fructose is a monosaccharide found naturally in fruits, grains, and root vegetables and is one of the essential energy sources for the body. Fructose, also known as fruit sugar, is the sweetest of all-natural sugars. High-fructose corn syrup is produced by converting glucose molecules into fructose using enzymes. It is used in food and beverage manufacturing. (Forshee et al., 2007).

An increase in the consumption of high-fructose corn syrup has occurred nowadays. Since fructose increases the taste and delays the feeling of satiety, it reveals health risks with excessive food consumption (Angelopoulos et al., 2009). The digestion, absorption, and metabolism of fructose are different from glucose. Fructose is absorbed from the intestines with the glucose transporter GLUT5 and then diffused into blood vessels via GLUT2. Unlike glucose, the absorption of fructose from the intestines does not require ATP hydrolysis and is independent of sodium absorption. This results in excessive fructose uptake by the liver (Rizkalla, 2010). It has been demonstrated that a diet high in fructose leads to glucose intolerance and insulin resistance, type 2 diabetes, obesity, hypertension, and cardiovascular diseases (Ross et al., 2009).

Adiponectin is among the many proteins secreted by adipocytes that are involved in the regulation of glucose and lipid metabolism and can change insulin sensitivity and energy balance. It increases the sensitivity of tissues to insulin, has protective impacts against the development of type 2 diabetes mellitus and has several anti-inflammatory and antiatherogenic features (Berg et al., 2002; Brochu-Gaudreau et al., 2010).

Adiponectin exerts its metabolic impacts, at least partially, by stimulating protein kinase activated with adenosine 5-monophosphate, which increases both glucose uptake and fatty acid oxidation rates (Tomas et al., 2002) and nuclear receptor peroxisome proliferator-active receptor (PPAR) and promotes lipid oxidation. All these impacts are thought to be mediated by two receptors, called adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) expressed in most tissues, such as the liver, adipose tissue, skeletal muscles (Chinetti et al., 2004), pancreatic β-cells (Kharroubi et al., 2004), and macrophages (Tomas et al., 2002).

AdipoR1 is commonly expressed in skeletal muscle and AdipoR2 in the liver. AdipoR1 receptors provide AMP-activated protein kinase (AMPK) activation in skeletal muscle, increase glucose uptake and fatty acid oxidation, and AdipoR2 causes AMPK activation in the liver (Ma et al., 2015). AdipoR1 and AdipoR2 are integral membrane proteins, in contrast to G-protein-coupled receptors, the N-terminal part is intracellular, and the C-terminal part is extracellular. Globular and full-length adiponectin binds to AdipoR1/R2, and AMP-kinase affects PPARα ligand activity, fatty acid oxidation. The N-terminal cytoplasmic part of AdipoR1 interacts with adaptor protein part with pleckstrin, phosphotyrosine-binding part and leucine zipper motif (APPL). The interaction of APPL with AdipoR1 is stimulated by the binding of adiponectin to the C-terminal part of AdipoR1. The mentioned interaction takes an essential part in adiponectin signalling and adiponectin-mediated lipid oxidation and glucose uptake (Kadowaki et al., 2006).

Obesity decreases AdipoR1/R2 expression as well as adiponectin levels. This decreases adiponectin sensitivity in obese patients and leads to insulin resistance. The expression of AdipoR1 and AdipoR2 in muscle and adipose tissue and AMP-kinase activation in skeletal muscle decreased in ob/ob insulin-resistant mice. A vicious circle develops by increasing hyperinsulinemia. It has been revealed that adiponectin receptor expression also decreases in type 2 diabetic patients. In a study, AdipoR1 mRNA expression was determined to be an independent determinant of first-phase insulin secretion independent of insulin sensitivity and body fat. Since AdipoR1/R2 is also expressed in pancreatic β cells, it is thought that it may also be involved in insulin secretion (Mao et al., 2006; Tsuchida et al., 2004).

The disruption of the expression of the said receptors affects the whole-body metabolism and the metabolic impacts of adiponectin (Yamauchi et al., 2003). Thus, the expression level of AdipoR1 and R2 in target tissues may take part in controlling metabolism and insulin sensitivity. Little is known about regulating AdipoR1/R2 expression. Studies have shown that the expression of adiponectin receptors decreases in ob/ob and db/db mice (Tsuchida et al., 2004; Inukai et al., 2005) and skeletal muscles of individuals with a family history of type 2 diabetes mellitus (Civitarese et al., 2004) but does not change in obese Zucker rats (Beylot et al., 2006). Thus, the exact role of AdipoRs in insulin-resistant situations continues to be discussed, and it is thought that it may differ depending on the model studied and the cause of insulin resistance. On the other contrary, Debard et al. (2004) revealed no decrease in AdipoR1 and AdipoR2 messenger mRNA concentrations in the skeletal muscle of type 2 diabetic patients in comparison with healthy subjects. Furthermore, taken together, the above-mentioned findings support the idea that the increased expression of AdipoR1/R2 increases insulin sensitivity and reduces the risk of developing type 2 diabetes mellitus.

Adiponectin and adiponectin receptors are multiple potential therapeutic targets for combating obesity-related diseases that are characterized by insulin resistance (Kadowaki et al., 2006). The present research aimed to examine serum adiponectin levels and liver adiponectin receptor (AdipoR1 and AdipoR2) gene expressions in rats with metabolic syndrome under different nutritional conditions (high-fructose diet).

Table 1 Rat qPCR primer sequences.

Gene	Primers
Gapdh	**F**orward ATGGTGAAGGTCGGTGTGAAC
	Reverse GGTCAATGAAGGGGTCGTT
AdipoR1	**F**orward GTACCAGCCAGATGTCTTCCC
	Reverse CGCTTACCCTTCTCCTCCAG
AdipoR2	**F**orward GTAACAATGACAACCACCACGG
	Reverse TCCCACACCTTACAAACAAACTC

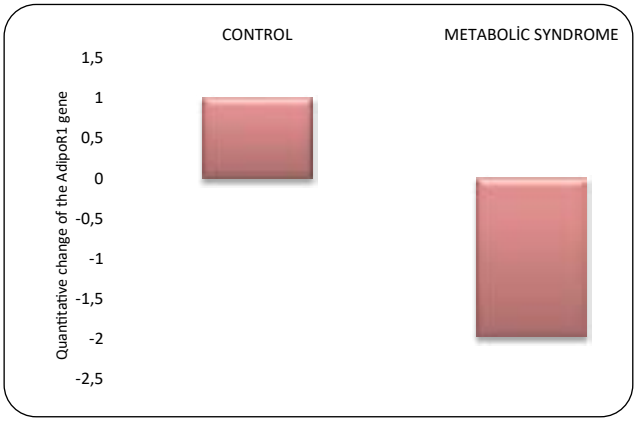

Figure 1 Change in AdipoR1 gene expression in liver tissue.

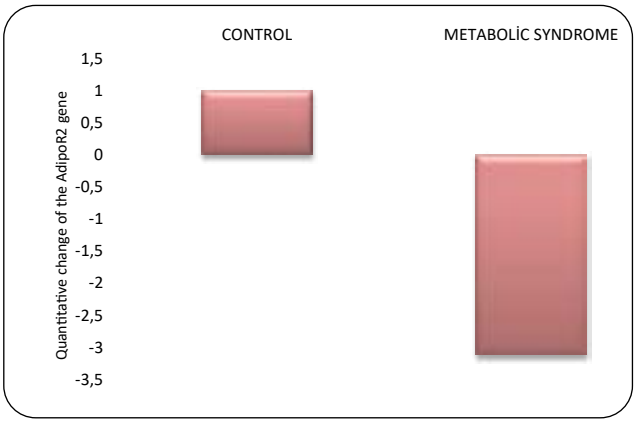

Figure 2 Change in AdipoR2 gene expression in liver tissue.

Material and Method

Animals

For this study, 24 male Sprague Dawley rats, 8 weeks old (~245 g b.w.) were used. They were kept in the animal house of Aydın Adnan Menderes University, Faculty of Veterinary Medicine. They were kept at 22 ± 1ºC temperature at 12 h dark-light cycles. Food and water were given as *ad libitum*. The experiments were conducted by Ethical Guidelines for investigations of laboratory animals and were approved by the Ethical Committee of Aydın Adnan Menderes University (2015/081).

The animals were adapted to the environment by placing them in standard cages in the experimental animal unit one week before the study. Animals were randomly divided into 2 groups (each, n = 12): (1) the control group received regular rodent diet (2) Metabolic syndrome group was fed on a regular rodent diet and 20 % of drinking water was composed of fructose solution. The fructose solution was prepared fresh each day. The body weight of each animal was measured every week from the start of the experiment. The body weight difference between control and metabolic syndrome group animals was calculated statistically with the data obtained at the end of the experiment. The abdominal circumference of the animals was measured and recorded on the experiment start and end day to determine the weight change of each animal during the experiment period. The average abdominal circumference measurement difference between the control group and the metabolic syndrome group with the data obtained at the end of the experiment was statistically calculated.

After 16-weeks feeding period, rats were euthanized under anaesthesia by taking blood from the heart. The samples were centrifuged and stored at -20˚C until time of assay of serum levels of Adiponectin. The animals were sacrificed by a blow to the head and tissue samples from the liver were dissected, frozen in liquid nitrogen and kept at –80°C until they were used to assess the AdipoR1 and AdipoR2 gene expression.

Biochemical Analysis

Serum level of Adiponectin was determined by using rat adiponectine ELISA kit (Boster Biological Technology, USA) according to the manufacturer's instruction.

Total RNA was isolated from the livers using a Total RNA extraction Kit (Geneall, Cat no: 305-101), according to the manufacturer's protocol. The samples were stored at -80ºC until they were used. RNA concentrations were determined by optical density measurement at 260 and 280 nm. Purity was assessed by the 260/280 nm ratio. Complementary deoxyribonucleic acid was synthesized from total RNA with the cDNA Synthesis kit (Transcriptor High Fidelity cDNA Synthese kit, Roche, Version 8, 05091284001).

Real-time PCR analysis was performed using SYBR green (Roche, Fast Start Essential DNA Green Master), by the manufacturer's procedure using a Light Cycler Nano Real-Time PCR. All primers were design program Primer3 and synthesized by Atlas Biotechnology (Ankara/Turkey).

Primers are listed in Table1. Gapdh mRNA levels were measured as internal standard and the data were expressed as relative units as a ratio of Gapdh mRNA concentrations. RT-PCR condition was an initial incubation at 40ºC for 10 minutes that was followed by a 10- minutes incubation at 95ºC then 40 cycles at 95ºC (10 s), 60ºC (10 s) and 72ºC (15 s), and 20-second melting at 58ºC. The Pfaffl method was used to determine the relative levels of AdipoR1 and AdipoR2 gene expression. The fold of AdipoR1 and AdipoR2 was normalized about the reference genes expressed (Livak and Schmittgen, 2001).

Statistical Analysis

Data were processed using the statistical package SPSS version 22 (Statistical Package for the Social Sciences). The compliance of the data to normal distribution was evaluated using the Shapiro Wilk test. Difference between groups showing normal distribution was made by Student's T test. Values of $p < 0.05$ were considered significant from the results obtained from the statistical analysis. Data were given as mean ± standard deviation. Significance levels were shown as *** $p < 0.001$.

Results

At the beginning and end of the experiment, the weights of threats in the control and metabolic syndrome groups were called and weight differences between the weightings were taken. Accordingly, the difference between the weights of threats in the control group was 36.84 ± 2.64 g and in the metabolic syndrome group was 40.93 ± 1.27 g. The weight difference values between the groups were found to be statistically significant ($p<0,001$). At the end of the experiment, abdominal circumference measurements of rats in the control and metabolic syndrome groups were taken and the abdominal circumference of threats in the control group was 18.59 ± 1.44 cm and the abdominal circumference of threats in the metabolic syndrome group was measured as 21.28 ± 1.3 cm. Statistical significance was found at the $p < 0.001$ between the groups. Serum adiponectin levels were measured as 11.72 ± 0.56 ng/ml in the control group and 8.34 ± 0.33 ng/ml in the experimental group. Fructose administration caused a statistically significant decrease in serum adiponectin level compared to the control group ($p<0,001$).

At the end of the fructose application period in the liver tissue, according to the RT-PCR results, in the quantitative change of the AdipoR1 gene compared to the control was observed a 1.97-fold decrease, while a 3.11-fold decrease was observed in the quantitative change of the AdipoR2 gene (Figure 1 and 2).

Discussion

It was reported that insulin resistance and hypertension were observed in laboratory animals fed with a high-fructose diet, glucose metabolism and uptake pathways were turned upside down, triglyceride synthesis and lipogenesis increased significantly, and the mentioned results were largely parallel to the metabolic syndrome criteria in humans. Thus, diets containing high fructose are critical for inducing changes in metabolic syndrome in experimental animals, identifying the causative mechanisms, and developing new strategies for disease prevention/treatment (de Moura et al., 2009; Cardinali et al., 2013).

It is known that the pathophysiological changes observed in fructose-mediated metabolic syndrome models show differences that may arise from the study plan, such as the breed, age of the animal used, the amount of fructose, the route and duration of administration (Dai and McNeill, 1995; Roglans et al., 2007). In our study, the use of Sprague Dawley rats, which were reported to be more sensitive to the administration of fructose via drinking water, was preferred, and 20% fructose was added to their drinking water for 16 weeks.

Fructose and glucose added to the diet cause weight gain and a significant increase in fat mass. However, while there is an increase in visceral adipose tissue in those fed with a fructose-added diet, an increase in subcutaneous fat accumulation is observed in those fed with a glucose-added diet. Important data are suggesting that an increase in visceral adipose tissue is associated with cardiovascular diseases and metabolic diseases such as type 2 diabetes compared to subcutaneous fat accumulation (de Moura et al., 2009).

In the study conducted by Bocarsly et al. (2010), mice were given high-fructose corn syrup and sucrose for certain periods, and their effect on the body weight, fat and triglyceride of the mice was investigated. As a result of the study, it was stated that mice fed with high-fructose corn syrup showed abnormal weight gain, high triglyceride level, and fat accumulation. Therefore, it was emphasized that the excessive consumption of high-fructose corn syrup is an essential factor in obesity.

In a study in which the metabolic effects of diets containing free or bound fructose were compared, Sheludiakova et al. (2012) reported that although the total energy intake of animals was 25% higher, there was no change in body weight, whereas, in animals fed with fructose predominantly, there was abdominal fat, increased plasma triglyceride level and decreased glucose tolerance. Novelli et al. (2007) observed a significant increase in body weight after 30 days when they gave 30% sucrose to rats.

In our study, the differences in weight gain at the beginning and the end of the experiment and abdominal circumference measurements were found to be higher in rats fed with fructose diet. This suggests that a diet high in fructose may be an important risk factor for obesity.

Adiponectin secreted from adipose tissue is inversely proportional to adipose tissue mass and visceral adipocytes, unlike other adipocytokines, and its amount in the circulation decreases in obese individuals. Metabolic syndrome is a condition characterized by obesity, hyperinsulinemia, hyperlipidaemia, and hypertension and represents a serious risk factor for cardiovascular diseases and type II diabetes. The studies conducted have shown that a decrease in serum adiponectin level plays a role in the development of insulin resistance and metabolic syndrome (Matsuzawa et al., 2004; Okamoto et al, 2006). It has been determined that the adiponectin form, which is in the form of a high molecular weight complex, is the active form in the serum and is better correlated with metabolic syndrome (Hanson et al., 2002).

Adiponectin takes an essential part in energy haemostasis by regulating glucose and fatty acid metabolism in peripheral tissues, including muscles and the liver (Berg et al., 2002). The biological functions of adiponectin depend on the function and expression of its specific receptors (AdipoR1, AdipoR2) as well as its serum levels. Some studies have demonstrated that the lack of adiponectin receptors causes hyperglycaemia and hyperinsulinemia (Parker-Duffen et al., 2014). It has been observed that the loss of metabolic effects of adiponectin in AdipoR1 and AdipoR2 knockout mice causes an increase in the tissue triglyceride content, inflammation, oxidative stress, insulin resistance, and glucose intolerance (Yamauchi et al., 2007).

The mechanisms that cause low adiponectin concentrations in insulin resistance are unclear. TNF-α is one of the molecules thought to cause insulin resistance. The studies conducted in adipose tissue and adipose culture cells have shown that (IL)-6 and TNF-α inhibit adiponectin secretion and expression (Bruun et al., 2003; Macda et al., 2001).

In a screening study carried out in India, it was observed that the rate of type 2 diabetes was low in individuals with high blood adiponectin levels and high in those with low blood adiponectin levels (Lindsay, 2002). In their study performed on obese individuals, Koca et al. (2006) reported that adiponectin levels decreased. Arita et al. (1999) reported a negative correlation between the decrease in adiponectin levels and body mass index in obese individuals. They stated that the reason for this might be related to the increase in insulin resistance, increase in the arteriosclerotic lesion and/or adipose tissue, and the increased TNF-α level.

Although mice with adiponectin deficiency showed almost normal insulin sensitivity when fed with a standard diet, they developed severe insulin resistance when fed with a high-fat and high-sucrose diet for two weeks (Kubota et al., 2002; Maeda et al., 2002). Another group of researchers observed increased fatty acid oxidation in the skeletal muscle of mice with adiponectin deficiency but did not observe any impact on insulin sensitivity or glucose tolerance in mice fed with a standard or high-fat diet (Ma et al., 2002).

Atanasovska et al. (2009) found that chronic fructose administration caused a significant increase in systolic blood pressure, body weight, serum triglyceride, free fatty acids, and insulin levels and decreased HDL adiponectin concentrations compared to the control group. The researchers reported that rosiglitazone treatment applied for four weeks could reverse these results and increase serum adiponectin levels two-fold.

Alzamendi et al. (2009) reported that plasma free fatty acids, leptin, and adiponectin levels increased and insulin resistance developed in laboratory animals fed with fructose for three weeks. Nakagawa et al. (2006) found that metabolic syndrome developed in rats fed with fructose, but not in rats fed with the same amount of glucose. Aksoy et al. (2016) observed that adiponectin levels increased significantly in rats fed with high-fructose and high-sucrose diet for eight weeks. They concluded that a diet rich in fructose was an important risk factor for obesity and mediated changes in Na^+/K^+-ATPase activity. Meeprom et al. (2011) reported that a high-fructose diet caused insulin resistance by considerably reducing insulin receptor, GLUT4 protein expression and adiponectin, AdipoR1 mRNA expression in the skeletal muscle. Mostafa-Hedeab et al.

(2017) showed that decreased AdipoR1/R2 expression levels in the liver, pancreas, kidney, and heart tissues of rats fed with a high-fructose diet for eight weeks were accompanied by an increase in blood sugar and insulin levels and that adiponectin activity, which led to insulin resistance, decreased.

Bonnard et al. (2008) found that AdipoR1 mRNA levels in the liver tissues of mice fed with a high-fructose diet for 16 weeks and AdipoR2 mRNA levels in the muscle tissues decreased and were inversely correlated with insulin levels. Choi et al. (2017) showed that AdipoR2 gene expression decreased in the liver tissues of mice fed for three weeks by increasing the fructose rate (35%) in the diet. Our results are in line with reports stating that high fructose consumption significantly impairs adiponectin receptor expression. Furthermore, AdipoR1 and AdipoR2 expression and the agonism of these receptors may be new targets in the treatment of insulin resistance and type 2 diabetes.

In our study, a decrease was observed in the serum adiponectin levels and the expression of AdipoR1 and AdipoR2 receptor genes expressed in the liver tissue of rats administered with a 20% fructose diet with drinking water for 16 weeks. Our results will contribute to the literature to examine the effects of the longer-term consumption of fructose as in humans and to develop new treatment strategies in future studies.

To obtain more comprehensive data on this subject, it is recommended to conduct in vivo and in vitro studies to compare the consumption of different amounts of sugar and sugar products in the long term and to better explain the mechanism of action of fructose.

Conflict of interest

The authors declare that they have no competing interests.

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Review

Bacterial Toxins

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ABSTRACT

Poisonous molecules produced by bacteria inside or outside the organisms are generally called “toxins”. The ability of bacteria to synthesize toxins is called “toxicity”. Toxigenicity is an important factor that increases the virulence of bacteria. The most important structure of bacteria in damage formation is toxin. Toxins created by bacteria are mainly described as two groups, exotoxins and endotoxins. Exotoxins are extracellular structures that bacteria secrete into the external environment. Endotoxins are found in the cell wall structure and released when bacteria are broken down. Exotoxins cause damage in several different ways. Some of them cause paralysis by affecting the neuromuscular system, some cause oedema by breaking down blood cells, some cause food poisoning and some cause enterotoxaemia. Endotoxins, on the other hand, cause inflammation through indirect ways. These inflammations can lead to septic shock. In this review, information about the toxins formed by *Escherichia coli Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp., *Clostridium* sp. are given briefly.

Key words: Enterotoxin, exotoxin, endotoxin, bacteria, intoxication

Bakteriyel Toksinler

ÖZET

Bakteriler tarafından vücut içinde ya da vücut dışında meydana getirilen zehirli moleküllere genel olarak “toksin” denmektedir. Bakterinin toksin sentezleme yeteneğine ise “toksijenite” adı verilmektedir. Toksijenite bakterinin virulensini arttıran önemli bir faktördür. Bakterinin hasar oluşumunda en önemli yapısı toksinleridir. Bakteriler tarafından oluşturulan toksinler ekzotoksinler ve endotoksinler olmak üzere başlıca iki gruptur. Ekzotoksinler bakterilerin dış ortama salgıladıkları ekstraselüler yapılardır. Endotoksinler ise hücre duvarı yapısında bulunmaktadırlar ve bakteri parçalandığında açığa çıkarlar. Ekzotoksinler birkaç farklı yolla hasar oluştururlar. Bazıları sinir- kas sistemini etkileyerek felçler oluşturur, bazıları kan hücrelerini parçalayarak ödemlere sebep olur, bazıları gıda zehirlenmelerine, bazıları da enterotoksemilere sebep olmaktadır. Endotoksinler ise indirekt yollarla yangı oluşturur. Bu yangısal olaylar, septik şoka sebep olabilmektedir. Bu derlemede *Escherichia coli*, *Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp ve *Clostridium* sp., etkenlerinin oluşturduğu toksinler hakkında bilgi verilmektedir

Anahtar kelimeler: Enterotoksin, ekzotoksin, endotoksin, bakteri, intoksikasyon

Introduction

The microorganisms can multiply in the digestive system and cause disease when they are ingested in high amounts. The food poisoning caused by *Salmonella* sp. agents can be given as an example. Enteric diseases related to food poisoning are arising due to the production of exotoxins and endotoxins. In some types of food poisoning (*Clostridium perfringens*) living organisms must be digested for the disease to occur. But the microorganism does not reproduce in the digestive system. After digestion, vegetative cells sporulate and enterotoxins are released into the environment when the spore cells break down. Because living cells are required for this type of food poisoning to occur. The process of enterotoxin release into the environment after vegetative cells become spore cells can be considered as an infection with food. However, not all infections that occur in this way cause enteritis. Ingested living organisms pass through the digestive system mucosa and reach the vascular system and invade other body tissues (Tayfur, 2009).

After the foodstuff is contaminated with a pathogenic microorganism and this microorganism multiplies and releases the toxin, the poisoning caused by the consumption of this food item is called "Foodborne Poisoning". In other words, the disease does not occur after not only ingestion of the pathogenic microorganism with food, but also after taking the toxin secreted by the microorganism (Tunail, 2000; Karapınar and Aktuğ, 1998). Foodborne diseases generally show clinical pictures that occur with the consumption of foods contaminated with pathogenic microorganisms or microbial toxins and progress with gastrointestinal symptoms (CDC, 2007; Carrique-Mas and Bryant, 2013). These diseases spread by food and they can occur in the form of foodborne infections and foodborne microbial intoxications (Scallan et al., 2011). Among these diseases, enteritis caused by *Salmonella* and *Campylobacter* species take the first place (Scallan et al., 2011; Håstein et al., 2014). Although there are many bacteria that cause foodborne infections and intoxications, the most important and common ones are known as *Escherichia coli*, *Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp. and *Clostridium* sp. (CDC, 2007; Carrique Mas and Bryant, 2013).

Escherichia coli

In developing countries, ETEC (Enterotoxigenic *Escherichia coli*) is an important cause of diarrhoea, especially among young children. The most obvious symptoms of this disease are diarrheal and abdominal cramps, sometimes accompanied by nausea and headache. However, vomiting and fever are rare symptoms. While ETEC generally causes diarrhoea, some recent ETEC outbreaks have manifested themselves in chronic duration (Murray et al., 2009). Enterotoxigenic *E. coli* strains bind to the small intestine mucosa via adhesins called colonization factor antigens (CFA). The small intestine is not actually a region where *E. coli* is found in large numbers. These structures, historically called CFA or *E. coli* surface antigens, consist mainly of bacterial pilus. After such attachment to small intestinal mucosa cells, ETEC strains have two important toxins encoded by plasmids and they cause diarrhoea by releasing heat-labile toxin: LT and heat-stable toxin (ST). ETEC strains secrete at least one of two different toxin groups, thermolabile (LT) and thermostable (ST) (Nataro et al., 1987).

ETEC origins mostly secrete ST (35%), some secrete both ST and LT, and a lesser part only LT (Orskov, 1984; Puente and Finlay, 2001; Donnenberg et al., 2005; Forbes et al., 2007). Labile toxin is closely related to cholera toxin biologically and antigenically. These toxins consist of an A subunit with catalytic properties and a subunit of B (A-B toxins) that bind receptors in a pentamer structure. LT binds to the host cell with the B subunit, and the A subunit enters the enterocyte, activating the adenyl cyclase enzyme. The increase in intracellular cAMP (cyclic adenosine monophosphate) activates protein kinase A, which in turn enables a molecule called "cystic fibrosis transmembrane transition regulator (CFTR)" to be phosphorylated and activated. Thus, with the activation of a highly complex cascade, there is active secretion of chlorine, passive secretion of sodium and water into the intestinal lumen. Stable toxin is a completely different molecule, a small peptide and similar to a mammalian hormone called guaniline. ST also binds to GTP (Guanosine triphosphate), which is located in the enterocyte apical membrane, which is a guaniline receptor. As a result, intracellular GMP (Guanosine monophosphate) level increases and this time protein kinase G is activated, in conclusion CFTR phosphorylation, electrolyte and fluid secretion increases into the intestinal lumen (Orskov, 1984; Clarke, 2001; Puente and Finlay, 2001; Donnenberg et al., 2005; Forbes et al., 2007). Stable toxin, unlike labile toxin, causes diarrhoea in the intestine similar to labile toxin by affecting not adenylate cyclase, but guanylate cyclase (Töreci, 2002). Labile toxin is mostly synthesized in calf and bovine strains, while stable toxin synthesis differs according to species (Gülhan, 2009). Two different exotoxins produced by ETEC affect the intestinal mucosa, causing fluid loss and watery diarrhoea. Although two toxins have different molecular mechanisms, they both interfere with the passage of sodium and chloride ions across the cytoplasmic membrane. Thus, abundant watery diarrhoea is observed (Batzing, 2002; Gülhan, 2009; Altındiş, 2010).

Enterohemorrhagic, Vero or Shiga toxin-producing *Escherichia coli* (VTEC/STEC/EHEC)

Shiga toxin-producing *E. coli* was first discovered in 1977. It is one of the pathotypes of *E. coli* that cause disease in humans and animals. While the role of STEC has been determined only in endemic disease of pigs, its roles in disease formation in lambs, calves and dogs are not clear. The reason *E. coli* is named as STEC is that the cytotoxin it secretes is largely similar in genetics and protein structure to the Shiga toxin produced by *Shigella dysenteriae*. There are different other synonyms used for STEC. These are VTEC (Verotoxin producing *E. coli*) or EHEC (Enterohemorrhagic *E. coli*). Shiga / Vero toxin (Stx) is the main virulence factor and identifies STEC strains. This potent cytotoxin is the cause of many symptoms and deaths in STEC infections. There are two immunoreactive subgroups in Stx toxin subgroup. These are Stx1 and Stx2. A STEC strain can produce only Stx1 or only Stx2 or both toxins. STEC's Stx1 toxin is the same as *Sh. dysenteriae* 1 Shiga toxin (Olivier et al., 2013).

Enteropathogenic *Escherichia coli*

Enteropathogenic *E. coli* virulence factors are under the control of genes on chromosomes and plasmids. In order for EPEC to show all the features of its pathogenicity, it must have two genetic elements: first; EPEC carries plasmids that encode plasmids called adherence factor (EAF) plasmid type IV fimbria or bundle forming pilus (bfp). The adhesion and disruption effect, called as the "enterocyte effacement" (LEE) is governed by T3SS (Type 3 secretion system: an island of pathogenicity encoding the type 3 secretion system), the outer membrane adhesion structure, the translocated intimin receptor (Tir) and other proteins. The typical EPEC refers to both the EAF plasmid and the LEE pathogenicity island of the organism. These strains are attributed to enterocytes by showing local adherence. Atypical EPEC strains do not carry EAF plasmids, therefore they do not

have bfp and they are less pathogenic (Puente and Finlay, 2001; Donnenberg et al., 2005).

Enteroinvasive *Escherichia coli*

Enteroinvasive *E. coli* strains are usually dormant and invade colon cells, causing inflammation and often watery diarrhoea. Rarely, it can cause bloody diarrhoea with a pathogenic mechanism similar to that of *Shigella* sp. It carries a large invasion plasmid encoding T3SS. It invades the epithelium using T3SS, escapes from the phagosome and moves with actin flagella in the cytoplasm of the cell, finally reaches and enters the neighbouring cell. In the same way, it reaches the submucosa by being transported and apoptosis mechanism of the macrophages. The release of interleukin 1 from the macrophages initiates the inflammatory response. Leukocyte migration occurs, leukocytes come to the region by passing between cells, and they open a second path for bacteria to pass. After bacteria cross the epithelium in both ways, they easily infect epithelial cells (Salyers and Whitt 1994; Nataro and Kaper, 1998; Donnenberg et al., 2005; Forbes et al., 2007; Murray et al., 2009).

Enteroaggregative *Escherichia coli*

EAEC, which is typically defined by its specific aggregative adherence feature in Hep-2 cell cultures, is known to cause microvilli loss and cell death by adhering to the human colon mucosa by aggregation. EAEC have plasmid dependent piluses known as aggregative adherence fimbriae (AAF / I and AAF / II). Some EAEC strains are known to secrete cytotoxins. This toxin is called as enteroaggregative cytotoxin (EAST). This toxin is also encoded by the plasmid. This toxin has been shown to cause damage in cells, cell rupture in cell cultures, dilatation and cell destruction in crypts in the human intestinal model (Clarke, 2001; Campos, 2004; Donnenberg et al., 2005; Forbes et al., 2007; Murray et al., 2009). The pathogenicity of enteroaggregative *E. coli* has been confirmed by the diarrhoea outbreaks reported to be due to EAEC. While the relationship between paediatric diarrhoea and EAEC has not been shown in previous studies, this relationship has been shown with the development of molecular techniques that can distinguish the real pathogen with aggregative properties (Doğancı and Vidinlisan, 1988; Murray et al., 2009). Enteroaggregative *E. coli* is responsible for diarrhoea-related conditions such as endemic, epidemic diarrhoea in children in both poor and industrialized countries, travel diarrhoea in developing countries, and persistent diarrhoea observed among HIV-infected or AIDS patients (Murray et al., 2009).

Campylobacter jejuni

C. jejuni has the ability to produce cytotoxin and enterotoxin. *C. jejuni* toxin (CJT), which is an enterotoxin, has been defined as cholera-like toxin (CLT) because it resembles cholera toxin structurally and functionally and is inactivated by cholera antitoxin (Ruiz-Palacios, 1983). The interaction between *C. jejuni* and host intestinal epithelial cells occurs in four stages: In the first stage, the agent can adhere to the host cell by passing through the mucus layer with its mobility feature. Here, adhesin receptor interactions [(major OMP, lipo-oligosaccharide, capsular oligosaccharide, fibronectin binding protein (CadF), surface lipoprotein A (JlpA) and polyoma antigenic protein (PEB1)] play an important role. After that, the cytolethal distending toxin (CDT) with DNase enzyme, which is secreted by bacteria, inhibits the development of host cells. Although the release time of CDT is not known exactly, it is thought that it begins to be released from the intestines following ingestion by the host. It is observed that the cytotoxin produced by *C. jejuni* has different properties in terms of cell selectivity and is different from *Shigella*-like toxin, CDT and hemolysin. It has been found that this toxin is not related to cholera toxin genetically but causes watery diarrhoea similar to cholera toxin (Wassenaar, 1997). It has been reported that cdt ABC genes, commonly known as cytotoxins and produced by *E. coli*, are also encoded by *C. jejuni* (Pickett, 1996).

***Shigella* sp.**

Shigella strains secrete 3 different enterotoxins: *Shigella* enterotoxin 1 (shET1), *Shigella* enterotoxin 2 (shET2) and Shiga toxin (Stx). The shET1 encoded by the chromosome is secreted by all *Sh. flexneri* 2a strains. This toxin is rare in other Shigella species. shET2 is located on the large plasmid responsible for *Shigella* virulence and is encoded by many different *Shigella* serotypes (Fasano et al., 1995). Stx is a neurotoxic, cytotoxic and enterotoxic toxin and is encoded by genes located in the chromosome. Only *Sh. dysenteriae* serotype 1 produces this toxin. It consists of Stx A and B subunits. It binds with the Shiga toxin B subunit to a glycolipid receptor found in target cells. Subunit A inhibits protein synthesis by irreversibly binding to the 60S subunit of the ribosome in the host cell and causes cell death (Niyogi, 2005). Basically, as in *E. coli* (STEC) that produces Shiga toxin, except for bacterial strains causing oedema, Stx genes in *Sh. dysenteriae* are carried on chromosome. However, unlike STEC, *Sh. dysenteriae* serotype 1 does not carry intact Stx transforming phages. This is thought to be the result of the loss of essential phage genes caused by transposition and recombination events. Stx causes severe vascular lesions in the colon mucosa, renal glomeruli and other organs resulting in haemorrhagic colitis and in some cases haemolytic uremic syndrome (HUS) (McVey, 2013).

Staphylococcus aureus

S. aureus enterotoxins are heat-resistant, antigenic extracellular proteins, some of which have been held responsible for staphylococcal food poisoning for many years (Argudin et al., 2010). Five types of enterotoxins, which are also considered to be superantigens, were first defined as A, B, C (C1, C2, C3), D and E, and then F, G, H, I, J, K, L, M, N, O, P, Q, R and U types were added, and finally, S and T types were named. Among these, staphylococcal enterotoxins A and D are the most common in food poisoning (Ortega et al., 2010). Among the enterotoxin-producing staphylococci species, the most important species for foods is *S. aureus* (Roberts, 1990). Staphylococcal enterotoxins are a water-soluble heterogeneous group of simple proteins with molecular weights ranging from 28-35 kDa weight. Among the serologically separable toxin types (A, B, C1, C2, C3, D, E), staphylococcal enterotoxin A (SEA) and SED mostly in food poisoning, SEB in clinical isolates, SEC and SED types in mastitis milk (Halpin-Dahnalek et al., 1989). Since SEA is produced in the first period of the logarithmic reproduction phase of *S. aureus*, it can reach the level of intoxication even if the environmental conditions are not optimal. Therefore, SEA is the most common type of toxin in food poisoning. It has been reported that staphylococcal enterotoxins with thermostable character are not destroyed at pasteurization temperatures, and SEC is more resistant to high temperature than SEA and SEB. In a study, it was determined that SEA could not be detected serologically after 3 minutes at 80 °C and 1 minute at 100 °C (Bergdoll, 1989). Baird Parker (Baird-Parker, 1990) reported that staphylococcal enterotoxins are not degraded at 121 °C in 3-8 minutes and are resistant to drying, cooling and freezing processes.

Almost all strains of *S. aureus* produce a group of enzymes and cytokines. Among these secreted enzymes and cytokines are four haemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase and collagenase. The main task of these proteins is to make the host tissues suitable for the growth of bacteria. Some other strains also have additional exoproteins such as toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEH, and SEI), exfoliative toxin, and leukocidin. Among these, haemolysins and leukocidin, exfoliative toxin, TSST-1, staphylococcal enterotoxins are the toxins of *S. aureus*. Among these toxins, TSST-1 and staphylococcal enterotoxins are also known as pyrogenic toxin superantigens (PTSAGs) (Dinges et al., 2000).

Due to the stimulation of toxins local nerve receptors in the intestine, the impulses passing through the vagus nerve and sympathetic nerves reach the subcortical vomiting centre of the brain resulting in an emetic response (Sutherland and Varnam, 2002). The second most common symptom in staphylococcal food poisoning is diarrhoea. The direct effect of staphylococcal enterotoxins on intestinal cells is unclear and they are apart from classical enterotoxins such as cholera toxin or *E. coli* enterotoxins (Halpin-Dohnalek and Marth, 1989; Sutherland and Varnam, 2002).

The development phases with enterotoxigenic *Staphylococcus* sp. cells have an important effect on the formation of staphylococcal enterotoxin types. In relation to this, SEA is formed in the logarithmic phase of the cell, SEB, SEC and SED in the late logarithmic phase or early stationary phase (Bergdoll, 1989). In addition, secondary metabolites can also have an inhibitory effect on the bacteria. In this context, it has been determined that the *S. aureus* strains that constitute SEB are suppressed by the toxin during the development cycle (Halpin Dohnalek and Marth, 1989). The agr (accessory gene regulator) gene plays an important role in the regulation of virulence factors of *S. aureus*. Mutations in the agr locus result in reduced formation of many SE and other exoproteins. The gene can be transcriptionally and translationally regulated by the agr. It has been reported that agr regulates SEB and SEC at the transcriptional level, and the activation of agr and the formation of SEB and SEC and SED coincide with the bacterial growth cycle. Not all SEs are regulated by agr gene. For example, SEA is not affected by agr mutations (Tremaine et al., 1993). Agr is most highly activated at neutral pH values. It has been reported that genes targeted by agr are negatively regulated and exoprotein formation is reduced or not formed at all in glucose containing low pH media (Jablonski and Bohach, 1997). However, in environments where the bacterial density is low, agr cannot be activated, so that the formation of toxins and enzymes is prevented by the upregulation of adhesins. It has been reported that toxins and enzymes can be released by regulating agr up and adhesins down in cases where the bacterial density is high (Wisell, 2000).

Bacillus cereus

Food poisoning can be seen in individuals with taking large numbers of *B. cereus*. Especially when foods contaminated with *B. cereus* are not cooled sufficiently and quickly after cooking, or when the time between the preparation and consumption of food is prolonged, the microorganism can multiply and form a toxin that can cause food poisoning as a result of germination of live and heat-resistant spores. Food poisoning occurs when the number of bacteria in the food is higher than 10^6 cfu / g (Pichhardt, 2004).

B. cereus produces two different toxins. One of them is enterotoxin, a heat-resistant protein (Stable Toxin; ST) weighing 40 kDa. This toxin, which causes food poisoning, becomes inactive in 90 minutes at 126 °C. The other toxin is a peptide with a weight of 5–7 kDa, resistant to heat (Labil Toxin; LT). It is destroyed in a few minutes at 60oC. It is known that all strains of *Bacillus cereus* do not produce toxins and only certain serotypes produce toxins. It is not possible to say for sure that this bacterium causes disease through intoxication. It has also been shown that the bacterium known to develop in the intestine and form toxins creates toxins in anaerobic conditions *in vitro* (Tunail, 2000). The extracellular toxin that causes *B. cereus* food poisoning, can be detected after the number of bacteria in the environment reaches a certain level. *B. cereus* also secretes lecithinase C enzyme such as *C. perfringens*, but it has been reported that toxic activity is not involved in the same molecule. It is known that the toxin formation of *B. cereus* depends on the presence of some nutrients in the environment. Bacteria synthesize and release the toxin during the logarithmic phase. Studies have found that this bacterium synthesizes toxins between 18-44 °C and stops at 45 °C. The toxin of *B. cereus* is sensitive to pronase enzymes and becomes inactive with a dose of 0.01% of these enzymes at 37 °C in 60 minutes. The toxin does not lose its activity in 30 minutes at 45 °C, but becomes inactive at 56 °C (Pichhardt, 2004). *B. cereus* synthesizes two different types of enterotoxins, emetic and diarrheal enterotoxin, and causes two different types of poisoning. One of these is the "acute onset vomiting type syndrome" and is mostly associated with the toxin produced in cooked rice and rice foods. This toxin, called emetic toxin, is resistant to heat and low pH as well as trypsin and pepsin enzymes. The other type of disease is known as "prolonged diarrheal syndrome" and is associated with a wider food group. Among these foods; cereal-containing foods, especially corn and corn starch, mashed potatoes, vegetables, minced meat, pudding and soups. This toxin, known as diarrheal toxin, is in protein structure and is sensitive to heat with trypsin and pronase enzymes (Kaleli and *Özkaya*, 2000).

Bacillus anthracis

Anthrax has two important virulence factors. One is the antiphagocytic PGA capsule encoded by pX02 plasmid and the other is two exotoxins encoded by pX01 plasmid. The pX01 plasmid encodes three biologically inactive components: protective antigen (PA), oedema factor (EF) and lethal factor (LF). When PA and EF come together, it creates oedema toxin. Oedema toxin increases intracellular AMP level by showing adenylate cyclase activity. This causes fluid and electrolyte loss and impairs innate and acquired immune functions, including neutrophil chemotaxis. Together with PA, LF forms lethal toxin. This toxin is in the calmodulin-dependent zinc metalloprotease structure, inactivating protein kinases activated by mitogens and disrupting signal transduction (Baldari et al., 2006; Sherer et al., 2007).

Clostridium difficile

C. difficile strains, an anaerobic, sporulated Gram-positive bacterium, were first shown in 1977 to cause antibiotic-associated diarrhoea (Bartlett, 2008). According to classical knowledge, toxin A (enterotoxin) and toxin B (cytotoxin) encoded by the tcdA and tcdB genes located in the pathogenicity locus (PaLoc) of the *C. difficile* chromosome are responsible for the pathogenesis of the disease. Toxigenic strains of *C. difficile* often produce both toxins together. In recent years, differences in toxin A and toxin B production or variants in toxin genes (such as toxin A- / B +) have been identified. Also, in some countries, origins producing binary toxin different from toxins A and B,

causing hospital outbreaks have been reported (Rupnik, 2001). *C. difficile* origins that cause antibiotic-associated diarrhoea produce two different toxins called Toxin-A (Tox-A) and Toxin-B (Tox-B). These toxins, whose mechanisms of action are similar, enter the intestinal epithelial cell by endocytosis and cause cell death by affecting the actin skeleton in the cell. It has also been found that toxins lead to the secretion of certain cytokines, thereby the development of inflammatory response and the formation of pseudomembranes. In the past, it was thought that Tox-A caused damage to the intestinal epithelium, then Tox-B showed its effect, so Tox B could not be active alone, with the development of molecular techniques, it was understood that Tox-B without Tox-A had a cytotoxic effect (Poxton et al., 2001). In recent studies, strains producing only Tox-B have been identified, as well as some other variant strains have been reported. It has been found that some strains secrete large amounts of Tox-A and Tox-B, and there is a defect in the tcdC gene that negatively regulates toxin expression. It has been determined that the highly virulent 027 / NAP1 / BI origin, which causes hospital outbreaks in various countries, has a deletion in the tcdC gene and therefore secretes high levels of Tox-A and Tox-B. The 027 / NAP1 / BI origin has also been shown to produce a third toxin identified as binary toxin (B-Tox) (O'Connor et al., 2009).

Clostridium botulinum

C. botulinum exotoxin, commonly used as botulinum toxin (BTX), was shown by Van Ermengem in 1897 to be a spore-forming Gram-positive, anaerobic neurotoxin produced by *C. botulinum* bacteria (Van Ermengem, 1979) For the release of acetylcholine (Ach) BTX, denervation in muscles by binding necessary proteins, is the most effective biological toxin known. After BTX is synthesized as a single chain polypeptide, it takes the form of double chain with the help of endogenous bacterial proteases. These two chains, which are separated as "heavy" weighing 100 kDa and "light" weighing 50 kDa, are held together by disulphide bond (Simpson, 1981). There are 7 different serotypes of BTX (A, B, C, D, E, F, G). Of these, A is the strongest and the first form that entered medical use. Serotypes A, B, E are responsible for food poisoning in humans (Dertzbaugh, 1996).

Botulinum toxin is injected into the submandibular and parotid glands for hypersalivation treatment, and is also used in postoperative salivary fistulas (Rohrbach and Laskawi, 2003). In 2001, another serotype of neurotoxin, botulinum toxin type B (Btx B) Myobloc, was licensed in America and European countries for its use for cervical dystonia. Acetylcholine needs many transport proteins for release from nerve endings to the synaptic space. BTX-A irreversibly cuts the SNAP-25 protein, while BTX-B irreversibly cuts the VAMP protein (Moore and Naumann, 2003).

C. botulinum produces several proteins with "toxic" activity (botulinum toxin, C2 toxin and C3 exoenzyme), but only botulinum toxin plays a central role in the production of botulism. Although new "mosaic" toxins have been recently identified, there are seven types of botulinum toxin (BoNT for botulinum neuroxin) that differ by antigenic differences. Letters from A to G indicate the types. The type of neurotoxin characterizes the *C. botulinum* species that produces it. Therefore, the *C. botulinum* strain producing a type A BoNT will be identified as *C. botulinum* A type. All seven BoNT types are zinc endopeptidases with the same activity, i.e. the hydrolysis of the insertion proteins required for the fusion of neurotransmitter-containing vesicles. Although the result is the same with the presynaptic membrane (inhibition of neurotransmitter release), various BoNT types hydrolyze different docking proteins. Types A and E hydrolyse SNAP (synaptosomal associated protein); Types B, D, F and G hydrolyse VAMP (vesicle-associated membrane protein, also known as synaptobrevin) and type C hydrolyses SNAP and syntaxin. BoNT is a "di-chain" molecule (binding to nerve cells) consisting of a light chain (with zinc endopeptidase activity), a heavy chain consisting of a translocation domain (responsible for a pore through which the light chain passes), and a binding domain. Several "accessory" proteins thought to help the toxin survival in the gastrointestinal tract are secreted by BoNT. BoNT binds to cholinergic nerve cells of BoNT, each of which binds to a different receptor. Once bound, the toxin is internalized through receptor mediated endocytosis. BoNT-containing vesicles remain at the neuromuscular junction. After a cleavage event, the light chain (zinc endopeptidase) passes to the cytosol of the nerve cell where it hydrolyses docking proteins across the vesicle membrane. Both C2 toxin and C3 exoenzyme are ADP ribosyl transferases, C2 toxin and C3 exoenzyme ribosylate G-actin and Rho, respectively, and cause cytoskeleton degradation. Both enzymes do not appear to play a role in the disease process (McVey et al., 2013).

Conclusion

Symptoms and disorders about the digestive system such as nausea, vomiting, severe abdominal pain, weakness, muscle pain, are caused by eating foods contaminated with microorganisms and toxins produced by microorganisms. The most common food poisoning is caused by *Staphylococcus* sp. The bacterium is not the main disease factor itself, but the toxin of the bacteria causes disease. Exotoxins, one of the most important virulence factors of bacteria, cause damage in the body by several different mechanisms. Some exotoxins are produced by bacteria that contaminate feed and are taken into the body through digestion. This is a form of food poisoning. Food poisoning can occur in the same way in humans. Botulism disease, which occurs with feeds that produce toxins in animals, and ingestion of such foods in humans, is the best example for this disease. Botulinum toxin produced by *C. botulinum* is a very powerful toxin, even a small number of bacteria is enough for disease formation. Some exotoxins are synthesized by bacteria that grow in body wounds or abscesses and spread from these damaged tissues to the entire organism. Bacteria can also produce exotoxins while they are on mucosal surfaces in the body. For example, *E. coli* causes diarrhoea by producing enterotoxins in the intestine. Exotoxin-producing bacteria can cause systemic shock and death in animals by indirectly stimulating excessive cytokine production as in Anthrax disease.

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Case Report

First clinical case of leishmaniosis due to Leishmania infantum in a domestic cat from Turkey

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ABSTRACT

A 12-year-old, female domestic cat was admitted with a history of suffering from pruritic, recurrent crusting-ulcerative skin lesions on neck for several years. Physical examination revealed lesions with hair loss, mild crusting and ulcerative plaques on the neck. Two weeks before the presentation, an attempt to treat the cat in a private clinic was unsuccessful. Based on history and dermatological signs, leishmaniosis was suspected. No *Leishmania* amastigotes were found on microscopical examination of the skin, blood and lymph node smears stained using Giemsa, and serological tests for detecting FeLV and FIV gave negative results. The presumptive diagnosis was confirmed by the detection of *L. infantum* DNA by C-nPCR in EDTA blood samples. Treatment with enrofloxacin and supporting drugs for two weeks provided complete recovery of skin lesions one month after the treatment, and the direct detection of pathogen DNA was negative. At a yearly follow-up for 3 years, no clinical recurrences were observed. To the authors' knowledge, this is the first clinical case of *L. infantum* infection diagnosed in a domestic cat in Turkey.

Keywords: Cat, Leishmania infantum, clinical case, Turkey

Türkiye'de bir kedide Leishmania infantum'dan ileri gelen ilk klinik leishmaniosis olgusu

ÖZET

Oniki yaşlı, dişi bir kedi kaşıntı ve boyun bölgesinde birkaç yıldır tekrarlayan kabuklu-ülseratif deri lezyonları şikayetiyle getirildi. Fiziksel muayenede boyun bölgesinde kıl dökülmesi, hafif kabuklanma ve ülseratif plaklarla karakterize lezyonlar belirlendi. Hasta getirilmeden iki hafta kadar önce özel bir veteriner kliniğinde uygulanan tedavi denemesinden sonuç alınmadığı belirtildi. Anamnez ve dermatolojik bulgular temelinde leishmaniosisden şüphelenildi. Giemsa ile boyanan deri, kan ve lenf yumrusu örneklerinin mikroskopik incelemesinde *Leishmania* amastigotlarına rastlanmadı; FeLV ve FIV'in belirlenmesine yönelik serolojik testler negatif sonuç verdi. Olası tanı EDTA'lı kan örneklerinde C-nPCR yöntemiyle *L. infantum* DNA'sının belirlenmesiyle doğrulandı. İki hafta süreyle enrofloksasin ve destekleyici ilaçlarla uygulanan tedaviden bir ay sonra deri lezyonlarında tamamen iyileşme sağlanırken, kanda etken DNA'sına rastlanmadı. Üç yıl boyunca gerçekleştirilen yıllık takipte klinik bulguların tekrarlamadığı görüldü. Yazarların bilgisine göre bu Türkiye'de *L. infantum* enfeksiyonu belirlenen bir kedide tanımlanan ilk klinik olgudur.

Anahtar kelimeler: Kedi, Leishmania infantum, klinik olgu, Türkiye

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Introduction

Leishmaniases are a group of arthropod-borne diseases including different clinical presentations caused by *Leishmania* spp. Leishmaniosis caused by *Leishmania (L.) infantum* is endemic in the countries of The Mediterranean Basin and dogs are accepted as the main reservoir of the parasite (Gramiccia, 2011; Baneth et al., 2012). In Turkey, *L. infantum* was determined as a causative agent of both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in humans (Toz et al., 2013). It is also primarily responsible for canine leishmaniosis (CanL) with a seroprevalence ranging between 3.6% and 25% in western Turkey (Toz et al., 2005).

In contrast to dogs, leishmaniosis in cats is sporadically reported in various parts of the world (Simoes-Mattos et al., 2004; Penissi et al., 2013, 2015), although the first record of infection by *Leishmania* spp. dates back to 1912 (Sergent et al., 1912). Recently, Pennisi et *al* (2015) have reviewed a total of 46 clinical cases of feline leishmaniosis (FeL) published between 1989 and 2014 from European countries, where the diagnosis of FeL was confirmed by serological and/or parasitological methods. Clinical cases are uncommonly in cats, but recent epidemiological investigations have confirmed that subclinical infections are not rare. The prevalence rates of *L. infantum* infection in cats in serological or molecular-based surveys range from 0 % to 68.5 % in CanL endemic regions of the Old World (Pennisi et al., 2015). Therefore, domestic cats are suggested as possible alternative reservoirs of *L. infantum* (Maia et al., 2010; Gramiccia, 2011; Pennisi et al., 2015).

Human VL and CanL caused by *L. infantum* are endemic in western Turkey and they have been investigated widely whereas information on FeL are very scarce. A recent study (Pasa et al., 2015) in the Aegean Region where CanL is endemic, revealed 13 (8.84%) out of the 147 cat blood samples positive for *Leishmania* by Real-Time (RT)-PCR (4 *L. major* and 9 *L. tropica*). Another seroprevalence study of *Leishmania* infection also revealed the molecular detection of *L. tropica* and *L. infantum* in stray cats of Izmir, Turkey (Can et al., 2016). To our knowledge, no clinical cases of FeL caused by *L. infantum* have been published to date in Turkey. Case reports of FeL are useful for gathering more data on the natural disease in this species. The aim of this study was therefore to present, for the first time, a clinical case of leishmaniosis due to *L. infantum* in a domestic cat in Turkey.

Case Description

In October 2012, a 12-year-old unneutered female domestic cat living in Kusadasi Town, western Turkey, was admitted to Adnan Menderes University Veterinary Teaching Hospital for further examination because of pruritic and recurrent ulcero-crusted skin lesions on the neck for several years. The owner noticed that the cat has been household with two dogs in which leishmaniosis had been diagnosed as CanL based on a positive IFAT and detection of amastigotes in lymph node two years ago. Two weeks before the presentation, the cat had been lastly treated with parenteral amoxicillin and dexamethasone, topical antifungal cream along with *Microsporium canis* vaccination in a private clinic. There was no response to this therapy. Conversely, the lesion became worse and ulcerative.

Physical examination was revealed the presence of pruritic skin lesions with diameters of 3x5 cm along with hair loss, mild crusting and ulcerative plaques on the neck (**Fig 1A, 1B**) without ocular, and systemic signs that have been reported in the cutaneous form of FeL (Pennisi, 2002; Poli et al., 2002; Gramiccia 2011; Pennisi et al., 2015). Emaciation was not observed, and there was no evidence of systemic disease. Based on the medical history and physical examination, FeL was suspected. In order to confirm the infection, the cat was submitted to laboratory evaluation.

Whole blood samples (EDTA anticoagulant) for complete blood cell (CBC) count and PCR testing, and serum samples for bio

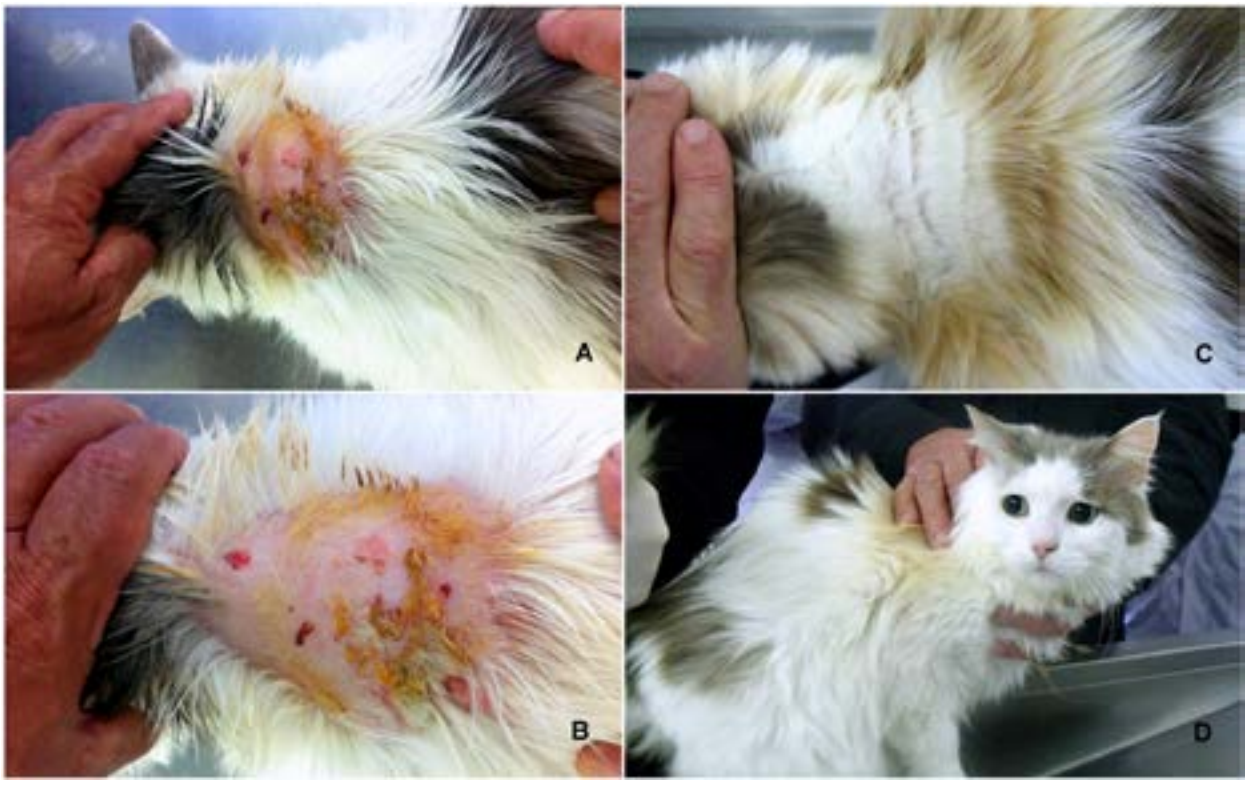

Figure 1 A, B. Ulcero-crusted skin lesions on neck of the cat.
Figure 1 C, D. Appearance of neck (C) and general appearance (D) of the cat one month after treatment.

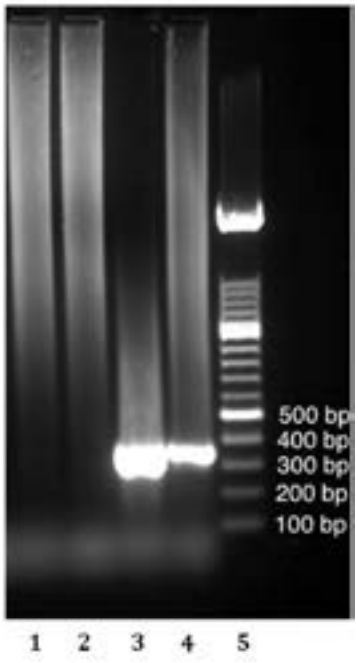

Figure 2. Results of C-nPCR identifying Leishmania spp. Lane 1, negative control; lane 2, one month after treatment; lane 3, before treatment; lane 4, Leishmania (L. infantum) positive control; lane 5, DNA marker 100 bp.

chemical parameters and investigation of the feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were collected. Skin, blood and lymph node smears were prepared and stained using Giemsa to confirm the presence of *Leishmania* amastigotes, but no *Leishmania* spp. amastigotes were observed. Results of CBC and serum biochemical profile were within normal limits, and ELISA results for FeLV and FIV were negative.

DNA extractions were performed by Qiagen Dneasy Blood & Tissue kit using 200 µl of peripheral blood samples obtained before the treatment and one month after the treatment. Classic Nested PCR (C-nPCR) was performed using primers R221-R332 and R223-R333 as described before (van Eys et al., 1992). The target for amplification of the small subunit ribosomal RNA (SSU rRNA) gene was 358 bp. PCR products were visualized by 1.5 % agarose gel electrophoresis (**Fig 2**). Positive PCR product was sequenced commercially by Biyomer with ABI 3130 Genetic Analyser. Sequence analysis of the positive PCR products revealed a 99% similarity with *L. infantum* isolate KC347301 from Iran in BLAST. The GenBank accession number provided for the nucleotide sequence reported in this case is KM408430.

Treatment consisting of enrofloxacin (Baytril®, Bayer, 3 mg/kg, SC, SID), 0.1% rivanol solution (twice a day, topically) and cream containing pantothenic acid with chlorhexidine HCl (Bepanthene Plus®, Bayer, twice a day, topically) was applied for two weeks. One month after the treatment, the skin lesions of the cat improved completely **(Fig 1C, 1D)** and the C-nPCR test was negative. At a yearly follow-up for 3 years, no clinical recurrences were observed.

Discussion

So far, clinical leishmaniosis in cats has never been reported in Turkey and this case, therefore represents the first clinical report of a domestic cat naturally infected with *L. infantum*. The cat was born and stayed permanently in Turkey; therefore, *L. infantum* infection has to be considered as sporadic autochthonous appearance. Clinical leishmaniosis is uncommon in cats although significant numbers of cats might be subclinically infected in the same areas where the disease occurs in dogs or humans (Maia et al., 2010; Gramiccia, 2011; Pennisi et al., 2015). Kusadasi town located in western Turkey, where the infected cat described herein was identified, is an endemic region for CanL with a seroprevalence rate reaching up to 9.1% and autochthonous human and/or canine cases of leishmaniosis have been reported previously (Toz et al., 2005). The cat presented herein lived in the same house where two dogs were affected by *L. infantum*. Therefore, these dogs are suspected to play a reservoir role for the presented cat.

Leishmaniosis due to *L. infantum* in cats can cause both cutaneous and visceral forms of the disease, although the cutaneous form is recorded most frequently (Simoes-Mattos et al., 2004; Gramiccia, 2011; Pennisi et al., 2013, 2015). Ulcerative, crusty, nodular, or scaly dermatitis found mainly on the head and neck and alopecia with unspecific clinical characteristics are the most frequent clinical manifestation (Hervás et al., 1999; Poli et al., 2002; Spickler et al., 2010; Gramiccia, 2011; Pennisi et al., 2013, 2015), and sometimes the only finding on physical examination (Pennisi et al., 2013, 2015). The cat with possible FeL in this report presented clinical signs compatible with cutaneous abnormalities described in the literature (Spickler et al., 2010; Gramiccia, 2011; Pennisi et al., 2013, 2015). Clinical disease and visceral signs developing in some cats seem to be related to concurrent immunosuppressive viral infections such as FIV and FeLV (Poli et al., 2002; Pennisi et al., 2013), although this association has not been confirmed (Leiva et al., 2005). In our case, it can be suspected that a concomitant immunosuppressive event might have caused clinical disease. FIV and FeLV testing yielded negative findings, but the cat was treated several times with systemic glucocorticoids, the immunological status may have been impaired (Pennisi et al., 2013, 2015). Information on clinicopathological abnormalities are FeL is scarce. Mild nonregenerative anemia, thrombocytopenia, and hyperproteinemia are reported as the main laboratory findings (Pennisi, 2002; Leiva et al., 2005) which were not detected in our case.

Diagnosis of FeL is fairly difficult since clinical signs can mimic other infections in cats and culture is not routinely available (Poli et al., 2002; Simoes-Mattos et al., 2004; Gramiccia, 2011; Pennisi et al., 2013, 2015). However, FeL should be suspected in cats living or traveling in endemic areas with clinical presentation of skin, ocular, or systemic signs (Pennisi et al., 2015). The serological diagnosis usually confirms the direct diagnosis but it is not standardized in cats as for dogs (Maia et al., 2010). Many problems of serology are circumvented by the use of PCR that enables detection and accurate quantification of the parasite (Gramiccia, 2011; Chatzis et al., 2014; Pennisi et al., 2015). Definitive diagnosis can also be made by the detection of *Leishmania* amastigotes in different tissues by cytological examination (Pennisi et al., 2013), although the sensitivity of this method may not be high enough (Maia et al., 2010; Chatzis et al., 2014). The diagnosis in this case at presentation was suspect leishmaniosis because of information on case history and unspecific clinical findings. Thereafter, C-nPCR reaction confirmed the suspected diagnosis, and the etiologic agent was identified as *L. infantum* (**Fig 2**), a *Leishmania* species that had been previously reported to infect cats from The Mediterranean Basin (Ozon et al., 1998; Hervas et al., 1999; Pennisi, 2002; Poli et al., 2002).

Treatment of FeL has not been standardized due to the small number of reported cases. Successful treatment of cats with clinical recovery can be achieved with allopurinol (Leiva et al., 2005; Pennisi et al., 2013, 2015) and meglumine antimonate (Hervas et al., 1999). Both fatal cases and spontaneous cures have also been reported (Spickler, 2010). In the present study,

the skin lesions recovered completely and *L. infantum* DNA was not detected from the blood, as documented by PCR after one month of the treatment. Clinical and parasitological improvement of the case could be explained by the efficacy of enrofloxacin as described early in CanL (Bianciardi et al., 2004). Alternatively, the cat could experience spontaneous clearance of infection, as evidenced by the negative PCR assays in blood tested; similar to what has been documented previously in dogs (Koutinas and Mylonakis, 2010). Lastly, the negative PCR status of the treated cat in the present study may be due to the suppression of tissue *Leishmania* loads to a level not detectable by the applied assays. This possibility was demonstrated in dogs (Francino et al., 2006). The cat presented herein is still living healthy.

FeL has to be included in the differential of ulcerative and crusty dermatitis for cats living in *L. infantum*-endemic areas. Further studies are necessary to demonstrate the possible epidemiological role of domestic cats in leishmaniosis in Turkey.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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