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EDITORIAL

Dear Readers;

We are very pleased and honored to present the first issue of our journal for 2024. In this issue, there are a total of 15 articles, comprising 12 research articles, 2 case reports, and 1 review article.

Dear readers, I would like to provide you with brief information about the statistics of our journal in 2022. Throughout the year, a total of 286 articles were submitted to our journal, out of which 75 were accepted. During this process, our article rejection rate stood at approximately 74%. Of the 211 rejected articles, 8 were withdrawn by the authors, 32 were rejected following the "Referee Evaluation Process," and 171 did not pass the "Preliminary Evaluation Phase." In the Preliminary Evaluation Phase, the report prepared by the relevant field editors regarding the submitted articles is evaluated by the Editorial Board during the weekly meeting. At this stage, decisions are made on whether to initiate the "Referee Evaluation Process" for the article. Articles rejected at this phase are returned to the authors with a clear explanation of the reasons for rejection.

Dear readers, in the last quarter of 2023, as we prepared the first issue for 2024, we unfortunately faced a human tragedy. In the conflicts between Palestine and Israel, many innocent people and civilians lost their lives. Attacks on hospitals, universities, and schools in the Gaza Region deeply affected and wounded all of humanity. We sincerely hope that this human tragedy in the region will end and reach a resolution as soon as possible, and that such conflicts will never happen again.

Dear academicians, I would like to emphasize that we would be honored to publish your valuable articles, and we look forward to receiving your submissions. On this occasion, I express my gratitude to those who contributed to the preparation of our journal, our authors who contributed scientifically, and I extend my sincerest regards to all of you with the belief that it will significantly contribute to the world of science.

Sincerely,

Dr. Levent ALTINTAŞ
Editor in Chief
Ankara Üniversitesi Veteriner Fakültesi Dergisi

Dr. Vasfi Samim (1905-1981): A successful veterinarian, artist and sportsman in Albania

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ABSTRACT

This biographical research aims to reveal the professional life and scientific works of Dr. Vasfi Samim. Dr. Vasfi Samim (1905–1981) was an internationally renowned Albanian veterinarian registered in the Ottoman State Register, who has been considered as an accomplished veterinarian, zootechnist, artist as well as sportsman in Albania. After graduating from the Higher Veterinary School (*Baytar Mekteb-i Alisi*) in Istanbul in 1927, Dr. Vasfi Samim completed his doctorate in the field of veterinary zootechny at the Berlin Higher Veterinary School in 1931. Upon returning to his country, he provided the structuring and development of animal health, breeding and zootechnical disciplines at international standards through serving important administrative duties. Dr. Vasfi Samim has 27 scientific articles on veterinary infectious diseases, animal breeding, selection, and crossbreeding as well as 11 books with social, cultural and political themes, 16 stories, 43 essays, and 23 theater works. He also played in Fenerbahçe, one of Türkiye's well-established sports clubs, and in Albanian football teams, and became an exemplary role model by being a manager in the national and international promotion and development of Albanian sports. Dr. Vasfi Samim should be remembered as an influential veterinarian, and an intellectual personality for the region he was grown in and for the world.

Introduction

Veterinary medicine education in Türkiye started with the first veterinary school opened in Istanbul in 1842 (21). Since the graduates of the Military Veterinary School were insufficient in preventing the diseases of the animals in the hands of the public and could not meet the needs of the country, the necessity of establishing a Civil Veterinary School emerged and the first Civil Veterinary School was established in 1889 based on the regulations and programs from the Alfort Veterinary School, France. Military and Civil Veterinary Schools were combined under one roof in 1920 and teaching activities were carried out under the name of the Higher Veterinary School in 1921 (20). At that time, hundreds of veterinarians graduated from these schools. Among the graduates including Vasfi Samim, some scientists provide successful services in the field of medicine and veterinary medicine (6, 7, 10, 12, 19, 23, 24) as well as veterinarians who provide outstanding services in the fields of politics, bureaucracy, culture, art, and sports (2, 3, 13, 29, 30, 33). In this research it was aimed to reveal Vasfi Samim's biography as a prominent contributor to the history of science in general and to the history of veterinary medicine in particular of with his identity as a scientist, journalist, writer, bureaucrat, and athlete who was born in Albania as a citizen of the Ottoman Empire in the 20th century, then came to Istanbul, the capital of his state, for education, and pioneered the development of veterinary medicine and zootechny in his country with his knowledge of veterinary medicine.

Materials and Methods

The material of the study consists of personal interviews with Dr. Vasfi Samim's living son, Genci Samim,

documents and photographs obtained from the family archive, books and newspapers written about Dr. Vasfi Samim's life, scientific journals, articles, books, stories, essays, and theater works published by Dr. Vasfi Samim. The information obtained from the document analysis was written down in accordance with the chronology with a retrospective approach.

Results

Vasfi Samim was born on December 15, 1905, in the village of Visoka, Fier, Albania. When he was four years old, he came to his uncle who immigrated to Istanbul after his father's death and started school under his auspices. Vasfi Samim completed his primary, secondary and high school education free of charge at a private boarding school in Istanbul (8, 15). After his high school education, he enrolled in Higher Veterinary School (*Baytar Mekteb-i Alisi*) in Istanbul on September 23, 1923, with a scholarship (26) (Figure 1). He graduated from this school in 1927 as a civil veterinarian (5).

Figure 1. The record of student Vasfi Samim at the Higher Veterinary School - *Baytar Mekteb-i Alisi* (Courtesy of Genci Samim archive).

His Professional Life and Scientific Studies: After graduation, he was appointed to Samsun city as a state veterinarian with the decision of the Ministry of Agriculture dated 14 August 1927 and numbered 2264 (26) (Figure 2). He took part in the fight against many infectious animal diseases, especially rinderpest, for one year. Vasfi Samim moved from Samsun to Istanbul on May 24, 1928, and completed his technical internship and military service for one year (18 June 1928-18 June 1929) at the Military Practice Veterinary School. At the end of the exams, he received the rank of lieutenant veteran (26) (Figure 3).

Figure 2. Vasfi Samim's document showing to be assigned as a veterinarian in Samsun (Courtesy of Genci Samim archive).

Vasfi Samim was appointed to the Artvin province as a state veterinarian on 18 June 1929, after completing his internship at the Military Practice Veterinary School, but he did not accept this duty. Meanwhile, he received an invitation letter from his colleague Dr. Bilal Golem who is in Albania. In this letter, Dr. Golem stated that there is a need for veterinarians, doctors, and intellectuals for the development of Albania, which is a small country at the time. Upon this invitation from Dr. Golem, Vasfi Samim returned to his country in 1929 as a well-educated young veterinarian (8, 28). On his return to Albania, he first worked as an assistant Dr. Golem's in veterinary department in Tirana. Dr. Golem is a veterinarian with the rank of captain who graduated from the Military Veterinary School in Türkiye in 1920 (5) and received his postgraduate education at the famous Pasteur Institute in Paris. During the following period, he was assigned to the Vlora region by the Ministry of Agriculture and Forestry to fight the diseases (especially foot and mouth disease) spreading among large cattle herds. Vasfi Samim worked here from October 1, 1929, in the early spring of 1930 and was successful in controlling the epidemics (8). On June 13,

Figure 3. The record of trainee of Vasfi Samim at the Military Practice Veterinary School - *Askeri Baytar Tatbikat Mektebi* (Courtesy of Genci Samim archive).

1930, he was appointed Head of the Veterinary Department of Vlora province (11). With the suggestion of Golem, Vasfi Samim went to the Veterinary Higher School in Berlin (*Die Tierarztliche Hochschule Zu Berlin*) in September 1930 to receive his PhD. Between 1930 and 1932, he continued his education under the consultancy of Professor Valentin Stang and Carl Cronacher, and completed his thesis titled "Investigation of the effect of different types of defatted soybean meal on the blood count of cattle" (*Zur Kenntnis der Einëirkung verschiadenartig entfetteter Sojaschrote auf das Blutbild des Rindes*) in the field of zootechny on 19 December 1931 with the degree of very good (*sehr gut bestanden)*, and received the title Dr. Vet. Med. (26) (Figure 4).

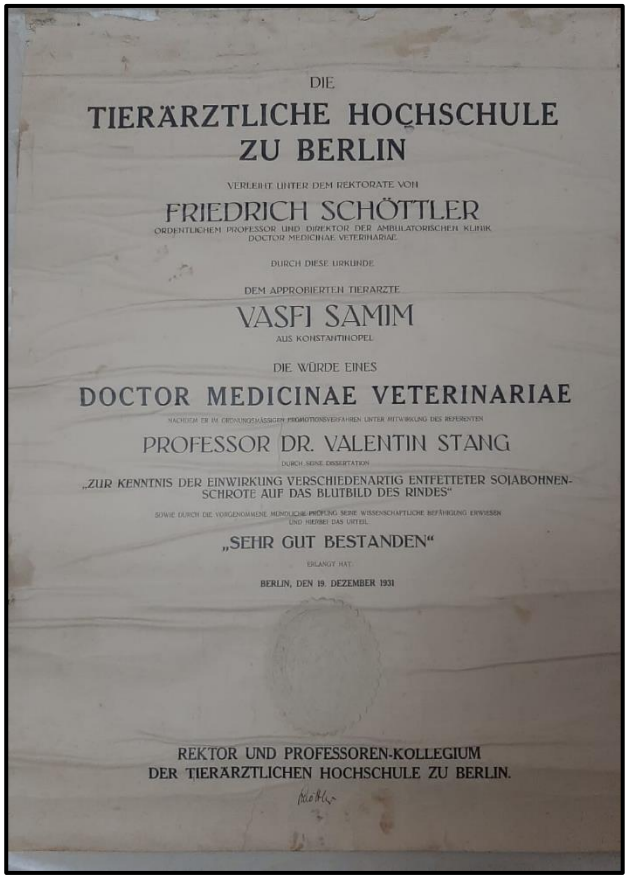
DIE
TIERÄRZTLICHE HOCHSCHULE
ZU BERLIN
VERLEIHT UNTER DEM REKTORATE VON
FRIEDRICH SCHÖTTLER
ORDENTLICHEM PROFESSOR UND DIREKTOR DER AMBULATORISCHEN KLINIK
DOCTOR MEDICINAE VETERINARIAE
DURCH DIESE URKUNDE
DEM APPROBIERTEN TIERARZTE
VASFI SAMIM
AUS KONSTANTINOPEL
DIE WÜRDE EINES
DOCTOR MEDICINAE VETERINARIAE
NACHDEM ER IN ORDNUNGSMÄSSIGEN PROMOTIONSVERFAHREN UNTER WÜRDIGUNG DES REFERENTEN
PROFESSOR DR. VALENTIN STANG
DURCH SEINE DISSERTATION
„ZUR KENNTNIS DER EINWIRKUNG VERSCHIEDENARTIG ENTFETTETER SOJABOHNEN-SCHROTE AUF DAS BLUTBILD DES RINDES"
SOWIE DURCH DIE VORGENOMMENE MÜNDLICHE PRÜFUNG SEINE WISSENSCHAFTLICHE BEFÄHIGUNG ERWIESEN
UND HIERBEI DAS URTEIL
„SEHR GUT BESTANDEN"
ERLANGT HAT
BERLIN, DEN 19. DEZEMBER 1931
REKTOR UND PROFESSOREN-KOLLEGIUM
DER TIERÄRZTLICHEN HOCHSCHULE ZU BERLIN.

Figure 4. PhD diploma of Vasfi Samim (Courtesy of Genci Samim archive).

Dr. Vasfi Samim came to Tirana, Albania, again, in June 1932, after completing his doctorate education. He was appointed as the Head of Animal Science at the Ministry of Agriculture and Forestry on 6 July 1932 and as the Director of Veterinary Affairs on 20 September 1933 (11). During these duties, Dr. Vasfi Samim prepared reports in which he evaluated the situation, problems, and solutions of Albanian livestock. It organized competitions, meetings, and conferences to educate and raise awareness of farmers on the solution of the basic problems of veterinary medicine and zootechny. He published his impressions during his travels to Austria, Bulgaria, Denmark, France, Germany, Greece, Hungary, Italy, Malta, Romania, Türkiye, and former Yugoslavia in a series of articles, reports, and notes in various newspapers (8).

Dr. Vasfi Samim was appointed as the Chief Inspector of Herd Management at the Ministry of Agriculture and Forestry in 1941. He served as the Secretary General of the Ministry of Culture between 1943 and 1944 (1). He was awarded the St. Sava medal by the King of former Yugoslavia for his work in the field of veterinary medicine on December 28, 1936, and he was rewarded with the Order of Skanderbeg with the Royal Decree on April 19, 1942, in Albania (8).

After World War II, Dr. Vasfi Samim was arrested for a book he wrote (Mother Kosovo-The Real Albania) by the Albanian Communist Party's seizure of power (27). Because of this book, he was prosecuted as an *enemy of the people* and arrested on 9 December 1944. Due to the political crime, his doctoral title was taken away by the government and he was appointed as a zootechnician at the Agricultural Enterprise in Sukth. He worked as a zootechnician at the Agricultural Enterprise in Sukth (1946-1949) and the Animal Science Center in Shkodër (1949-1956) (11). In the next period, he worked as a lecturer at Kamza Agricultural Institute, which is the only agricultural institute in Albania, for 15 years from November 1, 1959 to December 1974 (26).

Thanks to Dr. Vasfi Samim, a number of achievements have been completed, including the restructuring of the Ministry of Agriculture and Forestry, the establishment and reorganization of the veterinary (Higher Veterinary Council), and zootechnical organizations. Dr. Vasfi Samim, who can also speak German, Russian and Turkish languages, has published many scientific papers (Table 1) and books (Table 2) in the field of veterinary sciences. Dr. Vasfi Samim's veterinary services include training veterinary technicians, controlling and eradicating communicable animal diseases (foot and mouth disease, etc.), sending thousands of experts in the field of herd management and zootechny abroad, crossing local breeds, opening and rebuilding slaughterhouses, establishing veterinary dispensaries, organization of veterinary services in Kosovo, measures to protect public health, and the creation of legislation for the export of dairy and poultry products are included in. Dr. Vasfi Samim continued all these professional and academic achievement until his retirement on 31 December 1974 (8).

Table 1. Major manuscripts by Vasfi Samim (8).

Year	Title of the article	Title of the journal
1933	A study on the Infectious Diseases of Albanian Animals	Unknown
1933	The breeding and distribution of Angora goats in Turkey	Unknown
1936	Cross-breeding of Hungarian Rambuillet and Albanian Sheep	Unknown
1937	Skutariner Schafrasse in Nordalbanian	Tierzu Zuechbio
1937	Sheep milk production and sheep cheese - export from Albania	Unknown
1939	Overview of the current state of Albanian cattle breeding	Unknown
1940	Veterinary essay 1928-1938	Unknown
1940	A short Observation on the Zootechnology Activity in Albania during 1928-1938	Unknown
1940	Crossbreeding Greek Italian Sardo-Arab stallions and Albanian native mares	Unknown
1940	The improvement of Albanian horses	Unknown
1943	On Agricultural Education and Restoration of the Farmers	Shkolla Kombëtare
1950	Domestic Meat Production	Bulletin of Natural Sciences
1951	Industrial Value of Domestic Wool	Unknown
1952	Wool Quality and its Industrial Value	Unknown
1952	The Importance of Cow in the Betterment of the breeds	Unknown
1954	How can we Reduce the Death of Calves?	Unknown
1954	The Importance of Fodder in Tirana	Unknown
1955	My Work on Raising 125 Calves with One Loss	Unknown
1955	The Betterment of Domestic Cattle and The Results Obtained During 1948-1955	Bulletin Natural Sciences
1955	The Main Results on the Betterment of Domestic Cattle	Unknown
1955	The Proper Age of Breeding Domestic Lambs	Bulletin of Natural Sciences
1955	The Dynamics of Development among Domestic Calves	Unknown
1955	The Utility of Feeding Corn to Cows	Unknown
1955	Teeth Development in Domestic Cattle	Unknown
1957	Physical Development of Domestic Cattle	Bulletin of Natural Sciences
1957	How Can We Raise Calves with Reduced Milk Portions	Bulletin of Natural Sciences
1959	Milk Production from the Cows of Shkodra	Bulletin of Natural Sciences

Table 2. Major books of Vasfi Samim (8, 26).

Year	Title of the book	Name of the publisher
1936	The Important Issues of Herding and Recommendations for Improvement	Unknown
1937	Restoration of the Farmer	Unknown
1938	Raising Birds and Egg Production	Unknown
1940	Albanian Herding	Unknown
1940	Ankara Goats in Albania	Unknown
1943	Nena Kosove-Shqiperija E Vertete	Unknown
1955	Silo and Fodder	Unknown
1955	Milk Production and Milking Techniques	Unknown
1956	Feed-An Important Tool for Expanding the Food Base	Unknown
1958	Raising Cattle Dr. Bilal Golemi	Unknown
2000	Evliya Çelebi Beratı 300 vjet me pare	Botimet Dita

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Theatre, Authorship, and Journalism Activities: Dr. Vasfi Samim also excelled at journalism, authorship, and theatre activities besides his veterinary courses in his early years of studentship in Istanbul. Although the modern theatre was not developed in Türkiye in these years, he got to know closely some theatre giants such as Muhsin Ertuğrul, Bedia Muvahhit and Behzat who were mentioned with sagas in the country. He has four important dramas, of which he also played in *Çizgiler ve Lekeler* (Dr. Vasfi Samim played as Selahattin Bey), *Dert Ortağı*, *Kırık Hayatlar* and *Yaşasın Krallık* (17). Dr. Vasfi Samim's dramas, scenarios, monologues, and memoirs (Table 3) are important due to his care about theatre from different aspects. He performed playwriting, acting, directing, and criticism. He attended a drama course directed by a well-known director Max Reinhardt while studying his masters in Germany and attended Berlin Theatre School (15). Dr. Vasfi Samim focused on some important social and cultural issues in his dramas. He focused on love, illness, poverty, desolation, death, women, and social life subjects and themes that come from glitches and some shortcomings in social life that caused depressions (17).

Table 3. Theatre works by Vasfi Samim (17).

Theatre works			
Dramas	**Scenarios**	**Monologues**	**Memoirs**
Çizgiler ve Lekeler	Yastığımın Romanı	Bizim Hayatımız	İlk Operam: Aida Operası
Dert Ortağı	Toprak Sesleri	Asılacak Adam	Emil Jannings
Yaşasın Krallık		İnşad ve Hitabet Muallimi	İstintak
Kırık Hayatlar		İsyan	Kraliçe Elizabet
		Hiç	Büyük Jannings Sahnede
		Piç	Bir Hatıra
			Usanç
			Danton
			Herr Held'in Ölümü
			Faust
			Aleksander Moissi İkinci Oyunda

Besides theatre, he wrote essays and stories. He published 16 stories and 43 essays in some magazines and newspapers in Türkiye, Germany, and Albania (15, 16) (Table 4). He started journalism in Istanbul while he is a high school student and he continued to write in newspapers until the end of his life when he returned to Albania. He worked as a reporter of Istanbul and Ankara in *Türk Yolu* (1926), *Edirne Postası* (1927) and *Sakarya* (1927) newspapers (26).

Dr. Vasfi Samim gathered eight articles involving notes from his trip to Kosovo in 1938-1943 years in a book called *Mother Kosovo-The Real Albania*. That book mentioned earlier themed literature, philosophy, culture, history, and social issues (25).

Table 4. Some literary works by Vasfi Samim (15, 16).

Essays	**Short Stories**
Sanatkârın Evi	Harcırah
Artistin Ölümü	Mukaddes Yalan
Kalp Yıldızları	Hatıra Defterimdeki Sır
Buselerin Seyahati	Gurbette İlk Arkadaş
Sahipsiz Şehir	İskelet
Küçük Kürek	İzmit Hakkında Tahassürler
Dağlar Kurdu	Oyuncak
Amur	Minatori Tüneli
Dilek	Teneke Palas
Kadavra	Kadıköy'ün Meşhur Kadını…
Rozafa'nın Kayalıkları	Kadıköy'ün Meşhur Hacı Annesi
Hapishane Mazgalları	Şair Fuat Efendi
Mezarın Üzerinde Bayrak	Annesini Parçalayan Vahşi
Yastık	Öğrenci Evinde
Fotoğrafın Gözü	Hayattan Hikâyeler
Toprak	Sofyalı Derviş Saliha Abla
İstanbul'dan Ayrılırken	
Hatıra Defterimden Bir Tahassüs Sayfası	
Köyde İlk Aşk Hissi	
Baytarlar Nedir ve Kimdir?	
Gurbet	
Mesut Ümit	
Sükût	
Veda	
Bizim Hayatımız	
Sahnenin Şairi – 1	
Sahnenin Şairi – 2	
Kütüphanelere En Çok Kimler Devam Eder ve En Ziyade Hangi Eserler Okunur	
Türk Gençliğinin Tipi Hangisidir	
Miguen'den; Hayatı ve Eserleri	
Öldüren Güzellik	
Küçük Zeynel	
Luli	
Kömür İstiyor Musunuz?	
Mısır Efsanesi	
Memnu Elma	
Kirazlar	
Krizin Hikâyesi	
Cadılar ve Cinler	
Kilisede Gölgeler	
Allah Versin	
Başsız Tanrılar	
Bir Mecmuanın Programı	

Footballer, Coach, Organizer, and Sports Journalist: Dr. Vasfi Samim started his sports activities when he was a high school student in Türkiye. Between 1927 and 1928, he worked as a goalkeeper in Fenerbahçe, one of Türkiye's deep-rooted sports clubs (4), with registration number 1307 (26) (Figure 5).

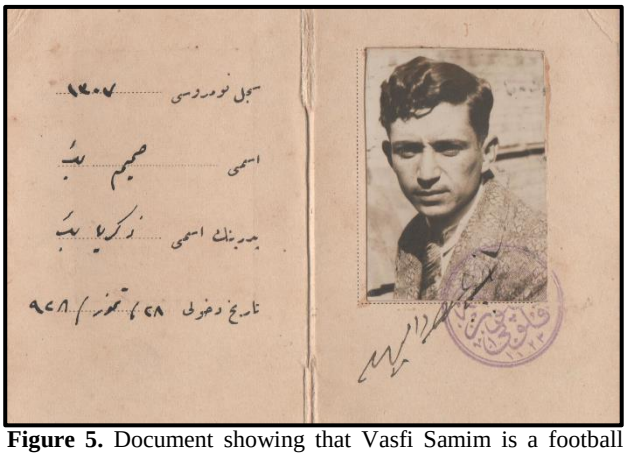

Figure 5. Document showing that Vasfi Samim is a football player in Fenerbahçe Sports Club (Courtesy of Genci Samim archive).

Dr. Vasfi Samim continued to play football as a goalkeeper in the Vlora Football Club in Albania. In 1933, he played as a goalkeeper in the Albanian Capital team Sport Club Tirana and was the goalkeeper coach of this team for a while (28, 32).

Dr. Vasfi Samim was appointed by the Ministry of Education as the chairman (1933-1936) of the Albanian Technical Committee, which was responsible for the activities of Albanian football clubs. He contributed to the establishment and chaired the Albanian Sports Federation called *Vllaznia Shqiptare* (Albanian Brotherhood). As a result of Dr. Vasfi Samim's work, national sports events (football, cycling, athletics and swimming championships) were organized for the first time in Albania. Albanian teams participated in the Olimpic Games in 1936 held in Berlin and Zagreb, and Albania became a member of two International Federations (Fédération Internationale de Football Association-FIFA, and International Association of Athletics Federations-IAAF) (8).

Discussion and Conclusion

It was envisaged to establish a Veterinary Surgery Hospital (*Baytar Ameliyat Hastanesi*) in 1873 for veterinarians who graduated from the Military Veterinary School in Türkiye to receive practical training for one year (9). In this process, this practice training continued and this school was united under the name of Military Practice Veterinary School and Hospital (*Askeri Tatbikat-ı Baytariye Mektebi ve Seririyatı*) (14, 18). The staff of the Military Practice Veterinary School, which was restructured with the proclamation of the Republic of Türkiye, was expanded and civilian veterinarians started to do their military service in this school (18). In this context, the fact that the course instructor and Ministry of Education approval information are included in the diploma (Figure 3), which shows that Dr. Vasfi Samim graduated as an intern from Military Practice Veterinary School in 1929, can be considered a historical document that sets an example for the information given by Melikoğlu and Osmanoğlu (18).

With the establishment of the civilian veterinary school by the Ottoman Empire, a teaching staff consisting of veterinarians trained in the light of developing modern science in Europe in the 19th century was needed. It is reported that most of the veterinarians, who were sent abroad, especially to France and Germany, which started in the Ottoman Empire and continued in the Republic of Türkiye, to meet this need, provided important services in the education, training and organization of veterinary medicine when they returned to Türkiye. On the other hand, it is seen that the attitude of the Ottoman Empire regarding the non-discrimination of language, religion and race in sending students abroad has not changed in the field of veterinary medicine (22). In addition, foreign citizens of the Ottoman Empire such as Nikolaki (Mavroğlu), Takfor, Samoel (Aysoy), Santor, Armenak, and Yorgi were sent to Europe for specialist training and were appointed to the staff of civilian and military veterinary schools after returning to Türkiye. Thus, they played a major role in the teaching and development of Turkish veterinary medicine (22). One of the veterinarians sent abroad in this process is Albanian national Dr. Bilal Golem, who invited Dr. Vasfi Samim to Albania and encouraged him to receive doctorate education in Germany. Dr. Golem, with the world-renowned French microbiologist Prof. Gaston Ramon at the Pasteur Institute, has discovered a serum for the complete detoxification of dysentery toxin applied in the veterinary medicine world (8, 31). In this context, considering the scientific studies of Dr. Golem and Dr. Vasfi Samim and their success in the development and organization of Albanian veterinary medicine, it can be said that the Turkish Government's policy of sending students abroad, regardless of their ethnicity and belief, is a correct decision and practice.

Dr. Vasfi Samim is a versatile writer who has been closely interested in more than one variety of literature, in addition to his features such as writing, acting and directing in the field of theater (Table 3), which started when he was a student in Istanbul. The profession of veterinary medicine, which required Dr. Vasfi Samim to be in constant contact with the public, helped him get to

know the outside world. The obstacles he faced when he first started his profession was also reflected in his stories. The places that Dr. Vasfi Samim visited due to his veterinary services, the people he encountered, and the distressing and dramatic state of the people made him get to know the people better. In the works written by Dr. Vasfi Samim, traces of the philosophy of some famous writers, from Turkish national literature to world literature can be seen. These are valuable in that they show Dr. Vasfi Samim's inclination and success in the world of literature at a younger age (15, 16). Furthermore, Hayber (17) reported that Dr. Vasfi Samim focuses on some important social and cultural issues in his dramas. As it will be understood from the years that the dramas had been written, and a chaotic environment is encountered from experiencing some social changes. It can be argued that the main dynamic that led to the emergence of these works in the personality of Dr. Vasfi Samim was the realist emotion, thought and scientific point of view, which was inspired by his veterinary medicine profession.

It is possible to get to know the scientific thought and development lines of important people who have served in veterinary medicine with their biography studies, the social and cultural structure of the period they lived in, and the effects of these elements on individuals (29). Dr. Vasfi Samim's reports, works, and articles on veterinary and zootechny science, literature, arts, and sports might be an invaluable legacy for the Albanian history of science, art, and sports. In this article, it was concluded that Dr. Vasfi Samim attracted great attention with his duties and responsibilities in administrative, social, and cultural positions, as well as his contributions to veterinary medicine in a general and the development of the zootechny discipline in particular. He was educated in Türkiye and after returning to Albania he was a scientist who contributed to veterinary sciences and the development process of science in general. He should be remembered as a successful veterinarian, and intellectual man for the region he was grown and for the world. Thereof, it is worth integrating Dr. Vasfi Samim's fame into the veterinary literature.

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The authors thanks Genci Samim, son of the late Dr. Vasfi Samim, for critically proofreading the manuscript. This article is dedicated to the cherished memory of Batuhan Şahin, whom we lost in the Kahramanmaraş earthquake on 06 February 2023.

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Conflicting Interests

The authors declared that there is no conflict of interest.

Author Contributions

ÇÇS designed the study. ÇÇS and BŞ contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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Ministry regulations on specialization training in veterinary medicine in Türkiye

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ABSTRACT

The first practices associated with specialization training in veterinary medicine in Türkiye were initiated in the field of military veterinary medicine in the 1880s. The first civilian veterinarians were sent abroad for specialization training in 1909. In the Republican era, specialization training in civil veterinary medicine was carried out in a planned and programmed system by the seven basic regulations issued by the Ministry of Agriculture from 1942 to the 1980s. In 1982, all graduate studies in the field of health sciences were included in graduate schools of health sciences, meanwhile, the Specialization High School, the main institution that trains specialists for civil veterinary services, was closed, thus specialization education in this field was terminated. In the following years, the legal regulations prepared for specialization training in civil veterinary medicine could not be put into effect, thus specialists could not be trained for about 40 years. In 2018, a new regulation was put into effect for specialization training in civil veterinary medicine. However, despite the end of the three-year transition period, the shortage of specialists in the field still continues, since education has not yet started. On the other hand, some details in the new regulation do not fully coincide with international practices. There is no doubt that specialization training in veterinary medicine in Türkiye should be carried out in line with national requirements; but it should also be of universal standards.

Introduction

The first examples of specialization training in veterinary medicine in Türkiye were initiated under the umbrella of the military veterinary organization; an operation hospital was opened in 1881 for the graduates of the Military Veterinary School to do practical training. In this institution, which was transformed into a school (*Baytar Ameliyat Mektebi*) in 1883 and renamed *Askeri Tatbikat-ı Baytariye Mektebi* in 1909; graduates were provided with internships for one year (23). In 1909, six civilian veterinarians were sent to Germany and France for specialization training for the first time; in the following years, a limited number of veterinarians continued to be sent abroad (24). Following the foundation of the Turkish Republic, *Askeri Tatbikat-ı Baytariye Mektebi* was restructured under the name of *Askeri Tatbikat-ı Baytariye Mektebi ve Seririyatı*. One of the founding purposes of this institution was determined as to train specialists as much as the army needed with two years of education and training period in the specialization branches (*internal medicine, infectious diseases, and bacteriology, external medicine and operation, podiatry and horseshoe technique, pathology and meat control, chemistry, sanitation, and anatomy*) of the school (1, 10). Thus, in this school, the name of which was changed respectively as *Askeri Veteriner Akademisi* (1949), *Askeri Veteriner Okulu* (1969), *Askeri Veteriner Araştırma Enstitüsü ve Eğitim Merkezi Komutanlığı* (1978) and *Askeri Veteriner Okulu ve Eğitim Merkezi Komutanlığı* (1992), specialist staff was educated for military veterinary organization. Specialists for military veterinary services were also trained in *Millî Savunma Bakanlığı Veteriner Bakteriyoloji ve Serum Aşı Evi*, which was established in 1922 and has continued its activities (2, 14, 15).

In the Republican period of Türkiye, specialization training in the field of civil veterinary medicine was

provided by the Ministry of Agriculture in cooperation with the Veterinary Faculty[1] (Faculties after 1970) and institutions/organizations affiliated with the Ministry of Agriculture by the regulations enacted in various dates. Since the second half of the 1970s, specialization training had been carried out mainly at the Specialization High School *(Ankara Üniversitesi Veteriner Fakültesi Hayvan Yetiştiriciliği ve Sağlık Bilimleri Uzmanlık Yüksek Okulu)*, which was established for this purpose at the Ankara University Faculty of Veterinary Medicine (13).

Dinçer (17) states that in the first 50 years of the Republic, 272 veterinarians completed their specialization training in institutions affiliated to the Ministry, in cooperation with the veterinary faculties. He also reported that 161 veterinary specialists graduated from the Specialization High School. Therefore, a total of 433 specialization studies were carried out in civil veterinary medicine until 1982.

This study aims to examine the Ministry regulations on the education of veterinary specialization in Türkiye in a historical framework and to evaluate discussions on the latest regulation published in the Official Newspaper in 2018. Thus, from one hand, an important contribution will be made to the existing literature on the history of veterinary medicine, on the other hand, the veterinary specialization training, which is envisaged to be restarted in near future will be better understood and monitored correctly.

Materials and Methods

The first-hand sources of the research were eight regulations issued by the Ministry of Agriculture and archived documents of the Department of Veterinary History and Deontology, the Faculty of Veterinary Medicine of Ankara University. In addition, the main sources of the history of veterinary medicine, the second-hand sources reached by scanning the keywords of the article were also used. As is seen in the article, eight basic regulations on the subject were examined. The regulation with a stay of execution decision, the regulations amending some articles of the basic regulations and the relevant legislation of other institutions and organizations were excluded. In the Results section, the data obtained through document analysis were presented in the tables. In the Discussion and Conclusion section, the findings are discussed and evaluated by referring to the relevant literature. Tags of the documents and additional explanatory information have shown in the footnotes. The tags of the Official Newspapers are given in the footnotes where they are first used in the text, and the relevant footnotes are referred to in subsequent uses.

Results

As it is known, shortly after the proclamation of the Turkish Republic, the Law numbered 3203[2] was put into effect and both the task of the Ministry of Agriculture was defined and the organizational structure of the central and provincial units of the Ministry was formed. The title of "*specialist*" was included for the first time in the "provinces" section of the General Directorate of Veterinary Affairs.

Following this Law, *the Specialization Instruction and Practice Program for Veterinary Organization* (*Veteriner Teşkilâtı İhtisas Talimatnamesi ve Tatbikat Programı*[3]) was issued by the Ministry of Agriculture on the 6th of March, 1942. Thus, to be employed in the services specified in the Article 9 of the Law numbered 3203, civilian veterinarians were provided with the opportunity to receive specialization training in nine specialization branches that were taught in three specialization areas (*Clinic*, *Laboratory*, and *Zootechnics*), at the Veterinary Faculty of the Higher Agriculture Institute and certain institutions of the Ministry.

On the 18th of March, 1954 the Law numbered 6343[4], which has been the legal basis for the execution of the veterinary profession in Türkiye and provided the establishment of the Turkish Veterinary Medical Association was brought into force. The subject of specialization was clarified in the Article 7 of this Law as follows: "*in order to have the title of specialist - to have specialized training in one of the branches written in the related Directive, to pass the exam to be held in accordance with the regulation to be prepared by the Ministry of Agriculture and to obtain a specialization certificate - are obligatory.*" Following this Law, a total of eight basic regulations were enacted, seven of which regulated specialization training in civilian veterinary medicine and one of which could not be implemented. All regulations, enacted and organizing specialization training in Türkiye, and the regulations amending some articles of these basic regulations are shown in Table 1; the educational principles of the basic regulations are given in Table 2, and the specialization areas and branches are presented in Table 3.

In 1968, a Specialization High School for Animal Breeding and Health Sciences at the Ankara University Faculty of Veterinary Medicine (*Ankara Üniversitesi Veteriner Fakültesi Hayvan Yetiştiriciliği ve Sağlık Bilimleri Uzmanlık Yüksek Okulu*), which was decided to

[1] Faculty of Veterinary Medicine of the Higher Agricultural Institute until 1948; Ankara University Faculty of Veterinary Medicine since 1948.
[2] Official Newspaper, Nr: 3630, June 14, 1937.
[3] Ziraat Vekâleti Veteriner Umum Müdürlüğü. Umumî Sayı:536, Veteriner Serisi:15. This regulation and its implementation program was later published in the Official Newspaper, Nr: 7307, September 15,1949.
[4] Official Newspaper, Nr: 8661, March 18, 1954.

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be established with the decision of the Senate in accordance with Article 2 of the Law No. 4936[5], begun its operations[6]. Thus, specialized training in civil veterinary medicine started to be carried out in close cooperation between this School and the Ministry. The school was also allowed to provide Ph.D. education within the framework of the "Doctoral Regulation" of the Veterinary Faculty of Ankara University (16).

Considering the Regulation[7] on teaching and examination principles of the Specialization High School, in the fifth Specialization Regulation (*Veteriner Hekimliği Uzmanlık Yönetmeliği*[8]) issued by the Ministry of Agriculture in 1968, the specialization areas were directly included without three main specialization branches specified in the previous regulations.

In the Regulation (*Veteriner İşleri Genel Müdürlüğü Veteriner Hekimliği Uzmanlık Yönetmeliği*[9]) issued on January 8, 1975, "*specialist veterinarian*" was defined for the first time as "*the veterinarian who adopted the foreseen qualifications of this regulation, successfully completed the training period and candidacy exam, gained proficiency in the final science exam, showed competence and merit to work in the field and received a diploma."*

Due to the advantages provided by the Universities Law numbered 1750[10], the Specialization High School became the leading institution in the 1970s. But in 1982, with a decree-law[11], issued for the organization of higher education institutions, the Specialization High School was closed and the specialization training in the field of veterinary medicine was terminated. With the enactment of the Higher Education Law[12] a new era began in higher education. In the meantime, the graduate studies in the field of health sciences including veterinary medicine were left to graduate schools of health sciences established within universities.

Table 1. Regulations on Veterinary Specialization.

Year	Regulations	Sources
1942	Veteriner Teşkilâtı İhtisas Talimatnamesi ve Tatbikat Programı	General Directorate of Veterinary Medicine, Ministry of Agriculture, Issue Nr: 536, Veterinary Series Nr: 15 (Official Newspaper Nr: 7307, 15th September 1949)
1955	Veteriner Hekim İhtisas Talimatnamesi	Official Newspaper Nr: 9018, 2nd June 1955
1958	Veteriner Hekim İhtisas Talimatnamesi	Official Newspaper Nr: 9897, 3rd May 1958
1963	Veteriner Hekimliği Uzmanlık Yönetmeliği	Official Newspaper Nr:11562, 22nd November 1963
1966	22 Kasını 1963 gün ve 11562 sayılı Resmî Gazete'de Yayınlanan 13/11/1963 gün ve 2338 sayılı Veteriner Hekimliği Uzmanlık Yönetmeliğinin 2nci Maddesinin b, c ve ç Fıkralariyle 13üncü Maddesinin Değiştirilmesine Dair Yönetmelik	Official Newspaper Nr:12286, 29th April 1966
1968	Veteriner Hekimliği Uzmanlık Yönetmeliği	Official Newspaper Nr: 12898, 14th May 1968
1969	Yürürlükteki Veteriner Hekimliği Uzmanlık Yönetmeliğine bir Teknik Uzmanlık Dalı'nın İlâve Edilmesi Hakkında Yönetmelik	Official Neswspaper Nr: 13147, 13th March 1969
1970	Veteriner Hekimliği Uzmanlık Yönetmeliği	Official Newspaper Nr: 13654, 1st November 1970
1975	Veteriner İşleri Genel Müdürlüğü Veteriner Hekimliği Uzmanlık Yönetmeliği	Official Newspaper Nr: 15112, 8th January 1975
1975	Veteriner Hekimliği Uzmanlık Yönetmeliğinin 12. Maddesini Değiştiren Yönetmelik	Official Newspaper Nr: 15427, 29th November 1975
1979	Veteriner Hekimliği Uzmanlık Yönetmeliğinin 12. Maddesini Değiştiren Yönetmeliğin Değiştirilmesine Dair Yönetmelik	Official Newspaper Nr: 16579, 15th March 1979
1995*	Tarım ve Köyişleri Bakanlığı Veteriner Hekimliği Uzmanlık Yönetmeliği	Official Newspaper Nr: 22362, 2nd August 1995
2018	Veteriner Hekimliğinde Uzmanlık Eğitimi Yönetmeliği	Official Newspaper Nr: 30409, 2nd May 2018

*This Regulation was not put into effect because of a stay of execution decision.

[5] Official Newspaper, Nr: 6336, June 18, 1946.
[6] The Decision of the Senate of Ankara University, Nr: 2919/Date: May 16, 1965. The School became operational with the Law numbered 1030, Official Newspaper, Nr: 12852, March 19, 1968.
[7] Official Newspaper, Nr: 12340, July 5, 1966.
[8] Official Newspaper Nr: 12898, May 14, 1968.
[9] Official Newspaper, Nr: 15112, January 8, 1975.
[10] Official Newspaper, Nr: 14587, July 7, 1973.
[11] Official Newspaper, Nr: 17760, July 20, 1982.
[12] Official Newspaper, Nr: 17506, November 6, 1981.

Table 2. Training Fundamentals of Veterinary Specialization.

Regulations[1]	Training Facilities	Entrance Exam	Number of Main Areas& Branches	Training Period	Graduation Thesis/Exam	Title
1942	Veterinary Faculty Bacteriology Institutes Stud Farms	+	3 & 9	min. 3 years	- /+	Level 1 Specialist Level 2 Specialist
1955	Veterinary Faculty Bacteriology Institutes Stud Farms Schools of Animal Health Officers	+	3 & 18	3 years	- /+	Specialist
1958	Not specified	+	3 & 19	2 years	- /+	Specialist
1963	Veterinary Faculty Schools of Animal Health Officers Bacteriology Institutes Livestock Institutions of the Ministry of Agriculture	+	3 & 19	3 years	- /+	Specialist
1968	Specialization High School for Animal Breeding and Health Sciences-Faculty of Veterinary Medicine, Ankara University Institutions affiliated to the General Directorate of Veterinary Affairs of the Ministry of Agriculture	+	14	3 years	+/+	Specialist
1970	Specialization High School for Animal Breeding and Health Sciences-Faculty of Veterinary Medicine, Ankara University Institutions affiliated to the General Directorate of Veterinary Affairs of the Ministry of Agriculture Military Veterinary Institutions	+	15	3 years	+/+	Specialist
1975	Specialization High School for Animal Breeding and Health Sciences- Faculty of Veterinary Medicine, Ankara University Others: Not specified	+	20	3 years	+/+	Specialized Veterinarian
2018	Authorized Higher Education Institutions and Other Institutes	+	26	4 years	+/+	Specialist

[1]: The Regulations were indicated by the years in which they were enacted. Due to the decision to stay the execution, the Regulation issued in 1995 was not included in the Table.

Table 3. The Specialization Branches According to the Regulations*.

Branches	Regulations**							
	1942	1955	1958	1963	1968	1970	1975	2018
Internal Medicine[1]	+	+	+	+	+	+	+	+
Surgery[2]	+	+	+	+	+	+	+	+
Foot Diseases		+						
Traumatology and Orthopedics							+	
Obstetrics and Gynecology[3]						+	+	+
Bacteriology[4]	+	+	+	+	+	+	+	+
Virology and Epidemics[5]						+	+	+
Parasitology[6]		+	+	+	+	+	+	+
Food Control[7]	+	+	+	+	+	+	+	+
Pathology[8]		+	+	+	+	+	+	+
Biochemistry[9]		+	+	+	+	+	+	+
X-ray and Physiotherapy[10]		+	+	+				
Pharmacology and Toxicology[11]				+	+	+	+	+
Fisheries and Diseases[12]				+	+	+	+	+
Zootechnics								+
Horse Breeding and Diseases[13, 14]	+	+	+	+	+	+	+	+
Cattle Breeding and Diseases[13]	+	+	+	+	+	+	+	
Small Ruminant Breeding and Diseases[13, 15]	+	+	+	+	+	+	+	
Poultry Breeding and Diseases [13, 16]	+	+	+	+	+	+	+	+
Farm Animals and Diseases								+
Pet Diseases								+
Fur Animal Breeding[17]	+						+	
Pig and Geological Garden, Wild, Fur and Laboratory Animal Breeding and Diseases[18]		+	+	+	+			+
Wool-Mohair-Feather and Hair [19]			+			+	+	
Beekeeping and Diseases							+	+
Herd Health and Management								+
Genetic		+	+					+
Hygiene and Animal Nutrition[20]		+	+	+				+
Reproductive Biology and Artificial Insemination[21]		+	+	+	+	+	+	+
Livestock Economics[22]		+	+	+	+	+	+	+
Animal Nutrition and Nutritional Diseases[23]			+	+	+	+	+	+
Dairying						+		
Milk Inspection-Control and Technology							+	
Biostatistics								+

*Changes in the titles of branches were shown in the footnotes, ** The Regulations were indicated by the years in which they were enacted. Due to the decision to stay the execution, the Regulation issued in 1995 was not included in the Table. [1] 2018: Veterinary Internal Medicine; [2] 1955: Surgery and Obstetrics, 1958: Surgery and Foot Diseases-Obstetrics, 1963: Surgery-Obstetrics-Orthopedics, 2018: Veterinary Surgery; [3] 2018: Veterinary Obstetrics and Gynecology, [4] 1955/1958/1963/1968: Bacteriology and Epidemic Diseases, 1968/1970: Bacteriology and Epidemics, 1975: acteriology-Mycology and Epidemics, 2018: Veterinary Microbiology; [5] 1975: Virology, 2018: Veterinary Virology; [6] 1958/1963/1968/1970/1975: Parasitology and Parasitic Diseases, 2018: Veterinary Parasitology; [7]1963: Food Control-Analysis and Technology, 1968: Foot Control and Preventive Medicine, 1970/1975: Food Control and Technology, 2018: Food Hygiene and Technology; [8] 2018: Veterinary Pathology; [9] 2018: Veterinary Biochemistry; [10] 1963: Radiology and Radiobiology; [11] 2018: Veterinary Pharmacology and Toxicology; [12] 1968/1970: Fisheries; [13]1942: Horse Breeding, Cattle Breeding, Small Ruminant Breeding, Poultry Breeding; [14] 2018: Horse Diseases; [15] 1963/1975: Sheep and Angora Goat Breeding and Diseases; 1968/1970: Sheep Breeding and Diseases; [16] 2018: Poultry Diseases; [17] 1975: Rabbit and Fur Animal Breeding and Diseases; [18] 1958: Fur and Laboratory Animals-Zoology and Wild Animals and Pig Breeding, 1963: Fur and Laboratory Animals-Pig and Wild Animal Breeding and Diseases, 1968: Leatherwork-Fur and Laboratory Animals, 2018: Wild Animal Diseases and Laboratory Animal Diseases took place as two separate branches in this Regulation; [19] 1968: Wool and Mohair, 1970: Wool-Mohair and Leatherwork, 1975: Wool-Mohair-Leather Inspection-Control and Technology; [20] 1958: Hygiene, 1963: Hygiene-Preventive Veterinary Medicine and Public Training, 2018: Veterinary Public Health; [21] 1958: Reproductive Biology-Pathology and Artificial Insemination, 1963: Genetic-Artificial Insemination-Reproductive Biology, 1968/1970: Genetic and Reproductive Biology, 1975: Artificial Insemination- Genetics and Reproductive Biology, 2018: Reproduction and Artificial Insemination; [22] 1968: Livestock Economics and Statistics, 1975: Marketing and Livestock Economics, 2018: Livestock Health Economics and Management; [23] 1963/2018: Animal Nutrition and Nutritional Diseases, 1968/1970: Feedstuffs and Animal Nutrition, 1975: Animal Feedstuffs-Nutrition and Nutritional Diseases.

In 1995, a new Regulation (*Tarım ve Köyişleri Bakanlığı Veteriner Hekimliği Uzmanlık Yönetmeliği*[13]) was issued for specialization training in veterinary medicine. However, this Regulation was suspended due to the lawsuit filed because it only organized the specialized training of the Ministry personnel and therefore it violated the Law numbered 6343 (3, 4).

After nearly twenty years, the Law numbered 6569[14] was enacted in 2014, and specialization training in veterinary medicine was included in graduate education along with specialization training in the fields of medicine, dentistry, and pharmacy. In this Regulation, "specialization in veterinary medicine" was defined as "*a higher education conducted in accordance with the principles regulated by the Ministry of Food, Agriculture and Livestock and aiming to provide veterinarians with special skills and authority in certain field*s". In addition, with the arrangements in the relevant article (50 (1) a) of the Law numbered 2547[15], the opportunity for specialization training in civil veterinary medicine was provided. Upon these developments, a draft regulation was prepared by the Ministry of Food, Agriculture and Livestock, sent to the Central Council of the Turkish Veterinary Medical Association, and submitted to the opinions of interested parties such as veterinary chambers, professional organizations, and institutes via the official website of the Central Council (6, 7). As a result of these efforts, the Regulation on specialization training in veterinary medicine (*Veteriner Hekimliğinde Uzmanlık Eğitimi Yönetmeliği*[16]) was published in the Official Newspaper in May 2018. Thus, the general principles of specialization education have been determined. Accordingly, both higher education institutions and other institutes with a sufficient number of trainers authorized by the Specialization Board would be able to provide specialization training.

With this Regulation, the Specialization Board has been assigned with issues such as specialization institutions and branches, student quotas, application conditions and criteria for specialization examination, evaluation principles of training, the appointment of supervisor, principles related to specialization thesis, and final examination. The "Field Commission", which consists of academic members and/or specialist veterinarians for the specialization branches, has been authorized to carry out studies on the specified subjects and submit them to the approval of the Specialization Board. The "Academic Commission", which will be chaired by the authorized person of the institute and will be composed of trainers in the relevant program and at least one specialization student, has been given the task of creating the core and extended training curricula for specialization education.

In the Regulation, the requirements for specialist candidates are determined as follows: "*To graduate from a veterinary faculty in Türkiye or to have a graduation document obtained from veterinary faculties in other countries and to get its equivalency, approved by the Council of Higher Education, to have the requirements of being a veterinarian as specified in the Law numbered 6343, to get at least 50 out of 100 points on foreign language exam (in English, French or German languages), of which validity period of 5 years has not expired as of the date of application to the veterinary specialization exam or to have an international document, of which equivalence is recognized by the Assessment Selection and Placement Center (OSYM).*" The OSYM has been tasked with conducting this exam and placing the specialist candidates in one of the five choices they have made regarding the scores they get from the exam. As it is seen in Table 3, in the Regulation, 26 specialization branches (*Veterinary Surgery, Veterinary Microbiology, Veterinary Internal Medicine, Veterinary Obstetrics and Gynecology, Zootechnics, Veterinary Biochemistry, Veterinary Pharmacology and Toxicology, Veterinary Parasitology, Veterinary Virology, Veterinary Pathology, Reproduction and Artificial Insemination, Food Hygiene and Technology, Animal Nutrition and Nutritional Diseases, Poultry Diseases, Beekeeping and Diseases, Fisheries and Diseases, Laboratory Animals and Diseases, Herd Health and Management, Veterinary Public Health, Farm Animals and Diseases, Wild Animals and Diseases, Horse Diseases, Pet Diseases, Biostatistics, Genetic, Livestock Health Economics, and Management*), each of which has four years training period, are included. The training period can not exceed six years in total, including the registration suspends made with the approval of the board.

In order to complete the specialization training, completion of the training period and rotations[17] of the specialization branch, preparation of the thesis under the supervision of the tutor, acceptance of the thesis by a jury consisting of at least three principal and two substitute members and passing the specialization training exam, which has two stages, include professional knowledge and practical skills are necessary.

[13] Official Newspaper, Nr: 22362, August 2, 1995.
[14] Official Newspaper, Nr: 29187, November 26, 2014.
[15] For the current Law text, please see: https://www.mevzuat.gov.tr/MevzuatMetin/1.5.2547.pdf
[16] Official Newspaper, Nr: 30409, May 2, 2018.
[17] When the compulsory rotation training periods determined by the Board are completed, whether the goals set by the trainers have been achieved or not is evaluated via the Veterinary Specialization Training Tracking System. Proficiency in rotation training is achieved if the objectives are achieved, otherwise, the training is repeated.

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According to the Regulation; those who have completed their Ph.D. education in one of the fields determined as a specialization branch in veterinary medicine before 26th of November, 2014 may exceptionally be granted a specialization certificate by the Ministry upon their requests. As a matter of fact, by the relevant provision, specialization certificates have been started to be given to those who applied to the Ministry with the Authority's Approval dated 26th of November, 2020 (8). On the other hand, it was foreseen in the Regulation that both the specialization training tracking system was going to make operational and the specialization training was going to start within three years following the enforcement of the Regulation. But, although this three-year period has expired, specialization training in the field of civil veterinary medicine has not yet started.

Discussion and Conclusion

Although the prototype examples of specialization training in veterinary medicine in Türkiye were seen in the Ottoman State (14), contemporary specialization training was initiated in the Republic of Türkiye and its legal framework was established for the first time with the Regulation issued in 1942 (*See footnote 3*). It is important that specialization training in veterinary medicine was started shortly after the enactment of the Law numbered 3203 (*See footnote 2*). In this Law, in which all public veterinary services were given the responsibility of the General Directorate of Veterinary Affairs, the fact that specialists veterinarians would be appointed in various units of the Directorate should have revealed the necessity of training these staff as soon as possible. Thus, specialization training was started and specialist veterinarians were trained uninterruptedly until the 1980s. Beginning in 1942, the organization and execution of specialization training were carried out by the regulations (*See Table 1*) issued throughout the history of the Republic. All these regulations (*See Table 1, Table 2, and Table 3*), including the institutions and organizations where the specialization training would be taken place, the entrance exams and conditions, the fields and branches of specialization, the duration of the training, the final exams and other details; show that specialization training in veterinary medicine was carried out within a planned and programmed system.

In almost all regulations (enacted in 1942, 1955, 1963, 1968, 1970, 1975), the fact that Ankara University Faculty of Veterinary Medicine (before 1948, the Veterinary Faculty of the Higher Agriculture Institute) was always among the institutions that would provide specialist training (*See Table 2*) shows the pioneering and important role of this Faculty in specialization training in its field. The Specialization High School, which was established in 1965 within Ankara University Faculty of Veterinary Medicine (*See footnotes 5 and 6*) and is the only example in Türkiye in the field of health sciences, continued its educational activities with the infrastructure and manpower support of this Faculty.

In the 1980s, both with the reorganization of the Ministry of Agriculture and the transformations in higher education after the enactment of the Law numbered 2547 (*See footnote 12*), a major blow was dealt to veterinary medicine in Türkiye (12). In such a period when the Law numbered 3203 (*See footnote 2*) was repealed and the General Directorate of Veterinary Affairs was abolished, the Specialization High School was closed and the specialization training in the field of veterinary medicine was terminated (5). In these years, while graduate education in health sciences was transferred to the institutes of health sciences, specialization training in medicine was excluded from this approach and preserved its current status (11). A similar approach - unfortunately - could not be applied to specialization training in veterinary medicine and it is not possible to find a justification for this attempt. As a matter of fact, this situation was met with the objections of the relevant professional organizations and scientists (5). But these efforts did not bear fruit and draft regulations prepared for the resumption of this training could not be brought into effect. In such a process, the shortage of specialists was tried to be eliminated by those who completed the training programs organized periodically by the Ministry of Agriculture (5, 17, 21). However, this approach also led to discussions in practice, since the programs in question are not equivalent to specialization training (13).

After nearly about 40 years of interruption, in 2014, the new legal arrangement (*See footnotes 14 and 15*) for specialization training in veterinary medicine was implemented and in 2018, the Regulation on the conduct of training was issued. Despite these important developments, some discussions have come to the fore with this Regulation. For example, in the Regulation; those who have completed their Ph.D. education in one of the fields determined as a specialization branch in veterinary medicine before October 26, 2014, were allowed to be given specialization certificates upon their request and this decision was begun to accomplish. On the other hand, shortly after the new Regulation, with an amendment to the Law numbered 2547 (*See footnote 15*), like the degree of Ph.D., the title "*Specialist*" was deemed sufficient for the titles and position assignments of "*Assistant Professor*" and "*Associate Professor*". In the Ph.D. education which is organized by the Higher Education Institution and carried out by the Health Sciences Institutes, it is aimed to gain the right to work in an academic institute by obtaining the title of "doctor" as a result of in-depth academic studies in a field (20, 25).

However, in the specialization training to be carried out in cooperation with the Ministry and educational institutions, it is aimed to train competent who have the knowledge, skills, and attitudes for the needs of the field.[18] Therefore, it is not correct to consider these two pieces of training, which have different aims, functions, processes, and outputs, as equal. And it is anticipated that this situation may cause various problems in practice. The provision in the new Regulation that "*the specialization student cannot work in any job other than the conditions required by the specialization training*" constitutes an obstacle for veterinarians working in the private sector to get specialization training. So, in such a case, the fact that private veterinarians will have to take a break from their work to receive specialist training is another topic of discussion. The extent to which the needs of the field are taken into account in the determination of the branches of specialization in the new Regulation and how the specialization training is implemented should be discussed separately, with reference to this training in the world. It is known that the first specialization colleges of veterinary medicine in the world began to be established in the USA in the 1960s. Today, specialization training in veterinary medicine is carried out in 41 disciplines in 22 specialization institutions under the guidance of the American Board of Veterinary Specialties (ABVS) in the USA (9, 18). In Europe, it is conducted in 27 specialization institutions with more than 38 disciplines under the guidance of the European Board of Veterinary Specialization (EBVS) (18, 19, 22). In these two systems, the average training periods for specialization programs, which are organ, species or discipline-oriented, are three years (maximum six years). In the new system that is envisaged to be implemented in Türkiye, there are 26 specialization areas, each of which has 4-year training period, and these areas are not the same as the areas and branches in Europe and in the USA. This incompatibility between the areas and disciplines brings to mind the question of how a veterinarian trained abroad in a specialization discipline that has no equivalence in Türkiye, can get diploma equivalence. Similarly, it is not known whether this incompatibility will pose a problem for Turkish veterinary medicine, which is trying to catch up with the European Union standards in the field of veterinary medicine.

Finally, despite the completion of the three-year transition period envisaged for the initiation of training from the date of publication of the new Regulation, specialization training cannot be started yet. Considering the lack of specialists in the field of veterinary medicine in country that have emerged in nearly 40 years, this is also a problem.

In conclusion**,** specialization training in the field of veterinary medicine in Türkiye was carried out within a planned and programmed system in accordance with the regulations issued from 1942 to the 1980s. With the reorganization of the Ministry of Agriculture and the transformations in higher education in the 1980s, the General Directorate of Veterinary Affairs was abolished, the Specialization High School was closed and specialization training in veterinary medicine was terminated. Thus, specialists in this field could not be trained for about 40 years. In 2018, the new Regulation for specialization training was put into effect, however, although the three-year transition period for the start of training has expired, the specialization training has not yet started. Therefore, the lack of enough number of specialists in this field is still an ongoing problem. The issues in the new Regulation regarding the organization, execution and areas and disciplines of specialization training do not exactly coincide with international practices. There is no doubt that specialization training in the field of veterinary medicine in Türkiye should be carried out in line with national requirements; but it should also be of universal standards at the same time.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

Author Contributions

RTBG and ÖK conceived the ideas of the study. ÖK prepared the preliminary research. First hand sources were

[18] Official Newspaper, Nr: 29690, April 20, 2016.

found by ÖK and NY. The draft text was written by ÖK. The final text was written and translated to English by RTBG. The tables were prepared by RTBG and NY. References were inserted into the text and the list of References by NY. All authors have checked and approved the final version of the article. As the PhD tutor of the first and second authors and a senior academician, RTBG preferred to be the third author of the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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Geometric analysis of mandible using semilandmark in Hamdani and Awassi sheep

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ABSTRACT

The aim of this study is to determine whether or not the breed and sex factors have an effect on the shape in the mandibles of Hamdani and Awassi sheep. A total of 31 mandibles were used. The samples were analyzed via geometric morphometric methods by using semilandmark. In the study, it was determined that the first principal component accounted for 36.52% of the total shape difference. According to principal component analysis, samples were clustered significantly in terms of breed; whereas, they were not clustered in terms of sex. In terms of the first principal component, the places where the shape differences were concentrated were the attachment sites of teeth to the mandible, between the second molar and ramus mandibulae, the processus coronoideus and the angulus mandibulae. The Mandibulae of Hamdani sheep had a higher body than the mandible of Awassi sheep. The mandibulae of male sheep was more voluminous than the mandible of female sheep, especially in the body area. Consequently, it is thought that the data obtained as a result of the study would serve as a reference for the ruminant mandible remains obtained from archaeological excavations.

Introduction

Hamdani sheep are reared in wide geography including primarily Northern Iraq as well as Iran and Southeastern Türkiye (2). This sheep is a strain of Karadi sheep. Karadi sheep is the largest size of a local breed raised in Iraq. Hamdani sheep are distinguished by a fat tail, white and wide-body, long ears, black-brown head, and high legs from other sheep breeds (1). Awassi sheep is a sheep breed that is widely reared in Türkiye, Iraq, Israel, Jordan, and Syria (28). This fat-tailed sheep breed has a medium-sized body covered with matte white wool, a brown and narrow head and medium-sized floppy ears (1).

Shape analysis is performed by using the geometric morphometry (GM) method based on a statistical analysis of landmark(LM) coordinates (3, 19, 29). Landmarks are the biological homolog points among the samples. Many structures cannot be examined by using classical landmarks, as landmark locations along the fold or surface cannot be homologized according to the individuals. Semilandmarks (SLM) enable quantifying two- or three-dimensional homologous curves and surfaces and analyzing them together with traditional landmarks (9).

In recent years, numerous studies have been conducted to reveal the shape differences of cranium or mandible via the geometric morphometric method in different species such as wolf (11), dog (23), quail (30), turkey (10), sheep (6), goat (5), and deer (17). However, no study comparing two different sheep breeds via semilandmarks was encountered. Thus, the aim of this study is to determine whether or not the breed and sex factors have an effect on the shape of the mandible over the samples of Hamdani and Awassi sheep.

Materials and Methods

Samples: In the study, heads of a total of 31 sheep including 12 (6 female, 6 male) Hamdani sheep and 19 (9 female, 10 male) Awassi sheep were used. They were older than one year old. After the materials were collected from the slaughterhouses in Siirt and Şanlıurfa, they were boiled and macerated. Attention was paid to ensure that the materials used in the study did not have any pathological and clinical conditions in the tooth and bone tissue. This study was approved by the Harran University Animal Experiments Local Ethics Committee (28.03.2022/01-13).

Imaging and Digitization: Left mandibles were photographed laterally by using a camera (18x55 lens, Canon Eos, 600D, Japan) in order to keep the focus (first molar tooth) on the same plane (camera resolution 890x1065 pixels). The distance between the lens and the material was detected as 30 cm. Among the photographs in the format of JPG were recorded on the computer, 10 homolog landmarks (Figure 1) and 132 semilandmarks were marked by using TpsUtil (Version 1.79) (27) and TpsDig2 (Version 2.31) (25) program, respectively. Thus, the *x* and *y Cartesian* coordinates of the general shape of the mandible were considerably determined, together with the homologous anatomical points (14) and the short-distance points (semilandmarks) between these points. Before the statistical analysis, a verification test was done in TpsSmall (Version 1.34) (26) program for semilandmarks. Accordingly, uncentred correlation and root mean square error values were detected as 1.000000 and 0.000006, respectively. These results revealed the accuracy of the semilandmarks.

Landmarks: LM1=SLM132: Oral caudodorsal end point of alveoli dentales of I4, LM2=SLM14: Rostroventral edge of PM1, LM3=SLM21: Caudoventral edge of PM3, LM4=SLM32: Caudoventral edge of M3, LM5=SLM60: Dorsal edge of processus coronoideus, LM6=LM72: Medioventral point of incisura mandibulae, LM7=SLM78: Caudal end point of condylus mandibulae, LM8=SLM90: Caudoventral corner of angulus mandible, LM9=SLM96: Incisura vasorum facialium, LM10=SLM130: Aboral rostroventral end point of alveoli dentalis of I1 (I: incisiv, PM: Premolar, M: Molar).

Statistical analysis: In the mandible photographs, General Procrustes Analysis (superimposition) was conducted due to the differences such as size, position, and direction (29). PAST (Version 4.02) (12) program was used for this analysis. By using the same program, principal components analysis (PCA) was performed on the new coordinates obtained as a result of the Procrustes Analysis, and the components were calculated according to the breed and sex factors for the total shape variation. MorphoJ (14) program was used to analyze at which landmarks the shape differences are concentrated (PCA), proximity degree of individuals (Classical Cluster), allometry and grouping characteristics (Canonical variance analysis-CVA).

Results

Table 1 shows the results of the principal components analysis conducted on the semilandmark coordinates detected in the sheep mandible. Accordingly, the first principal component (PC1) explained 36.52% of the total shape difference and the first three principal components (PC1+PC2+PC3) explained 63.822% of the total shape difference. Among the principal components, a significant point of inflexion was observed between PC3 and PC4. The distribution of the samples according to PC1 is shown in the graph in Figure 2. Accordingly, the individuals were clustered significantly in terms of breed. However, the samples did not show any clustering in terms of sex.

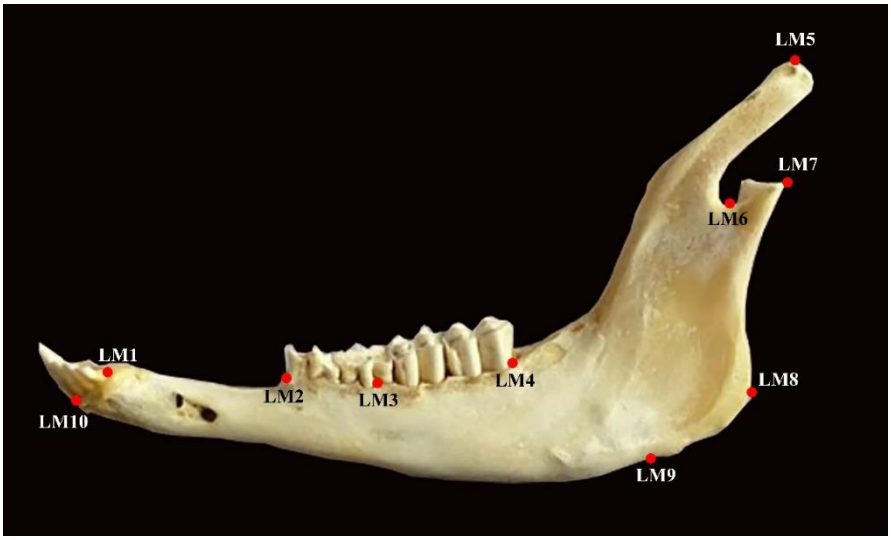

Figure 1. Landmarks for the mandible.

Table 1. Results of the principal component analysis, PC: principal component.

PC	Eigenvalue	% Variance	PC	Eigenvalue	% Variance
1	0.000956539	36.521	**16**	1.67996E-05	0.64142
2	0.000435134	16.614	**17**	1.43954E-05	0.54962
3	0.000279896	10.687	**18**	1.17449E-05	0.44843
4	0.000182373	6.9631	**19**	1.0266E-05	0.39196
5	0.000163238	6.2325	**20**	9.81998E-06	0.37493
6	0.000106542	4.0679	**21**	7.04139E-06	0.26884
7	8.27837E-05	3.1607	**22**	6.94329E-06	0.2651
8	6.40332E-05	2.4448	**23**	5.6035E-06	0.21395
9	5.68579E-05	2.1709	**24**	5.54077E-06	0.21155
10	4.13401E-05	1.5784	**25**	5.04472E-06	0.19261
11	3.85708E-05	1.4727	**26**	4.65318E-06	0.17766
12	3.32541E-05	1.2697	**27**	4.43753E-06	0.16943
13	2.63544E-05	1.0062	**28**	3.35491E-06	0.12809
14	2.30908E-05	0.88162	**29**	2.65676E-06	0.10144
15	1.82981E-05	0.69863	**30**	2.52151E-06	0.096273

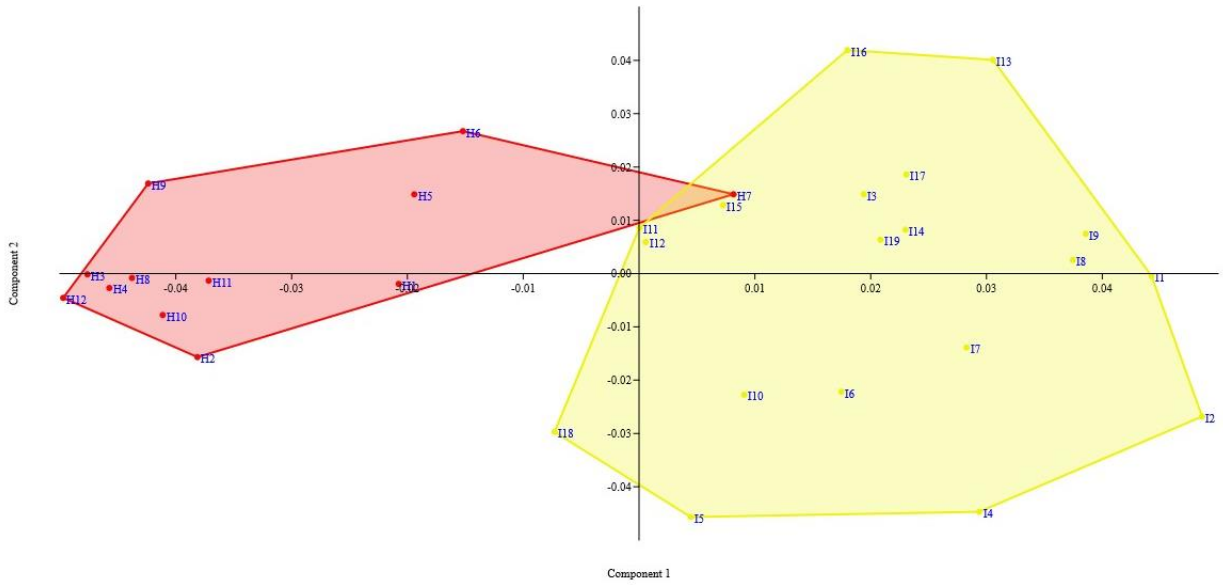

Figure 2. The distribution of individuals on the graph is based on the first principal component, Red: Hamdani (H), Yellow: Awassi (I), Individuals H1-H6 and I1-I9 are Female, H7-H12 and I10-I19 are Male.

Figure 3 shows the graph obtained as a result of the test performed on the Procrustes coordinates in order to show the proximity of the samples. While the samples were mostly grouped according to the breed factor, no significant proximity occurred according to the sex factor.

Regression analysis of shape over the centroid size (PCs) determined that 8.6402% of shape oversize was estimated according to the breed and 16.1168% according to the sex. This was significant at a confidence interval of 95% for both breed and sex (P: 0.0030 for the breed, P: 0.0002 for sex). In terms of breed factor, 38.8077% of the shape identified by PC1 was estimated by size (P: 0.0002). The shape identified by PC2 and estimated by size was smaller and insignificant in terms of this factor (6.1267%, P = 0.1733). The same values were determined as 38.2229% (P: 0.0004) and 7.8924% (P: 0.1309) for PC1 and PC2 according to the sex factor. According to these results, it was determined that the shape variations of the mandible according to the breed and sex factors used in the study did not depend on the size and thus there was no significant allometric component.

Figure 4 shows graphs showing at which semilandmarks the shape differences are concentrated. The locations where the shape differences concentrated in terms of PC1 are the attachment sites of teeth to the jaw bone (SLM128-132, SLM12-32), section starting from the

second molar tooth to the ramus mandibulae (SLM31-49), tip and caudal edge of processus coronoideus (SLM58-68), and angulus mandible including incisura vasorum facialium (SLM84-109). Among these concentration areas, the most significant semilandmark range was between SLM101-111 and SLM30-42. Thus, the high PC1 ventral edge defines the mandibles as having a significant convex line. PC1 also defines the mandibles with a shape deformity on the dental arch as of the last molar tooth and on the anterior margin of the ramus mandible. In terms of PC2, shape differences have completely concentrated on the mandibular dental arch level and the continuation of this arch towards ramus mandibulae (SLM1-43). Thus, PC2 only defines the mandibles with significant ventrocaudal (glenoid) edge at the anterior margin of the ramus mandibulae.

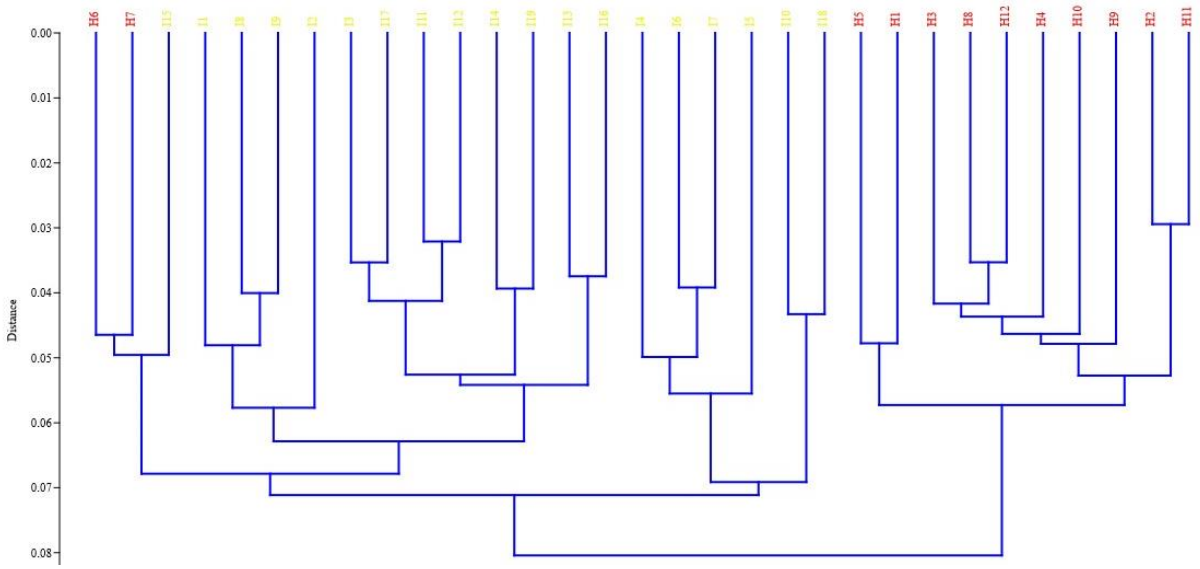

Figure 3. Graphical representation of hierarchical closeness of individuals, Red: Hamdani (H), Yellow: Awassi (I), H1-H6 and I1-I9 individuals are female, H7-H12 and I10-I19 individuals are male.

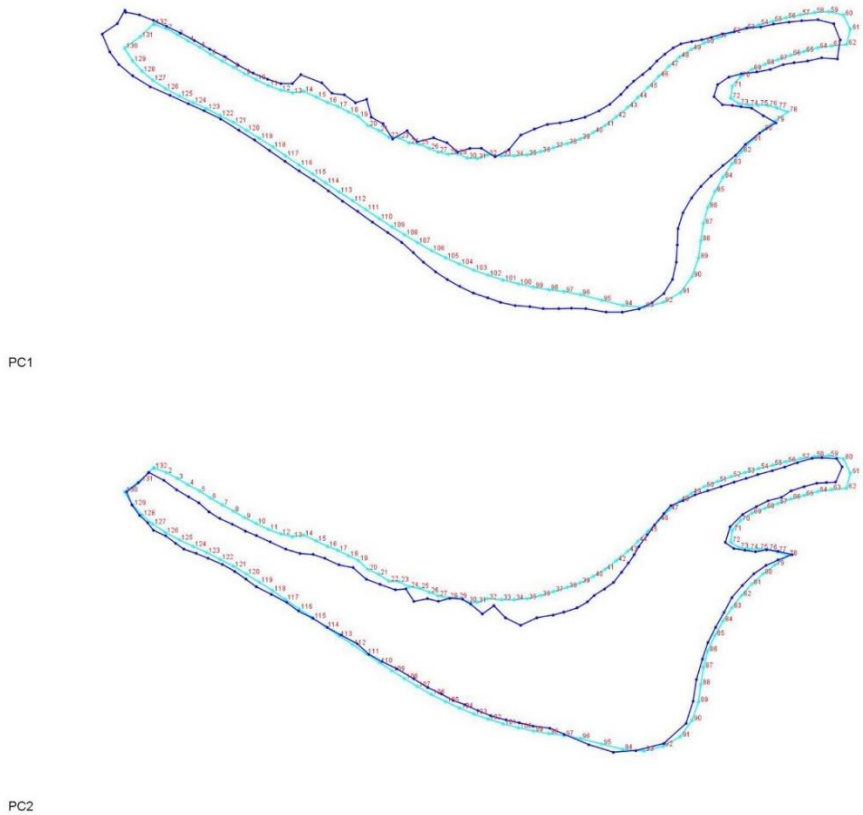

Figure 4. Wireframe graphical representation of shape differences concerning PC1 and PC2. Dark blue represents the positive bounds of principal component scores.

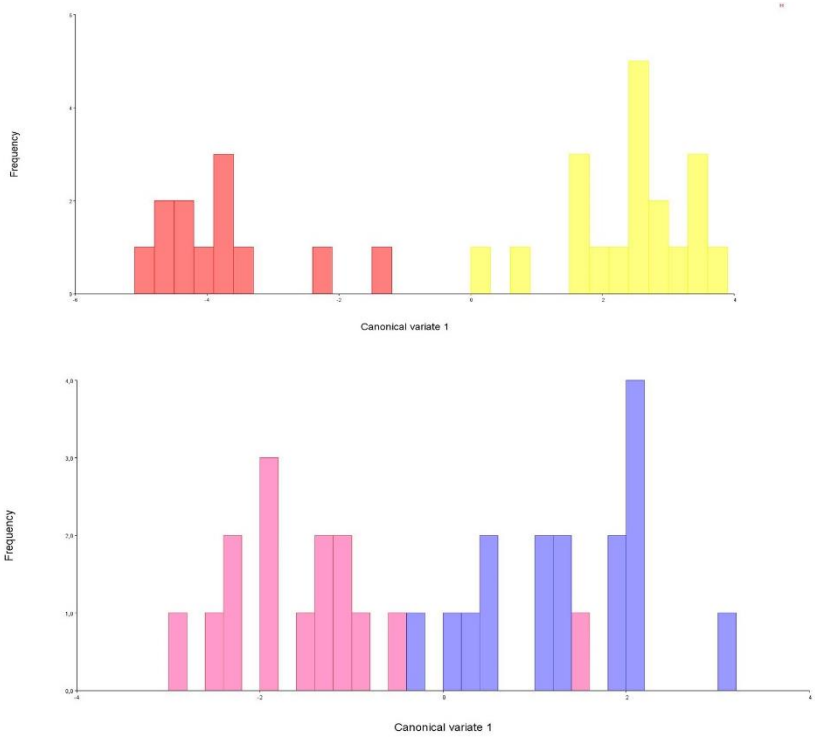

Figure 5. Wire-frame warp and frequency graphs of the mandible by breed (A) and sex (B). Red is Hamdani, yellow is Awassi for the breed. Pink is female and blue is male for sex.

Canonical variance analysis defined the sheep mandibles in a canonic variable in terms of breed or sex factors. Shape variations according to CV1 were similar to the anatomical points defined according to PC1. Table 2 shows Mahalanobis and Procrustes distances values according to breed and sex. Accordingly, distribution difference in terms of breed (<0.0001) showed a significant superiority compared to sex (0.1496).

Table 2. Mahalanobis distances (MD), Procrustes distances (PD) and P-value for PD (from permutation tests, 10000 permutation rounds) between sheep mandibles.

Breed			Sex		
MD	PD	P-value	MD	PD	P-value
6.1825	0.0543	<0.0001	2.7481	0.0224	0.1496

The wire-frame warp graph of sheep mandibles in Figure 5 shows the shape differences and frequencies in terms of breed and sex. While the frequencies were homogeneously distributed according to breed, no homogeneous distribution was found according to sex. In the breed-based comparison, it was found that the mandible of Hamdani sheep had a higher body than the mandible of Awassi sheep. In the sex-based comparison, the mandible of male sheep was found to be more voluminous than female sheep, especially in the body area.

Discussion and Conclusion

The development process of the mandible depends on various factors such as the growth hormones, growth factors, breed, and mechanical stress (13). In the study, the sheep mandible depending on breed and sex (hormonal) factors were analyzed by using semilandmarks. The most significant limitation of the study was the failure to standardize all the factors affecting the development of the mandible. This limitation can be neglected according to the scientific study methodology.

In their study, Pares-Casanova (21) examined allometry in the mandible of domestic sheep. In the present study, it was stated that the first three principal components explained 77.5% of the total shape variation. In the study, the mandible shape differences were primarily caused by the extraction of molar teeth with age and allometry in the margo ventralis . In the present study, the first three principal components explained 63.822% of the total shape variation. The shape differences were most significant in the arcus dentalis (premolar, molar), the section starting from the second molar tooth towards ramus mandibulae,processus coronoideus and angulus mandible. In addition, most of the shape variations according to the breed and sex factors of the sheep did not depend on the size and no significant allometric component was found. The researcher (21) suggested that the points showing variation in the mandible may be associated with a certain morphofunctional difference since they correspond to the adhesion sites of significant

masticatory muscles. The findings obtained in the present study also support this opinion.

Sexual dimorphism is common in different animal groups including goats and sheep (24). Rensch's rule defines the sexual dimorphism model by asserting that for large-sized species there is often a more significant male body size than female (7, 24). Demiraslan et al. (5) stated that sexual dimorphism was present in the mandibles of Honamlı and hair goats. Likewise, it has been highlighted that sexual dimorphism is not seen in the mandibles of Anatolian wild (32) and Awassi sheep (6).

Understanding the differences related to sex is important to learning ecology, behavior, generational mobility, and evolution (15). In the literature (18), it is stated that sexual dimorphism is important in sheep. Also, it is important to extensively analyze the features other than the cranium and mandible shape and the presence of the horn in terms of sexual dimorphism (6). In the present study, the curves affecting the general shape of the sheep mandible were analyzed by semilandmarks in order to see the details. In Iranian fallow deer (17) and Awassi sheep (6), the significant differences in the mandibles of male and female individuals are present at angulus mandibulae and molar teeth arch levels. In the current study, shape differences by sex were mostly concentrated on margo ventralis mandibulae and molar teeth arch level.

In Anatolian Wild and Akkaraman sheep, first-degree shape differences took place at LM1, 3, 8, 9 and 10 (32). In Honamlı and hair goats, first-degree shape differences were observed at LM4, 7, 8, 9, and 10. In both studies, as in the current study, the first-degree differences represented arcus dentalis, processus coronoideus, and angulus mandible. This data showed that shape differences of the mandible by species or breed factor concentrated on specific points.

The geometric morphometric method can be used to reveal the phylogenetic relationships from the cranium or mandible of current mammalian forms (16). The data of this study can be used for estimation of the morphologic properties, fauna determination, or some socio-economic implications in ancient period mammals (4, 8, 20, 22). As a result of the study, it was detected that the general shape of the sheep mandible was significantly affected, especially from the breed factor by using semilandmark. In addition, detailed shape analysis was performed on the mandibles of Hamdani and Awassi sheep, which are also remarkable in terms of geographical proximity. Consequently, it is thought that the data obtained as a result of the study would serve as a reference for the ruminant mandible remains obtained from archaeological excavations, especially in Mesopotamia region.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

YD and ID conceived and planned the experiments. YD and ID carried out the experiments. YD and ID planned and carried out the simulations. BCG contributed to sample preparation. YD, ID and BCG contributed to the interpretation of the results. YD, ID and BCG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Harran University Animal Experiments Local Ethics Committee (28.03.2022/01-13).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Near-infrared light transillumination for occlusal caries detection in dog teeth: A comparative study

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ABSTRACT

The aim of this study is to compare the effectiveness of visual, radiologic, and near-infrared light transillumination caries detection methods on caries of dog teeth in-vitro. A total of 237 dog molar teeth were evaluated with three different methods; visio-tactile examination with a probe, radiographic assessment, and NIR-LT. Each tooth was evaluated with all of the methods; the absence or presence of occlusal caries was scored as either 0, caries not present, or 1, caries present. All caries detection methods yielded the same outcomes for each tooth. Among all caries detection methods positive Spearman's rho value (ϱ=1) indicated that there was a strong positive correlation between the caries detection methods. Since NIR-LT is such a comfortable and easy caries diagnosis method, it can be used without sedation, especially in calm dogs.

Introduction

Dental caries is a term that refers to both the disease and the lesion that occurs as a result of this disease, which is a major healthcare problem as it is the most prevalent disease worldwide for humans. Dental caries is not only seen in human teeth but also develop on the teeth of other animals such as dogs. Dogs have 42 teeth, and these teeth are divided into three main categories with respect to their function and morphology: incisors, premolars and molars, similar to human teeth. Dental hard tissues such as enamel, dentin and cementum; surrounds the pulp tissue consisting of vessels and nerves. Although the prevalence of dental caries in dogs is lower than that of the humans because of the natural resistance of their teeth, it is still a serious oral health problem for dogs. The incidence of dental caries in dogs is between 3.1-5.3% and most commonly occurs on the occlusal surfaces of the molar teeth (3). The maxillary first molar tooth is particularly prone to caries (4).

Early diagnosis and treatment of dental caries are important. If left untreated, caries can finally lead to pulp infections, lodged abscesses, or tooth loss (12). However, the diagnosis of caries lesions on dog teeth could be challenging. The conventional examination method involves checking the pits and fissures of the teeth with a pointed probe. This method is highly subjective and affected by the expertise of the operator, especially for the detection of the initial caries lesions (11). Only after the lesion advances to a certain level, it can be detected easily otherwise could easily be missed. Unfortunately, by that time, caries has usually extended into the pulp dentin complex of the tooth and there is such extensive loss of tooth structure that extraction remains the only viable

option (3). The radiological examination could be a gold standard for the diagnosis of early dental caries in dogs (5), but it is almost impossible to perform without sedating the dogs. Therefore, intra-oral dental radiographs cannot be part of routine oral check-ups for dogs.

A near-infrared light transillumination (NIR-LT) approach which is used for caries diagnosis for human teeth (8) could be an alternative to radiographic and visio-tactile evaluation for caries detection for dogs. Currently, there are two devices available on the market that employ this method (DIAGNOcam, KaVo, Biberach, Germany/CariVu, DEXIS, Hatfield, PA, USA) to visualize enamel and dentin caries lesions in molars and premolars. The intraoral camera of the devices emits near-infrared light with a wavelength of ~780 nm from two light emission windows, each arranged on the buccal and oral branches of the handpiece (Figure 1). The light is transmitted through the alveolar bone and into the dental hard tissue. The image of the transilluminated tooth is captured with a CCD sensor over the occlusal surface (7). It is a radiation-free caries detection method and it can be performed on dogs without the need for sedation or anesthesia. There are many studies that suggest the NILT method is reliable for detecting caries on human teeth (7-11). However, to the author's knowledge, there is no study in the literature comparing the effectiveness of this method with other routine caries detection methods in dogs.

Figure 1. Diagnocam caries detection device.

Therefore, this study aimed to compare the effectiveness of visual, radiologic, and NILT caries detection methods on caries of dog teeth in-vitro. The null hypothesis tested is that the NILT method would not differ from radiological and intraoral examinations regarding the detection of caries on the occlusal surface of dog molar teeth.

Materials and Methods

For this study, 60 canine mandibles and maxillas were randomly selected from the collection of the anatomy department of the Veterinary Faculty of Ankara University. A total of 237 dog molar teeth on the jaws were evaluated with three different methods; visio-tactile examination with a probe, radiographic assessment, and NIR-LT method. Each tooth was evaluated with all of the methods as described below; the absence or presence of occlusal caries was scored as either 0, caries not present or 1, caries present. Radiographs and NIR-LT photographs were taken by single operator and two blinded dentist observers (Restorative dentistry specialist, 15 years of experience and maxillofacial radiologist, 12 years of experience) were involved in the visio-tactile examination, radiographic assessment and NIR-LT photograph evaluation. Before the evaluations, the teeth in question were cleaned with a slurry of pumice and prophy cup using a low-speed rotary handpiece. After rinsing the pumice with water, surfaces were thoroughly dried with compressed air.

Visio-tactile examination: A ball-ended explorer was then manually used under light pressure for assessment of the occlusal surfaces of the teeth under dental operating light. The tooth had score 1 (caries present). When the probe was stuck in the fissures, the surface had a cavity or demineralized surface. Otherwise, the tooth was scored 0 (caries not present).

NIR-LT examination: The NIR-LT device (DIAGNOcam, KaVo) was used in a room with ambient lighting and without dental operating light. The device was positioned on the occlusal surface of the tooth and images were obtained with the integrated software (KaVo Integrated Desktop/version 2.4.1.6374, KaVo, Biberach, Germany) of the NIR-LT device. Changes are displayed as dark shades in contrast to the healthy tooth substances and were scored. If the image had no change in light transmission throughout the surface, a tooth was scored 0 (caries not present); but if the visible shadow was observed on the occlusal surface, a tooth was scored 1 (caries present).

Radiographic examination: Digital periapical radiographs were taken with the paralleling technique. A Size 2 (31x41 mm) photostimulable phosphor plate attached to a sensor holder (XPP-DS Digital Sensor Holders for Sirona, Dentsply, IL, USA) was exposed with a digital dental x-ray unit (Expert DC, Gendex Dental Systems, Des Plaines, IL, USA) operated at 70 kVp and 7 mA with a 0.05 s exposure time. The focal spot distance was 30 cm and 1.5 mm Al equivalent at 20 kV, with a constant working distance, and images with magnification ranging from x5000 to 30000 were taken. Images were

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then evaluated by the observers, and each tooth was scored 0 (caries not present) or 1 (caries present).

Statistical analysis: Kappa values were calculated for the assessment of inter-observer agreement. The Spearman's rank-order correlation test was used to measure the association between the caries detection methods. A computer software (Jamovi 1.6, The Jamovi project, jamovi.org) was used for statistical analysis (α=0.05).

Results

There was a perfect agreement between the two observers for visio-tactile, radiographic and NIR-LT caries detection methods (Cohen's kappa=1) All caries detection methods yielded the exact same outcomes for each tooth. Between all the caries detection methods, positive Spearman's rho value (ϱ=1) indicated that there was a strong positive correlation between the caries detection methods (P<0.001) (Table 1). In other words, if one test scored 0 (caries not present) in a particular tooth, other two tests also scored 0 in the same tooth; similarly, if one test scored 1 (caries present) in a particular tooth, other two tests also scored 1 in the same tooth. This was true for all teeth evaluated.

Discussion and Conclusion

This study compared the outcomes of visual, radiographic, and NIR-LT approaches for the detection of occlusal caries in dog molar teeth. The null hypothesis was accepted, as there was no difference between the tested caries detection methods; all methods yielded the exact same outcomes for each tooth examined.

Although dental caries in dogs is a rare disease compared to the prevalence of dental calculus and periodontal disease (2), without early detection and appropriate treatment, caries almost always results in tooth loss (3, 4). However, early diagnosis of dental caries in dogs is quite challenging. In the early diagnosis of caries lesions, diagnostic methods are as important as the clinician's experience. Although the visio-tactile examination with a probe is the most commonly used diagnostic method, its reliability is debatable. Diagnosing early caries lesions by inspection may not always be accurate, and small lesions can be easily missed. Although the radiological examination is reliable, it is almost impossible to include it in a routine dental check-up due to the necessity of sedation.

Table 1. Correlation Matrix of caries diagnosis methods.

		Visio-tactile	Radiography	NIR-LT
Visio-tactile	Spearman's rho	—		
	P-value	—		
Radiography	Spearman's rho	1.000	—	
	P-value	< .001	—	
NIR-LT	Spearman's rho	1.000	1.000	—
	P-value	< .001	< .001	—

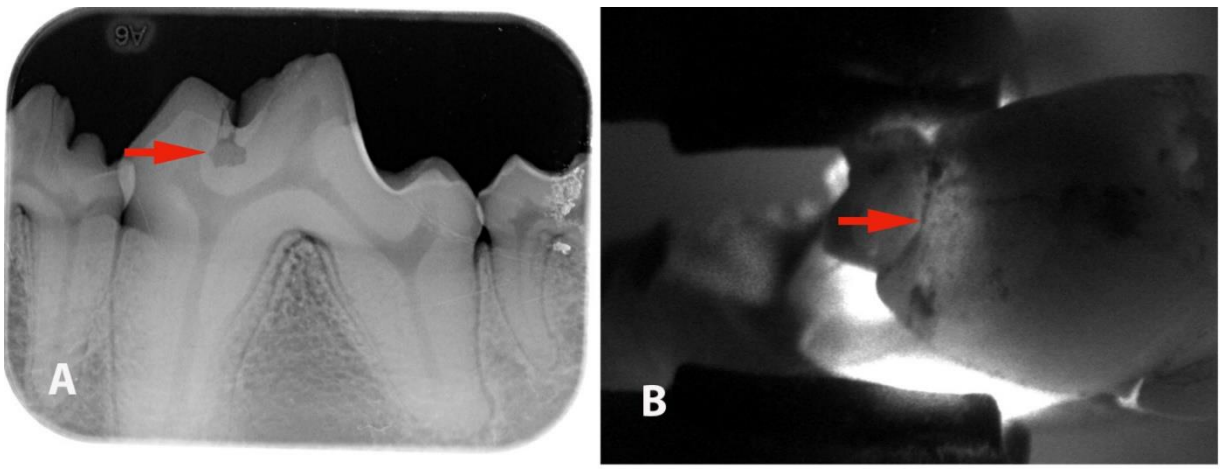

Figure 2. A: Representative radiographic image of caries dog tooth. Red arrow indicates caries. B: Diagnocam image of the same tooth. Red arrow indicates the caries.

The other technique for caries detection is using light. NIR-LT method uses invisible long-wavelength light. The camera of DIAGNOcam based on photo-optical principles, uses an illuminating wavelength of 780 nm (6). This device consists of a USB connection to a computer and specific software is used for the detection of dental caries (1). The use of this device allows capturing different stages of caries lesions and could be used effectively for the detection of occlusal caries without cavitation (6, 9). Nevertheless, the diagnostic accuracy of DIAGNOcam exhibited similarity with bitewing in human teeth (6-9, 11).

In this study, three different diagnostic methods were compared, and the result of this study showed that the NIR-LT method could be used reliably for the detection of dental caries in dogs. The NIR-LT device is portable and very easy to use. It does not cause pain during its use, and therefore it is widely used in pediatric dentistry as well as in adult human patients. Another advantage of the NIR-LT method is that there is no exposure to ionizing radiation. Furthermore, NIR-LT can be repeated as often as necessary, and occlusal and proximal surfaces can be evaluated simultaneously with this method.

This study has some limitations. Experiments were performed in vitro conditions. Especially in vivo NIR-LT and radiography may yield different outcomes, therefore results of this study should be verified with further in vivo setup. Investigating the use of the NIR-LT method for the detection of caries in dogs in vivo will be an important step towards introducing this method in routine dental check-ups in dogs.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MEK, GD and ÖI conceived and planned the experiments. GD and CE arried out the experiments. MEK, OE and CB contributed to sample preparation. MEK, OE, ÖI and GD contributed to the interpretation of the results. MEK and GD took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

No examination, research, manipulation or experimentation was performed on any animal species during this study.

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Investigating the presence and antibiotic susceptibilities of *Escherichia coli* O157 and *Listeria monocytogenes* in ruminant feces and feed in Balıkesir province

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ABSTRACT

The aim of this study is to determine the presence, virulence factors, and antibiotic susceptibilities of *E. coli* O157 and *L. monocytogenes* in ruminant feces and feed. This study was carried out for the first time in Balıkesir province. Feces, pellet feed, and silage samples were analyzed simultaneously for *E. coli* O157 and *L. monocytogenes* using feces of one gram and feed of twenty-five grams according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1, respectively. 38 (38%) *E. coli* O157 strains were isolated and identified from a total of 100 ruminant feces. A total of 3 (3%) *E. coli* O157:H7 strains were detected by PCR from one hundred ruminant fecal samples. In the study, resistance to antibiotics increased, especially in *E. coli* O157 isolates. In this study, enterohaemolysin was the predominant virulence factor among the *E. coli* isolates, and it was thought that it was important for pathogenesis. The *Sxt1* gene was higher than the *Stx2* gene. A total of 24 *L. monocytogenes* strains were isolated from a total of 100 ruminant fecal samples and 50 silage samples. Three of these strains were isolated from silage samples taken from the farms, where *L. monocytogenes* was isolated from sheep feces. As a result, poor quality silage could be an important source of infection for listeriosis in Balıkesir province. Epidemiologically, poor quality silage was thought to be one of the sources of listeriosis. It was thought that ruminant feces played an important role as a reservoir in the spread and transmission of *E. coli* O157. The antibiotic resistance status of *E. coli* O157 and *L. monocytogenes* isolates should be monitored with epidemiological studies.

Introduction

Verotoxigenic *Escherichia coli* infections are one of the most frequently reported zoonotic diseases in the European Union countries, according to 2020 data from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (19). *E. coli* O157:H7 is a member of the verotoxin-synthesizing *E. coli* group and was first identified in the 1970s (4, 9, 17, 23, 37, 49). *E. coli* O157:H7 has more than one virulence factor. The most important ones among them are Shiga toxin (Stx) 1 and 2 (encoded by the genes, *Stx1* and *Stx2*), intimin (encoded by the gene, *eaeA*), and the plasmid-encoded enterohaemolysin (encoded by the gene, *EhlyA*) (50). It is a zoonotic pathogen and causes various illnesses, such as Stx-producing *E. coli* (STEC). Stx is thought to be responsible for causing life-threatening conditions such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis in humans (4, 9, 17, 23, 37, 49). Previous studies reported that the first *E. coli* O157:H7 outbreak occurred as a result of the consumption of beef and dairy products that did not undergo adequate heat treatment (5, 28).

It is well known that cattle are the most important reservoir of *E. coli* O157 (5, 11, 26, 46, 47). Other reservoir animals are sheep, goats, wild deer, pigs, and birds (2, 11, 43, 46). Infection occurs due to the oral consumption of contaminated cattle feces, and foods through direct contact with reservoir cattle (5, 25, 44, 46, 47). *E. coli* O157:H7 can survive in feces for approximately 50-99 days (16, 26). Feces are also used as a fertilizer. Silage could be contaminated with feces during the silage preparation and cause infections in

ruminants and humans (16). Drinking water contaminated with cattle feces as a result of consumption of contaminated products such as milk and meat plays a key role in contamination. Carcass contamination in slaughterhouses also causes transmission of *E. coli* O157 to humans (33, 35, 37). The bacteria that pass to the inner surfaces during the processing of meat can maintain their vitality when adequate heat treatment is not applied and can cause infections that are important for public health (20, 44, 47, 49).

The prevalence of the *E. coli* O157 disease increases during the summer months and at the beginning of autumn (2, 5, 11, 43, 46). Another important issue for this zoonotic bacterium is that it shows a wide variety of multi-antibiotic resistance with the complex interaction of different mechanisms (21).

Isolation of *E. coli* O157 can be detected from feces and contaminated materials with feces. Conventional and molecular methods are used for detection of *E. coli* O157. Conventional ones are immunomagnetic separation, culture, serological verification with antisera, and biochemical tests. CT-SMAC (cefixime tellurite-sorbitol MacConkey) agar and CHROM agar can be used as differential agars. In molecular methods, various gene regions of the bacterium and its toxin can be identified by polymerase chain reaction (PCR) (5).

Şeker and Kuş (49) isolated *E. coli* O157 in 16 of 417 fecal samples collected from adult ruminants. In addition, they found high resistance to ampicillin (68.7%), neomycin (68.7%), tetracycline (68.7%), trimethoprim/sulfamethoxazole (62.5%), and amoxicillin/clavulanic acid (56.2%) in these isolates. Birdal and Ak (8) reported that they isolated *E. coli* O157:H7 in 2 of 576 fecal samples collected from dairy cattle reared in several provinces in the Marmara region. McCabe et al. (42) isolated *E. coli* O157:H7 in 55 of 1317 rectal mucosal swab samples from dairy and beef cattle. Khalifa et al. (37) reported that they isolated 3 *E. coli* O157:H7 strains in fecal samples taken from 3 month-old calves with diarrhea, and these isolates were susceptible to amoxicillin and intermediate to cefotaxime and tetracycline. Umar et al. (53) reported that they isolated 4 *E. coli* O157:H7 strains in 50 fecal samples of cattle. Jacob et al. (32) reported that they isolated *E. coli* O157:H:7 in 33 of 296 goat fecal samples.

Listeria monocytogenes causes various infections in humans and animals (5, 7, 15, 18, 24). *L. monocytogenes* is found in the gastrointestinal tract of ruminants. Its fecal shedding is associated with the spread of the disease in the herd, especially in small ruminant farms (15, 27, 34, 55). For this reason, listeriosis can be found in soil, water, and associated food, primarily as a result of fecal contamination (1). *L. monocytogenes* cannot survive below pH 5. Cattle and sheep, which consume poorly prepared silage, may become infected as a result thereof (15). In addition, due to its psychrophilic character, it can grow in frozen foods (1). *L. monocytogenes* can be detected in feces. Following pre-, selective, and cold enrichment, *L. monocytogenes* can be isolated in both selective and differential agars. *L. monocytogenes* is identified through Gram stain, catalase, motion examination, aesculin, Voges-Proskauer tests, CAMP, and hemolysis tests. In addition, the agglutination test is performed serologically with O and H antisera (29, 53).

Oyinlore et al. (46) isolated a total of 176 colonies from 30 bovine fecal samples and identified 27 of these colonies as *L. monocytogenes*. Kalorey et al. (36) isolated *L. monocytogenes* in 8 of 50 fecal samples collected from 6 different mammals and one bird. Abay and Aydın (1) reported that they isolated *L. monocytogenes* from 27 of 400 fecal samples taken from healthy cattle and observed the highest isolation rate in January. Weis and Seeliger (56) isolated 15.2% of *L. monocytogenes* in 102 fecal samples collected from nature. Iida et al. (29) isolated *L. monocytogenes* from 189 of 9539 bovine large intestine contents.

In this study, which was carried out for the first time in Balıkesir province, it was aimed to determine the presence, virulence factors, and antibiotic susceptibilities of *E. coli* O157 and *L. monocytogenes* in ruminant feces and feed.

Materials and Methods

Sample collection: A total of 100 ruminant feces (80 from calves and 20 from sheep) and 100 animal feed samples (50 from silages and 50 from pellet feeds) were collected from 4 farms located in the Balıkesir province of Türkiye between January 2020 and May 2021 in order to examine the presence of *E. coli* O157 and *L. monocytogenes*. Fecal samples were collected from the animals' rectum by veterinarians working on these farms. The sampled calves were male and 11-15 month-old Charollois, Simmental, Blonde, and Limousin calves. The sampled sheep were female and 4-year-old Merino sheep, which are on their fiftieth day during the lactation period. All sampled animals and feeds were randomly selected from the farms. The animals were apparently healthy and did not suffer from diarrhea. Feces and animal feed samples were transferred into sterile boxes and transported to the laboratory in a cold chain (2-8 °C) and they were analyzed promptly in the laboratory.

Isolation and identification of E. coli O157 and L. monocytogenes: Each feces and animal feed sample were analyzed simultaneously for *E. coli* O157 and *L. monocytogenes* using one gram of feces and twenty-five grams of animal feed according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1, respectively (30, 31). For feces and feed samples, 1/9 enrichment broth was used in both bacterial analyses according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1.

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For *E. coli* O157, fecal and feed samples were transferred into modified Tryptone Soy Broth (mTSB) containing Novobiocin (20 mg/L) (Merck, Germany) and homogenized by using a mixer for one minute. The homogenate was incubated at 37°C for 6 h. After incubation, immunomagnetic separation (IMS) of *E. coli* O157 was done by using a Captivate Magnetic kit in accordance with the manufacturer's procedure (LabM, Germany). After the IMS procedure, the particles were streaked into the Cefixim-Tellurite Sorbitol MacConkey agar (CT-SMAC, LabM, Germany). Agar plates were incubated at 37°C for 18-24 h. After incubation, colonies were examined and confirmed using conventional biochemical tests such as Gram staining, motility, oxidase, indole, etc., according to Şeker and Yardımcı (51). Serological confirmation was done with latex agglutination using the *E. coli* O157 antisera (Microgen, Germany). In all tests, the *E. coli* O157:H7 strain (ATCC 43895) and the *E. coli* (ATCC 25922) strain were used as positive and negative control strains, respectively.

Feces, pellet feed, and silage samples were analyzed for *L. monocytogenes* according to ISO 11290-1 (30). For the pre-enrichment process, fecal and feed (pellet and silage) samples were put into the half-fraser broth (Merck, Germany) and incubated at 30 °C for 24 h. Then, 0.1 ml of half-fraser broth was put into fraser broth (Merck, Germany) and incubated in 37 °C for up to 48 h. Afterwards, enrichment cultures were streaked onto two selective agars-PALCAM (Oxoid, UK) and Rapid' *L. mono* (Bio-Rad, USA) and incubated at 37 °C for 24-48 h. Presumptive *L. monocytogenes* colonies were also confirmed through Gram staining, motility examination on a microscope, catalase, oxidase, and CAMP tests for *Staphylococcus aureus* (ATCC® 25923™) strain and aesculine test (1, 3, 6). All confirmed cultures of both *E. coli* O157 and *L. monocytogenes* were stored in beads (Cryobank, Mast) at-20 °C.

Antibiotic susceptibility tests: Antibiotic susceptibilities were investigated in the Mueller- Hinton agar (Merck, Germany) by using disc diffusion method stated by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and in the Clinical and Laboratory Standards Institute (CLSI) standards (22).

Antibiotics, which were used in antibiogram tests for *E. coli* O157 and *L. monocytogenes,* were selected based on both previous studies (3, 40, 47) and antibiotics used for therapeutic purposes.

For *L. monocytogenes*, gentamicin (10 µg), streptomycin (10 µg), meropenem (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), ampicillin (10 µg), erythromycin (15 µg), trimethoprim/ sulfamethoxazole (25 µg), penicillin G (10 U), sulbactam/ ampicillin 1:1 (20 µg) and tetracycline (30 µg) antibiotic discs (Oxoid, UK, Liofilchem, Italy) were used.

For *E. coli* O157, ampicillin (10 µg), amoxicillin/ clavulanic acid (30 µg), ertapenem (10 µg), meropenem (10 µg), ceftriaxone (30 µg), cefotaxime (5 µg), cefotaxime/clavulanic acid (40 µg), gentamicin (10 µg), tobramycin (10 µg), tetracycline (30 µg), aztroneam (30 µg), oxacillin (5 µg), colistin sulphate (5 µg), doxycycline (5 µg), vancomycin (30 µg), erythromycin (15 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg), and trimethoprim/sulfamethoxazole (25 µg) antibiotic discs (Oxoid, UK, Liofilchem, Italy) were used.

For *L. monocytogenes*, ampicillin, penicillin G, erythromycin, trimethoprim/sulfamethoxazole, and meropenem were evaluated according to EUCAST guidelines (22). The other antibiotics were evaluated according to Aksoy et al. (3) and CLSI guidelines (12, 13). For *E. coli* O157, antibiotic discs were evaluated according to Lukasova et al. (40) and international standards (14, 22). Oxacillin was evaluated according to the BSAC standard (11). Vancomycin was evaluated according to Oxoid manufacturer's guidance (45). In antibiograms, *E. coli* (ATCC ® 25922™) strain and *L. monocytogenes* strain (GeneBank Accession Number: MN496429) from Department of Veterinary culture collection of Balıkesir University Kepsut Vocational School were used as reference strains.

PCR tests of E. coli O157 strains: All *E. coli* O157 isolates were inoculated in Nutrient Broth (NB, Oxoid, UK) and incubated at 37°C for 18 hours to obtain pure cultures. After incubation, 1 mL of NB broth culture was centrifuged at 5000 g for 10 minutes. After centrifugation, the supernatant was removed and DNA extraction was performed using the pellet according to the GeneJET Genomic DNA Purification kit (Thermo Scientific, USA) and the DNA Purification Protocol for Gram-negative bacteria.

The presence of H7 serotype, Shiga toxin, intimin, and hemolysis was analyzed by PCR using *fliCH7, Stx1, Stx2, eaeA,* and *EhlyA* gene specific primers, which were previously used by Şeker and Kuş (49) (Table 1).

A multiplex PCR reaction mix for *fliCH7, Stx1, Stx2,* and *EhlyA* genes was prepared in a total volume of 50 µl. The PCR mix contained 2 µl of DNA extract and was prepared using a 35 µL DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA), 12.8 µL DEPC water, 0.1 µL Primer F (100 pmol/µL), and 0.1 µL Primer R (100 pmol/µL) (49). Amplification conditions were applied according to Şeker and Kuş (49).

A PCR reaction mix for the *eaeA* gene was prepared in a total volume of 50 µl. PCR mix contained 2 µl of DNA extract and was prepared with a 35 µL DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA), 12.8 µL DEPC water, 0.1 µL Primer F (100 pmol/µL), and 0.1 µL Primer R (100 pmol/µL) (49). Amplification conditions were applied according to Şeker and Kuş (49).

Table 1. Primer sequences, target genes, base pairs and references for *E. coli* O157 virulence genes.

Primers	Sequences	Target genes	Base pairs (bp)	Reference
H7-F	GCGCTGTCGAGTTCTATCGAGC	*fliCH7*	625	Şeker and Kuş (49)
H7-R	CCACGGTGACTTTATCGCCATTCC			
Stx1-F	TGTAACTGGAAAGGTGGAGTATACA	*Stx1*	210	Şeker and Kuş (49)
Stx1-R	GCTATTCTGAGTCAACGAAAAATAAC			
Stx2-F	GTTTTTCTTCGGTATCCTATTCC	*Stx2*	484	Şeker and Kuş (49)
Stx2-R	GATGCATCTCTGGTCATTGTATTAC			
Int-F	GGGATCGATTACCGTCAT	*eaeA*	837	Şeker and Kuş (49)
Int-R	TTTATCAGCCTTAATCTC			
hlyA-F	GCATCATCAAGCGTACGTTCC	*EhlyA*	534	Şeker and Kuş (49)
hlyA-R	AATGAGCCAAGCTGGTTAAGCT			

Table 2. Antibiogram results of *L. monocytogenes* isolates.

Antibiotic discs	*Listeria monocytogenes* strains (n:24)		
	S (Susceptible) (%)	I (Intermediate) (%)	R (Resistant) (%)
Amikacin (30 µg)	20 (83.33)	4 (16.67)	0 (0)
Ampicillin (10 µg)	21 (87.50)	-	3 (12.50)
Erythromycin (15 µg)	24 (100)	-	0 (0)
Gentamicin (10 µg)	23 (95.83)	1(4.16)	0 (0)
Chloramphenicol (30 µg)	21 (87.50)	1 (4.16)	2 (8.33)
Meropenem (10 µg)	24 (100)	-	0 (0)
Penicillin G (10 U)	20 (83.33)	-	4 (16.67)
Ciprofloxacin (5 µg)	19 (79.16)	4 (16.67)	1 (04.17)
Streptomycin (10 µg)	24 (100)	0 (0)	0 (0)
Sulbactam/ampicillin 1:1 (20 µg)	0 (0)	2 (8.33)	22 (91.67)
Tetracycline (30 µg)	24 (100)	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole (25 µg)	24 (100)	-	0 (0)

S: Susceptible, I: Intermediate, R: Resistant.

All PCR amplicons were electrophoresed on a 1.5% agarose (Prona, USA) gel using Bluejuice dye (Thermo Scientific, USA) and DNA molecular weight marker (Gene Ruler 100bp DNA Ladder plus, Thermo Scientific, USA) and visualized on the gel imaging system (EBOX CX5 TS EDGE, Vilber).

Results

A total of 38 *E. coli* O157 (38%) strains were isolated from a total of 100 ruminant fecal samples. While 15 of them were isolated from sheep fecal samples, 23 of them were isolated from calf fecal samples.

A total of 18 *L. monocytogenes* (18%) strains were isolated from 100 ruminant fecal samples. While 13 of them were isolated from sheep fecal samples, 5 of them were isolated and identified from calf fecal samples.

L. monocytogenes was isolated from 6 (12%) of 50 silage samples. Three of these isolates were isolated from silage samples taken from the farms, where *L. monocytogenes* was isolated from sheep feces. *E. coli* O157 could not be isolated from a total of 100 silage and pellet feed samples.

All *L. monocytogenes* isolates were susceptible to trimethoprim/sulfamethoxazole, tetracycline, streptomycin, meropenem, and erythromycin. The highest resistance was detected to sulbactam/ampicillin (Table 2).

All *E. coli* O157 isolates were resistant to oxacillin and vancomycin. 3 isolates were resistant to gentamicin and 7 isolates were resistant to tobramycin. On the other hand, 21 isolates were resistant to erythromycin and 12 isolates were intermediate. Table 3 shows the detailed results.

According to PCR results, the *EhlyA* gene was found in 20 *E. coli* O157 isolates. Of these isolates, 4 were isolated from sheep fecal samples and 16 from calf fecal samples. The *Stx1* gene was detected in 5 *E. coli* O157 isolates, 1 from a sheep fecal sample and 4 from calf fecal samples. The *EhlyA* gene was also detected in all isolates with the *stx1* gene.

The *Stx2* gene was found in 3 *E. coli* O157 isolates, including 1 from sheep fecal samples and 2 from calf fecal samples. The *Sxt1* gene was found at a higher rate than the *Stx2* gene.

The intimin gene was found in 8 *E. coli* O157 isolates, including 2 from sheep fecal isolates and 6 from

calf fecal isolates. The *EhlyA* gene was detected in all isolates with the intimin gene (Table 4, Figure 1, Figure 2).

A total of 3 (3%) *E. coli* O157:H7 isolates were detected by a PCR test from 100 ruminant fecal samples. Two of them were isolated from calf fecal samples and one from sheep fecal samples. The intimin gene was also detected in the *E. coli* O157:H7 strain isolated from calf fecal samples (Table 4, Figure 1 and 2).

Table 3. Antibiogram results of *E. coli* O157 isolates.

Antibiotic Discs (µg)	*E. coli* O157 Cattle isolates (n:23)			*E. coli* O157 Sheep isolates (n:15)			Total *E. coli* O157 isolates (n:38)		
	S	I	R	S	I	R	S (%)	I (%)	R(%)
Cefpodoxime (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Ciprofloxacin (5 µg)	23	-	-	15	-	-	38 (100)	-	-
Colistin sulphate (5 µg)	23	-	-	15	-	-	38 (100)	-	-
Ertapenem (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Cephotaxime (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Cephotaxime/clavulanic acid (40 µg)	23	-	-	15	-	-	38 (100)	-	-
Meropenem (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Aztroneam (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Ceftriaxone (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Trimetoprim/sulphametoxazole (25 µg)	23	-	-	15	-	-	38 (100)	-	-
Oxacillin (5 µg)	-	-	23	-	-	15	-	-	38 (100)
Doxycycline (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Vancomycin (30 µg)	-	-	23	-	-	15	-	-	38 (100)
Gentamicin (10 µg)	20	-	3	15	-	-	35 (92.10)	-	3 (7.90)
Ampisilin (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Eritromycin (15 µg)	2	6	15	3	6	6	5 (13.16)	12 (31.58)	21 (55.26)
Tobramicin (10 µg)	19	-	4	12	-	3	31 (81.58)	-	7 (18.42)
Tetracycline (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Amoxicillin/clavulanic acid (30 µg)	23	-	-	15	-	-	38 (100)	-	-

S: Susceptible, I: Intermediate, R: Resistant.

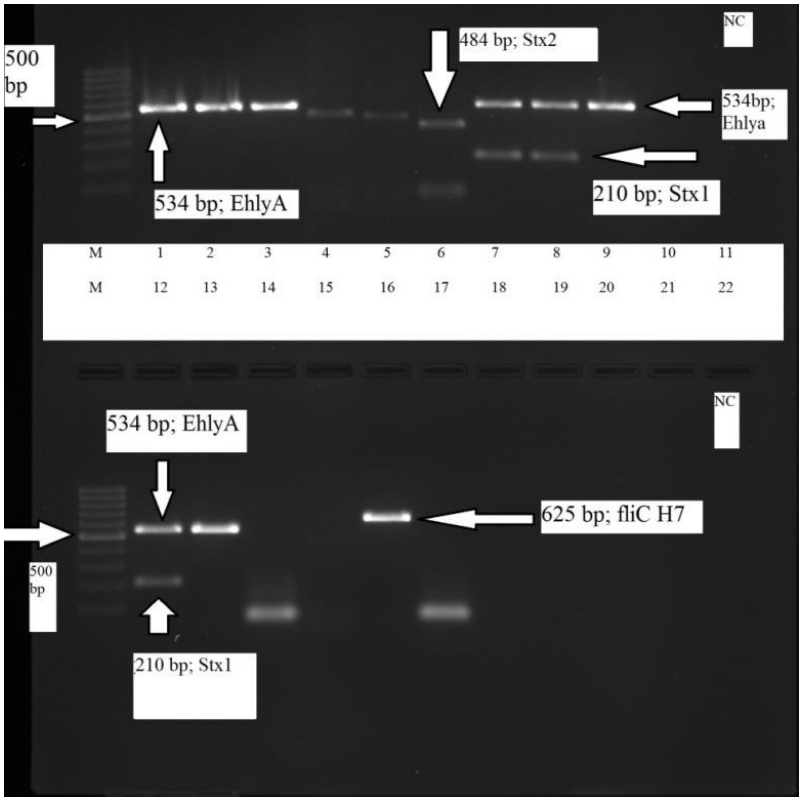

Figure 1. PCR image of virulence genes in *E. coli* O157 isolates.
M: Marker, Line 1,2,3: *E. coli* O157 isolates with *EhlyA* gene positive, Line 6: *E. coli* O157 isolate with *stx2* gene positive, Line 7,8,9: *E. coli* O157 isolates with *EhlyA* gene positive, Line 7,8,11: *E. coli* O157 isolates with *stx1* gene positive, Line 16: *E. coli* O157 isolate with *fliCH7* gene positive, Other lines: *E. coli* O157 isolates negative for the investigated virulence genes.

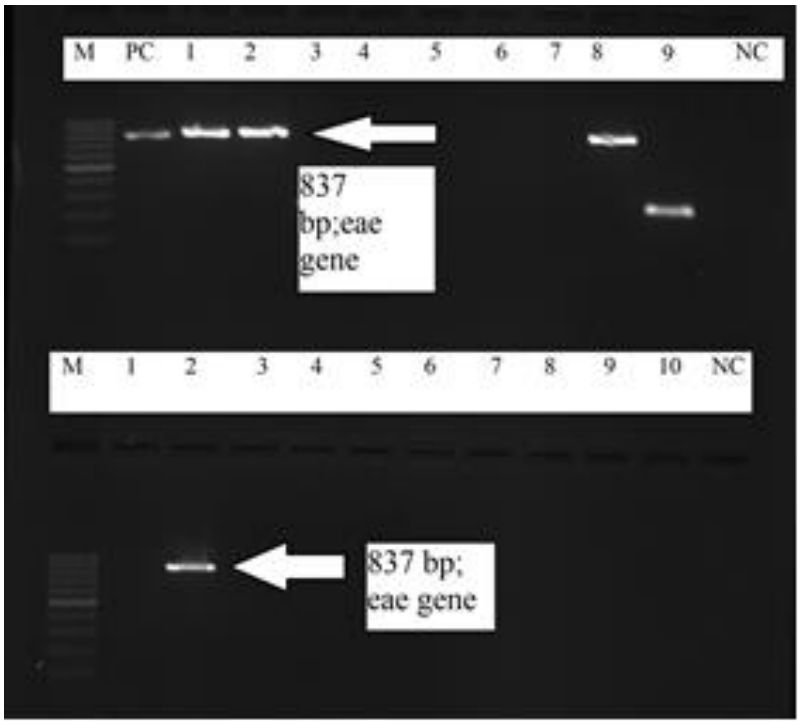

Figure 2. PCR image of *eae* (intimin) gene in *E. coli* O157 isolates.
M: Marker, PC: Positive Control, Line 2,3: *E. coli* O157 isolates with *eae* gene positive, Line 8: *E. coli* O157 isolate with *eae* gene positive, Bottom Line 2: *E. coli* O157 isolate with *eae* gene positive, Other Lines: *E. coli* O157 samples negative for the *eae* gene.

Table 4. PCR results of *E. coli* O157 virulence genes.

Virulence genes	*E. coli* O157 isolates from calf feces (n:23) (%)	*E. coli* O157 isolates from sheep feces (n:15) (%)	Total (n:38) (%)
fliCH7	2 (%8.69)	1 (%6.66)	3 (%7.89)
Stx1	4 (%17.39)	1 (%6.66)	5 (%13.15)
Stx2	2 (%8.69)	1 (%6.66)	3 (%7.89)
eaeA	6 (%26.08)	2 (%13.33)	8 (%21.05)
EhlyA	16 (%69.56)	4 (%26.66)	20 (%52.63)

Discussion and Conclusion

Cattle are the major reservoir of *E. coli* O157. Infection occurs due to the oral consumption of contaminated cattle feces and contaminated food and through direct contact with reservoir cattle (5, 11, 46, 47). *E. coli* O157 was much more intense in the feces of young cattle, especially after weaning, compared to adults (11, 16). In this study, it was thought that since the calves from which fecal samples were taken were 11-15 months old, the isolation rate might have increased. In addition, some studies reported that the number of *E. coli* O157 increased during the summer months (9, 11, 16). Since fecal samples were taken mostly during spring and summer in this study, it was thought that it might increase the isolation rate. Fecal contamination caused by poor hygiene is a risk factor in the contamination. Drinking water with fecal contamination is a suitable environment for this organism to live and grow and may cause cattle to get contaminated (11, 16). Apart from cattle, *E. coli* O157 has been found in domestic animals such as sheep, goats, pigs, dogs, and horses, as well as wild animals such as deer and birds. It is suggested that when domestic animals come into contact with wild animals, transmission between them may occur (5, 11, 16, 49).

In 2017, Alan et al. (4) detected *E. coli* O157 5.4% from two hundred and thirty seven cattle fecal samples. In this study, 15 *E. coli* O157 strains were isolated and identified from 20 sheep fecal samples. 23 *E. coli* O157 strains were identified from 80 calf fecal samples. 38 *E. coli* O157 (38%) strains were isolated from a total of 100 ruminant fecal samples.

When the results were compared, the isolation rates of *E. coli* O157 (38%) were increased as from 2017. The differences in the isolation rates of *E. coli* O157 between this study and previous studies were caused by factors such as the IMS method, age of the animals, geographical/seasonal conditions, hygiene conditions of farms, and contact with wild animals (5). Within the scope of one health concept, this result was thought to show the importance of ruminant feces and contaminated foods in human infections.

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In this study, the *EhlyA* gene was found in 20 *E. coli* O157 isolates with PCR. Of these isolates, 4 were isolated from sheep fecal samples and 16 from calf fecal samples. The *EhlyA* gene was also found in all the isolates with *Stx1* and intimin genes. Kuyucuoglu et al. (38) found the *eaeA* gene in 8 (57.1%) and the *EhlyA* gene in 13 (92.8%) of 14 *E. coli* O157:H7 isolates, and they also reported that enterohemolysin (*EhlyA*) was the dominant virulence factor. In this study, enterohaemolysin is the predominant virulence factor among the isolates, which is compatible with Kuyucuoğlu et al. (38)'s results.

Şeker and Kuş (49) reported that the *Stx2* gene was more common than the *Stx1* gene in *E. coli* O157 isolates. In this study, the *Sxt1* gene was found at a higher rate than the *Stx2* gene, which is opposite to the findings of Şeker and Kuş (49).

In 2011, Kuyucuoğlu et al. (39) reported that *E. coli* O157:H7 was detected at a rate of 3.1 % (14 out of 457 fecal samples) in calves and cattle. In this study, a total of 3 *E. coli* O157:H7 (3%) strains were detected from 100 ruminant feces by a PCR test. Isolation rates of *E. coli* O157:H7 were nearly the same compared to 2011. The intimin gene was also identified in *E. coli* O157:H7 strains isolated from calf feces.

Contamination of soil, water, and food with feces is of primary importance for the emergence of listeriosis (1). *L. monocytogenes* cannot survive below pH 5. Therefore, cattle and sheep which consume poorly prepared silage can develop various diseases as a result thereof (15). In 2019, Aydın et al. (6) detected *L. monocytogenes* 4% from 150 silage samples in Balıkesir province. In this study, six *L. monocytogenes* strains were isolated from 50 silage samples. Three of these isolates were isolated from silage samples taken from the farm, where *L. monocytogenes was* isolated from sheep fecal samples. Epidemiologically, poor quality silage was thought to be one of the sources of listeriosis. As a result, poor quality silage could be an important source of infection in Balıkesir province.

Unnerstad et al. (54) isolated *L. monocytogenes* from 6% of healthy dairy cow feces. Matto et al. (41) reported that there were identified clinically healthy dairy cows that shed *L. monocytogenes* via their feces, a situation previously described in studies conducted at dairy farms in other countries. Additionally, infected animals can shed *L. monocytogenes* via their feces. Also, fecal or soil contamination of silage is one of the sources of listeriosis (48, 52). In this study, *L. monocytogenes* was isolated from both feces and silage. The shedding of *L. monocytogenes* from clinically healthy cows shows that cattle feces could cause milk and carcass get contaminated and thus may contribute to foodborne listeriosis (54).

Resistance to antibiotics increased, especially in *E. coli* O157 strains isolated in the study. The antibiotic resistance status of *E. coli* O157 and *L. monocytogenes* isolates should be monitored with epidemiological studies.

As a conclusion, the presence and antibiotic susceptibility of *E. coli* O157 and *L. monocytogenes* were determined in this study, which was conducted for the first time in Balıkesir province of Türkiye. These results were presented as epidemiological data. It is thought that ruminant feces may be important as a reservoir in the spread and transmission of *E. coli* O157. Enterohemolysin was thought to be an important virulence factor in the pathogenesis of *E. coli* O157 infection. Contrary to the findings of other studies, the rate of the *Stx1* gene was higher than the rate of the *Sxt2* gene, especially in *E. coli* O157 isolates in this study, which is thought to be epidemiologically important. In addition, both feces and silage were an important source of listeriosis. The data of this study indicated that feces of calf and sheep are a potential source of infection and reservoir for both *E. coli* O157 infection and listeriosis in humans as well.

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Conflict of Interest

The author has no conflict of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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Türkiye's indigenous genetic resource: Muradiye Kelebek pigeon

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ABSTRACT

The current study was carried out to determine the morphological characteristics of the Muradiye kelebek pigeon, which has been reared for many years by breeders in the Muradiye region. The ages of pigeons were classified into two groups: 12-24 months (age group I) and 25-36 months (age group II). The male pigeons had a significantly higher trunk length ($P<0.001$), head width ($P<0.05$), beak length ($P<0.001$), beak depth ($P<0.05$), thoracic perimeter ($P<0.001$), and tarsus diameter ($P<0.001$) compared to the female pigeons. The body weight ($P<0.001$), chest width ($P<0.05$), and thoracic perimeter ($P<0.01$) values of age group II were higher than those of age group I. It was determined that most of the pigeons were grayish blue-eyed (69.90%) and non-crested (76.72%). It was determined that the head structure of the Muradiye kelebek pigeon was similar to the Edremit kelebek, Muradiye donek, Bursa oynarı, Thrace roller, and Alabadem pigeon genotypes. The spotted plumage color of the Muradiye butterfly pigeon and the other three genotypes (jackal plumage in the Thrace roller, speckled plumage in the Edremit kelebek pigeon, and red/black galaca in the Muradiye donek pigeon) may be a common trait. Since these genotypes share some phenotypic characteristics, the phylogenetic relationships between the Muradiye butterfly pigeon and the other five pigeon genotypes (Edremit kelebek, Muradiye donek, Alabadem, Bursa oynarı, and Thrace roller) should be determined by molecular studies.

Introduction

Domestic pigeon genotypes were domesticated from the rock pigeon (*Columba livia*) in the Mediterranean region. Pigeons are classified into seven species and approximately 350 breeds. They are grown for meat production, ornamental features, vocalization traits, aerial display, and racing performance (1, 18, 21). The geography of Anatolia, the cradle of many civilizations, has a historical and geographical structure that includes many indigenous genetic resources. One of these genetic resources is the pigeon, which has been traditionally bred in these lands for centuries. Türkiye has a large number of pigeon breeds as indigenous genetic resources. Although pigeon breeds are classified according to their body structure, body shape, color and pattern of plumage pigmentation, feather placement and structure, and number of wing and tail feathers in other parts of the world, they are classified according to their flight display characteristics in Türkiye (21-23).

Pigeon breeds in Türkiye have been classified into nine main groups as diver (dalıcı) (Azman, Bango, Baska, Dewlap, Dolapci, Domino, Donek, Kelebek, Misiri, Oynak, Spotted Kelebek, Şarabi, and Yoz), tumbler (taklacı) (Alabadem, Anatolian, Gogsu ak, Iç aglı, Ketme, Kizilbas, Taklambac), roller (makaracı) (Bursa roller, Cakal, Corum, Smyrna roller, Mulakat, Oriental roller, and Thrace roller), spinner (dolap dönücü) (Telkuyruk and Trabzon), fleet flyer (filo uçucusu) (Abu abse, Amberi, Bagdat, Bastankara, Bayramli, Buludi, Burmali, Cici, Damascene, Gullu, Halebi, Scandaroon, Ispir, Karakuyruk, Kespir, Kullu, Msawad, Shicki, Shafari, and Sirtikizil), high flyer (yüksek uçucu) (Katal, Van highflier, and Yasmakli), racing/homer (postacı) (Homer racing), ornamental/show (form/süs) (Gumuskuyruk, Hunkari, Karakan, Selcuklu, and Tavuskuyruk), and singer (ötücü) (Ankut trumpeter, Turkish whisperer, Demkesh, and Kumru trumpeter). Among the listed genotypes, the Dolapci, Donek, Kelebek, and Oynak pigeons can be

further classified as the spinner, and the Alabadem, Gogsu ak, Iç aglı, Ketme, Kizilbas, and Taklambac pigeons are also known as show birds (23).

The traditional structure of pigeon breeding in Türkiye has been transferred to future generations by experienced breeders for centuries. People breeding pigeons for many years and transferring their experience to each other are called pigeon fanciers (‘*meraklı*’ in Turkish). Although morphological studies have been carried out on some of these breeds (Alabadem, Ankara taklacısı, Filo uçucu güvercini, Muradiye donek, Bursa oynarı, Edremit kelebek güvercini, and Trakya makaracısı), there is still limited data on other breeds (Konya Selçuklu taklambaç), and no research has yet been carried out on Domino, Oynak, Çorum, and Manisa azmanı (1, 2, 6, 8, 9, 15, 16). Furthermore, some of these genotypes are registered as indigenous breeds (Alabadem, Bursa oynarı, Edremit kelebek, Manisa hünkârı, Muğla dalıcı güvercini, and Thrace roller) (11-14). Kelebek pigeons are bred in various regions of Türkiye and called "*Saya*" in İzmir, Manisa, Bursa, and Balıkesir provinces (22). Kelebek pigeons show morphological differences according to the regions where they are raised. The Edremit kelebek pigeon has black, blue, red or yellow marks that cover a long and wide area on the back of the neck. In addition, purple or black feathers can be found in their tail feathers (9). Kelebek pigeons are members of the diver-spinner group (23). Although they are primarily performance birds, they are also known for their variety of colors and ornamental features. Erdem et al. (9) stated that the name "*kelebek*" in Turkish (meaning ‘butterfly’) was given because their wings flutter during flight resembled that of a butterfly. The difference between the Muradiye kelebek pigeon and the Edremit kelebek pigeon is related to their flight styles. The Muradiye kelebek pigeon soars in groups in which many pigeons gather together (shoal-shaped high fly), and they descend perpendicularly to the ground during landing. Due to these differences, breeders in the Muradiye region insist that the Muradiye kelebek pigeon be considered a distinct breed from the other butterfly pigeons. Therefore, this study aimed to determine the morphological and morphometric characteristics of the Muradiye kelebek pigeon as one of the indigenous animal genetic resources of Türkiye, which has been reared for many years by breeders in the Muradiye region.

Materials and Methods

Birds: This study was conducted in Muradiye town in Susurluk county, Balıkesir. This town is located at 40°02'32" N 28°05'34" E. It is approximately 23 km from Susurluk county in the southern part of Marmara region. The study consisted of 73 pigeons (35 male and 38 female) from six different breeders (13, 14, 12, 15, 9, and 10 pigeons per breeder, respectively). The management, care, and nutrition of the pigeons were undertaken according to a routine program under the conditions provided by the breeders. The sex and age of the pigeons were determined according to the breeder’s records. The ages of pigeons were classified into two groups: 12-24 months (age group I) and 25-36 months (age group II). This study was carried out with the approval of Tekirdağ Namık Kemal University Animal Experiments Local Ethics Board (2017/09).

Morphological characteristics: Each pigeon was morphologically examined in detail concerning their plumage color, head type, eye color, head crest, presence or absence of head and body marks, wing and tail marks, and presence of muffs. Wing feathers are enumerated as primary, axillary, and secondary (p-a-s). Plumage color is traditionally entitled with body plumage color, head, wing, and tail marks in Türkiye. The plumage color names of the pigeons were recorded after consultation with local breeders. In light of this information and after much deliberation, we decided to classify the pigeons into two basic groups in terms of their plumage color. If the color of the feathers covering the whole body was uniform, it was classified as a basic plumage color, and if it was covered with feathers of two or more different colors, it was classified as an intermediate plumage color (8, 15, 16).

Morphometric characteristics: Body weight, body length, trunk length, head length, head width, beak length, beak depth, chest depth, chest width, thoracic perimeter, wing length, wing span, tail length, and tarsus diameter were individually obtained for each pigeon. The body weights of the pigeons were measured with a precision digital scale sensitive to 0.01 g (Vibra AJ-1200, Shinko Denshi Co. Ltd.). A metal ruler was used to determine body length, and a measuring tape was used to determine trunk length, wingspan, wing length, thoracic perimeter, and tail length. A digital caliper was used to determine the head length and head width, beak length and depth, chest width and depth, and tarsus diameter (500-155-30, Mitutoyo Co.Ltd.). Individual body weight and body measurements were undertaken by the same researcher in all the pigeons (Figure 1) (1, 8, 15, 16).

Statistical analysis: The general linear model (GLM) was used to identify the differences between the age and sex groups. If GLM showed an acceptable level of significance ($P<0.05$), Tukey's test was applied for post-hoc comparisons**.** Statistical analyses were performed using SPSS software version 22 for Windows. Data were presented as means ± standard error. A value of $P<0.05$ was considered statistically significant (7, 20).

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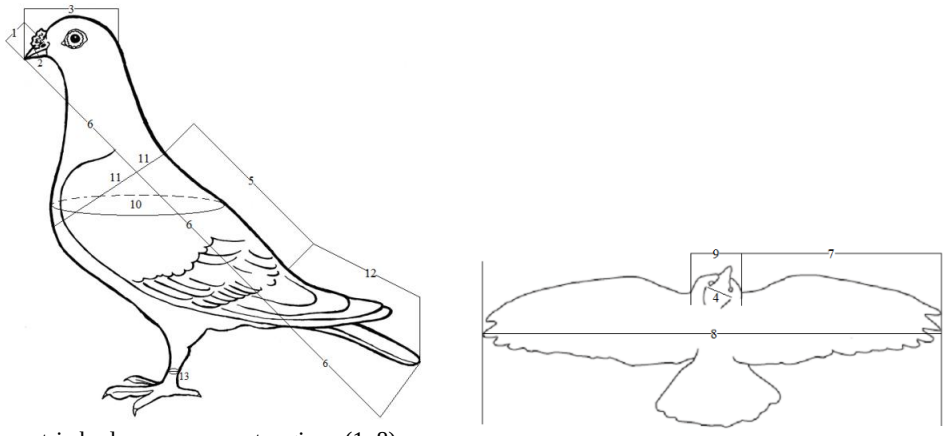

Figure 1. Morphometric body measurement regions (1, 8).
(1 - Beak length; 2 - Beak depth; 3 - Head length; 4 - Head width; 5 - Trunk length; 6 - Body length; 7 - Wing length; 8 - Wing span; 9 - Chest width; 10 - Thoracic perimeter; 11 - Chest depth; 12 - Tail length; and 13 - Tarsus diameter).

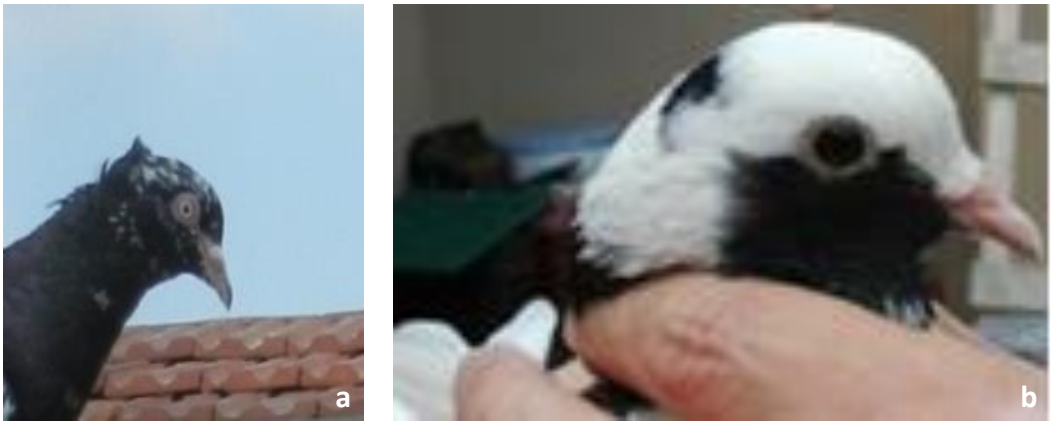

Figure 2. Eye colors of Muradiye kelebek pigeons.
a. grayish blue and b. black.

Figure 3. Basic plumage colors of the Muradiye kelebek pigeons.
a. white, b. black, and c. sable.

Results

Morphological characteristics: The descriptions and percentages of morphological characteristics of the pigeons are shown in Table 1. Based on our observations and conservations with the breeders, the head structure of the pigeons was determined to be large-sized and round-shaped (Figure 2a). Some of the pigeons (23.28%) had a crest in the form of a peak (Figure 2a and Figure 5c). The eye color of the pigeons was mostly grayish-blue (69.90%) (Figure 2a), followed by black (30.10%) (Figure 2b). Three basic plumage colors and eight intermediate plumage colors were identified in this pigeon genotype. Basic plumage colors were defined as white (*Ak*) (11.00%) (Figure 3a), black (*Arap*) (8.20%) (Figure 3b), and sable (*Samur*) (6.80%) (Figure 3c) (Table 1). In addition to these three basic plumage colors, there were eight intermediate

colors, including spotted (*Kaplan şeş*) (17.80%) (Figure 4a and Figure 4b), jackal (*Çakal*) (16.40%) (Figure 4c), black-tailed (*Kara kuyruk*) (13.70%) (Figure 5a), almond (*Bademli*) (6.85%) (Figure 5b), yellow-tailed (*Sarı kuyruk*) (6.80%) (Figure 5c), band-tailed (*Telli*) (5.50%) (Figure 6a), blue (*Mavi*) (4.20%) (Figure 6b), and black-headed (*Karabaş*) (2.75%) (Figure 6c).

The number of primary, axillary, and secondary wing primaries was determined as 10, 1, and 10, respectively in all the pigeons. The Muradiye kelebek pigeons were divided into four groups according to the number of tail primaries: 14 (24.70%), 15 (39.70%), 17 (19.20%), and 18 (16.40%) (Table 1). All the pigeons had feathers extending down from the metatarsus to cover certain digits, and they were therefore described as ʻmuffled' or 'feathered-legged' (paçalı) (Figures 4c, 5a, and 6a).

Morphometric characteristics: Body weight was significantly affected by sex. The body weight of the male pigeons was significantly higher than that of the female pigeons ($P<0.001$). Body weight was also significantly affected by age. Age group II was heavier than age group I ($P<0.001$). The male pigeons had a significantly higher trunk length ($P<0.001$), head width ($P<0.05$), beak length ($P<0.001$), beak depth ($P<0.05$), thoracic perimeter ($P<0.001$), and tarsus diameter ($P<0.001$) compared to the female pigeons. Age significantly affected body weight, chest width, and thoracic perimeter among the Muradiye kelebek pigeons. The body weight ($P<0.001$), chest width ($P<0.05$), and thoracic perimeter ($P<0.01$) of age group II were significantly higher than those of age group I (Table 2).

Figure 4. Intermediate plumage colors of the Muradiye kelebek pigeons.
a. spotted, b. head-spotted, and c. blue-jackal.

Figure 5. Intermediate plumage colors of the Muradiye kelebek pigeons.
a. black-tailed, b. almond, and c. yellow-tailed.

Figure 6. Intermediate plumage colors of the Muradiye kelebek pigeons.
a. band-tailed, b. blue, and c. black-headed.

Table 1. Morphological characteristics of the Muradiye kelebek pigeons (%).

Morphological characteristics	Description	Ratio (%)
Presence or absence of crest		
Crested	Presence of a peak crest	23.28
Non-crested	Absence of crest	76.72
Eye color		
Grayish-blue	Grayish-blue eye	69.90
Black	Black eye	30.10
Basic Plumage Colors		
White (*Ak/düz beyaz*)	White plumage	11.00
Black (*Arap*)	Black plumage	8.20
Sable (*Samur*)	Dark yellow plumage	6.80
Intermediate Plumage Colors		
Spotted (*Kaplan seş, kaplama,* or *bovma*)	The plumage was dark in color, usually black, with irregular-shaped black spots	17.80
Jackal (*Çakal*)	White plumage, with blue, yellow, red, or black feathers covering both wings	16.40
Black-tailed (*Kara kuyruk*)	The plumage color was white, and the entire tail was covered by black feathers, with large irregular-shaped black patches of identical color to those on the body. These patches were found mainly on the nuchae, neck, and under the beak.	13.70
Almond (*Bademli*)	The plumage color was white, and there were large irregular-patches on the body and a mark on the upper part of the head, which were identical in color.	6.85
Yellow-tailed (*Sarıkuyruk*)	The plumage color was white, and the entire tail was covered by yellow feathers, with large irregular-shaped yellow patches of identical color to those appears on the body	6.80
Band-tailed (*Telli*)	The plumage was white, and the middle parts of the tail feathers were colored in the form of long longitudinal thin strips, with white edges	5.50
Blue (*Mavi*)	Except for the wingtips and tail, the feathers covering the majority of the body were azuré-colored	4.20
Black-headed (*Karabaş*)	White plumage color, head and tail feathers are black	2.75
Number of wing primaries	The number of primary, axillary, and secondary wing primaries	
10, 1, and 10		100
Number of tail primaries	The number of tail primaries	
14		24.70
15		39.70
17		19.20
18		16.40

Table 2. Morphometric characteristics of the Muradiye kelebek pigeon ($X \pm S_{\bar{x}}$).

Variable	n	Body weight (g)	Body length (cm)	Trunk length (cm)	Head length (mm)	Head width (mm)	Beak length (mm)	Beak depth (mm)
Sex		***		***	-	*	***	*
Male	35	357.61±5.93	37.83±0.48	11.10±0.10	51.75±0.31	20.11±0.11	18.42±0.11	5.23±0.04
Female	38	320.06±4.76	36.69±0.43	9.91±0.13	51.36±0.32	19.65±0.17	17.53±0.16	5.04±0.06
Age group		***	-	-	-	-	-	-
I	42	325.52±4.70	37.44±0.36	10.39±0.16	51.69±0.31	19.74±0.15	18.03±0.14	5.16±0.05
II	31	355.06±7.03	36.96±0.60	10.60±0.13	51.34±0.33	20.05±0.15	17.86±0.18	5.09±0.05
Grand mean	73	338.06±4.35	37.24±0.32	10.48±0.11	51.54±0.22	19.87±0.10	17.96±0.11	5.13±0.04

Variable	n	Chest depth (mm)	Chest width (mm)	Thoracic perimeter (cm)	Wing length (cm)	Wing span (cm)	Tail length (cm)	Tarsus diameter (mm)
Sex		-	-	***	-	-	-	***
Male	35	58.96±0.94	54.49±0.57	19.69±0.15	30.61±0.21	64.92±0.84	13.92±0.15	3.77±0.07
Female	38	57.09±0.90	47.11±0.59	18.80±0.10	29.99±0.12	64.62±0.62	13.71±0.13	3.50±0.02
Age group		-	*	**	-	-	-	-
I	42	58.42±0.82	49.53±0.58	18.99±0.10	30.26±0.15	64.94±0.62	13.77±0.12	3.65±0.04
II	31	57.40±1.08	52.17±1.11	19.56±0.19	30.33±0.21	64.53±0.88	13.88±0.17	3.60±0.06
Grand mean	73	57.98±0.65	50.65±0.59	19.23±0.10	30.29±0.12	64.76±0.51	13.81±0.10	3.63±0.03

-: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ [a-c]

Discussion and Conclusion

Morphological characteristics: As we mentioned in our previous research, pigeons' head structure are described according to their shape (8). However, we observed that when describing the head structure of the Muradiye kelebek pigeon, breeders considered not only the shape but also the size. We determined that in artificial selection, breeders insisted that the head type of this genotype should be round. In recent years, there have been a growing number of studies on skull structure and beak development in birds (3-5, 10, 17). Bright et al. (4) drew attention to the variation in head and beak structures in birds, and stated that their beak morphology could evolve in harmony with their cranial morphology. We consider that head development is related to beak development in pigeons, and it can show changes due to genetic and environmental factors. In our study, the head structure of the Muradiye kelebek pigeon was defined as round-shaped, which is similar to the Edremit kelebek, Muradiye donek, Bursa oynarı, Thrace roller, and Alabadem pigeon genotypes (round-shaped) (2, 8, 9, 16, 19). Studies on skull shape in birds have shown that there may be kinship among genotypes with similar head shapes (3, 10, 24). Since these genotypes have mutual traits in terms of the head structure, molecular studies can be performed to determine the phylogenetic relationships between the Muradiye kelebek pigeon and the other five pigeon genotypes (Edremit kelebek, Muradiye donek, Alabadem, Bursa oynarı, and Thrace roller).

We determined two eye colors among the Muradiye kelebek pigeons: grayish-blue (69.90%) and black (30.10%). In the Muradiye donek pigeons, black, blue, and white eye colors were determined at the rates of 60%, 25%, and 15%, respectively. It was determined that the eye colors of the Bursa oynarı pigeon were dusty-rose (67.44%), white (23.26%), or dark dusty-rose (9.3%). In another study, it was determined that the eye color of the Alabadem pigeon was mostly black (76%), but some pigeons had grayish blue eyes (24%) (2, 8, 16). Soysal et al. (19) classified the eye color of the male Thrace roller pigeon as white (80%), dusty-rose (13%), and black (7%), and that of the female pigeon as white (58%), dusty-rose (24%), dark dusty-rose (5%), and black (7%). Edremit kelebek pigeons with black and greyish blue eyes were also described (9).

Most of the pigeons examined in the current study were non-crested (76.72%). In pigeons with a crest, this structure was described as peak-crest (23.28%) (19). The Muradiye donek pigeon was defined as non-crested (16). In previous studies, while some Edremit kelebek pigeons

were described as crested (46%), others were non-crested (54%). Similar to the Edremit kelebek pigeon, there were crested and non-crested pigeons for the Thrace roller breed (9, 19). Soysal et al. (19) described the crest structure of the Thrace roller pigeon as a straight and intact circle on the backside of the head. On the other hand, in the Edremit kelebek pigeon, this structure is not very large and consists of feathers turning upwards from the nape, and it is emphasized that the tips of the feathers forming the crest structure are in line with the highest point of the head (9). When we examined the crest shapes of the pigeons, we observed that the crest shape of the Muradiye kelebek pigeon was more similar to the Edremit kelebek pigeon than the Thrace roller pigeon. Özbaşer et al. (16) reported that according to their plumage colors, the Muradiye donek pigeons were divided into three groups as black galaca (70%), red galaca (16%), and blue galaca (14%). On the other hand, the body color was uniform in the Bursa oynarı pigeon. Since the latter is more uniform in terms of color (white, black, and white wing-white tail), so the pigeons of this breed can be named according to the distribution of the white feathers on the head (markless, browed, piebald, mottled, scarfed, and speckling) (2). In the Thrace roller pigeon, the basic plumage colors were black (*arap or mürakat*), white, red (*pal*), yellow (*kanarya*), and blue (*zavrak or küllü*), while intermediate colors described by breeders were olive-colored (*zeytini, kara pal or kara küllü*), chickpea (*nohudi or açık küllü*), and jackal (*çakal*) (19). For the Alabadem pigeon, three basic plumage colors (black, 15%; red, 6%; and yellow, 10%) and three intermediate plumage colors (citrine, 15%; ashy, 7%; chickpea, 25%; and scarlet, 22%) were identified. The Alabadem pigeon has an irregularly shaped mark on the upper part of the head, and the color of this mark is the same as the body plumage color (8).

In the current study, a similar mark was observed on the upper part of the head among the Muradiye kelebek pigeons with an almond-colored body plumage (Figure 5b). The main plumage colors of the Edremit kelebek pigeon were reported to be black-tailed (28%), purple-tailed (2%), black-neck (20%), yellow-neck (23%), red-neck (13%), black (2%), and tiger (*şeş*) (7%) (9). We consider that the spotted (kaplan-şeş) plumage color of the Muradiye kelebek pigeons, jackal (çakal) plumage color of the Thrace rollers, the speckled (şeş or tiger) plumage of the Edremit kelebek pigeons, and the red and black feather colors on the wings of the Muradiye donek pigeons (siyah galaca or kırmızı galaca) may be the common traits of these four pigeon genotypes (9, 16, 19). In previous studies, interindividual variation in terms of body color is less seen in the Thrace roller and Bursa oynarı pigeons than in other indigenous genotypes (2, 19), suggesting that these genotypes may be more uniform than the other genotypes. In the current study, it was determined that the number of primary, axillary, and secondary wing primaries was 10, 1, and 10, respectively in all the Muradiye kelebek pigeons.

The number of wing primaries (p-a-s) of the Alabadem pigeons varies between 8-1-10 (42%) and 9-1-10 (58%) (8). In a study on the Muradiye butterfly pigeon, the pigeons were divided into three groups as 10-1-12 (25%), 10-1-10 (48 %), and 10-1-9 (27%) according to the number of their wing primaries (16). In the Muradiye kelebek pigeons, we identified four groups according to the number of tail primaries (14, 24.70%; 15, 39.70%; 17, 19.20%; and 18, 16.40%). In previous studies, the number of tail primaries were reported as 14 (75%) and 13 (25%) for the male and female Muradiye donek pigeons, and 12-15 and 12-16, respectively for the Thrace roller pigeons. The Alabadem pigeon was reported to have 12 (63%) or 14 (37%) tail primaries. In another study, which was carried out on the non-crested Edremit kelebek pigeon, the number of tail primaries was 14.81 and 14.70 in the male and female pigeons, respectively (8, 9, 16, 19).

In our study, we observed that the legs of all the Muradiye kelebek pigeons were covered with feathers, called 'muffles'. Similarly, the Edremit kelebek pigeon has muffles. On the other hand, the Muradiye donek, Thrace roller, and Alabadem pigeons were found to be free from muffles (8, 9, 16, 19). In a study conducted on the Thrace roller pigeon, it was reported that breeders preferred a breed-specific eye color, medium-sized body, pearl-colored short beak, and non-muffled pattern in animals kept for breeding (19). This situation is completely different in pigeon genotypes with muffles. For the Edremit kelebek genotype, breeders prefer to breed pigeons with muffles in addition to proper eye color and body traits (9). In our field studies, we observed that artificial selection was applied to pigeons concerning some characteristics (eye color, beak color, plumage color, body traits, and the presence or absence of muffles) by breeders, and this led to morphological variation in these pigeons.

Morphometric characteristics: The beak length, thoracic perimeter, and wing span values determined for the Muradiye kelebek pigeons in age group II (17.86 mm, 19.56 cm, and 64.53 cm, respectively) were lower than those obtained from the Muradiye donek pigeons in age group II (19.25 mm, 19.65 cm, and 68.30 cm, respectively). However, the Muradiye kelebek pigeons had higher body weight, body length, head width, and chest depth values (335.06 g, 36.96 cm, 20.05 mm, and 57.40 mm, respectively) than the Muradiye donek pigeons (318.71 g, 35.03 cm, 18.23 mm, and 57.31 mm, respectively) (16). It was found that the Muradiye kelebek pigeons from age group I had a significantly lower body weight (325.52 g) and beak length (18.03 mm), and

significantly higher body length (37.44 cm), chest depth (58.42 mm), and wingspan (64.94 cm) values compared to the 17-26-month-old Bursa Oynarı pigeons (341.95 g, 26.70 cm, 26.00 mm, 56.00 mm, and 59.07 cm, respectively) (2).

In the current study, the values obtained for body weight, body length, head length, head width, beak length, chest depth, thoracic perimeter, wing span, and tarsus diameter from the Muradiye kelebek pigeons in age group II (355.06 g, 36.96 cm, 51.34 mm, 20.05 mm, 17.86 mm, 57.40 mm, 19.56 cm, 64.53 cm, and 3.60 mm, respectively) were higher than those obtained from the Alabadem pigeons of the same age range (309.50 g, 31.13 cm, 50.12 mm, 18.10 mm, 17.18 mm, 56.15 mm, 19.15 cm, 63.37 cm, and 3.30 mm, respectively) (8). The body weight and body length values obtained from the male (357.61 g and 37.83 cm, respectively) and female (320.06 and 36.69 cm, respectively) Muradiye kelebek pigeons were higher than those obtained for the same characteristics for the uncrested male (339.25 g and 36.04 cm, respectively) and female (310.50 g and 35.21 cm, respectively) Edremit kelebek pigeons (9). Shapiro et al. (18) emphasized that some morphological traits show similar patterns of variation in different breeds depending on genetic factors. They also noted the presence of variations in plumage color in the early stages of domestication, followed by plumage and structural (skeletal and soft tissue) characteristics, and finally behavioral changes. There were also variations among our indigenous pigeon genotypes in terms of structural characteristics due to domestication and artificial selection. The body weight, trunk length, head width, beak length, beak depth, thoracic perimeter, and tarsus diameter were significantly affected by sex. The male pigeons had significantly higher values for these traits than the female pigeons. These results are in line with previous studies that found statistically significant differences between the sexes in terms of body weight, head width, beak length, beak depth, thoracic perimeter, and tarsus diameter in the Alabadem and Muradiye donek pigeons (8, 16). In this study, the differences in body weight between the two age groups among the Muradiye kelebek pigeons suggest that growth continued after 24 months of age. In addition, the increase in the width and perimeter of the chest structure with age indicates that its volumetric development continues after 24 months. In bird species, the chest plays an important role in flight because it contains the heart, lungs, some parts of the air sacs, and large veins. We consider that breast development may occur more or less depending on the frequency of training, and this may play a role in the bird's performance. Erdem et al. (8) emphasized that the development of the Alabadem pigeon continued until the age of 47 months. However, to determine at what age growth and development are completed in indigenous pigeon genotypes, it is necessary to investigate their body weight and body characteristics during the entire growth period.

It is clear that there are morphological and morphometric variations among the indigenous pigeon genotypes in Türkiye. Although these differences show disparities according to the geographical regions in Türkiye, they are mostly related to the desire of breeders to obtain pigeon genotypes with different morphological and aerial-display characteristics. In terms of the head structure, we consider that the Muradiye kelebek pigeon shares some features with the Edremit kelebek, Muradiye donek, Alabadem, Bursa oynarı, and Thrace roller pigeons. The spotted (*kaplan-şeş*) plumage color of the Muradiye butterfly pigeon and the other three genotypes, the jackal (*çakal*) plumage color of the Thrace roller, the speckled (*kaplan-şeş*) plumage color of the Edremit kelebek pigeon, and the red/black galaca (*kırmızı/siyah galaca*) plumage color of the Muradiye donek pigeon may be the common traits of these pigeons. In terms of morphometric characteristics, the Muradiye kelebek pigeon can be evaluated somewhere between the Bursa oynarı and Alabadem pigeons. This genotype showed values close to the Muradiye donek pigeon. It was also determined that the Muradiye kelebek pigeon had higher body weight and length values than the Edremit kelebek pigeon. Since these genotypes share some phenotypic characteristics, the phylogenetic relationships between the Muradiye butterfly pigeon and the other five pigeon genotypes (Edremit kelebek pigeon, Muradiye donek, Alabadem, Bursa oynarı, and Thrace roller) should be determined by molecular studies.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

EE, FTÖ, EKG and MİS conceived and planned the experiments. EE and FTÖ carried out the experiments. EKG and MİS contributed to the interpretation of the results. EE took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Local Ethics Board for Animal Experiments of Tekirdağ Namık Kemal University, Türkiye (2017/09).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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The impact of peripheral blood cell ratios in dogs with diffuse B-cellsmall lymphocytic lymphoma treated with CHOP protocol

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ABSTRACT

In this study, pre-chemotherapy hematological values of 14 dogs diagnosed with diffuse B-cell small lymphocytic lymphoma were compared with the hematological data of 26 healthy dogs. Neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), platelet/lymphocyte ratio (PLR), and platelet/neutrophil ratio (PNR) were evaluated between two groups. Anemia and an increased total leukocyte count were observed in dogs with lymphoma compared to healthy ones. The PNR value was found to be significantly lower in dogs with lymphoma. It was concluded that more comprehensive studies are needed to clearly understand the diagnostic and prognostic importance of hematological parameters in B-cell small lymphocytic lymphoma of dogs.

Introduction

B-cell lymphoma has been defined asthe most common lymphoma histotype affecting dogs (25, 28). Diffuse small B-cell lymphocytic lymphoma (DSLL) is also very rare (<1%) among all canine lymphomas (8, 24).

Although B-cell lymphoma is a highly chemoresponsive neoplasm, several variations have been published in recent years with different outcomes (16, 33). Therefore, studies have focused on the prognostic markers such as stage, substage, immunophenotype, anatomical localization, hypercalcemia, histological type, and cell morphology (6, 14, 22, 27). Many of these prognostic factors are costly, difficult to perform, and data are not easy to evaluate. It is very important to determine cheap and easily applicable prognostic information before treatment in veterinary medicine.

In medicine, it is well-known the association of inflammation in lymphomagenesis and tumor progression (3, 12). So the evaluation of immune cell subsets from peripheral blood may reflect the inflammation and host-tumor interaction. In the veterinary literature, some studies showing prognostic importance of neutrophil-lymphocyte (NLR), lymphocyte-monocyte (LMR), platelet-neutrophil (PNR), and platelet-lymphocyte (PLR) ratio in dogs with lymphoma have been described (7, 11, 18). Nevertheless, B-cell DSLL is very rare, and more studies in dogs with B-cell DSLL are still required.

The purpose of the current study was to determine thepre-treatment immune cell subsets in dogs with B-cell DSLL that may be useful to manage the life quality of dogs compared to healthy individuals.

Materials and Methods

Animals: A total of 14 client-owned dogs with histopathologically confirmed B-cell DSLL diagnosed and treated at Ankara University Veterinary Training Hospital were included in this study. Inclusion criteria in dogs were a confirmed histopathological diagnosis of B-cell DSLL, available pre-treatment haematological data and, evaluation of WHO stage III/IV determined by full clinical examination, thoracic radiographs, abdominal ultrasonography and peripheral blood smears. Exclusion criteria were administration of previous chemotherapy or corticosteroids and central nervous system, cutaneousor leukemic involvement of B-cell lymphoma, and dogs having WHO stage I/II or V. Twenty-six clinically healthy dogs (control group) were used in the present study. No dogs in control group had also other inflammatory, infectious, immune-mediated, or neoplastic diseases. None of the dogs had receive medical or surgical treatment at least 3 months before CBC analysis.

Study Design: Clinical procedures including clinical examination, blood analyses, urinalysis, and imaging (abdominal and thoracic radiography and ultrasonography) were performed in all dogs. Medical data of signalment, history, tumor histopathological type, and hematological data were also recorded. CBC indices such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and platelet-to-neutrophil ratio (PNR) were calculated using absolute monocyte, neutrophil, lymphocyte and platelet values. Complete blood counts (CBC) were performed on an Exigo Eos Veterinary Hematology Analyzer with whole blood in EDTA. World Health Organization (WHO) classification of lymphoma was used in staging the dogs clinically (20). Histopathological examination was routinely performed from a lymph node extirpated totally in each dog (34). Lymphoma chemotherapy consisted of 19-week CHOP protocol including vincristine (0.5 mg/m^2 IV), cyclophosphamide (200 mg/m^2 IV), doxorubicin (30 mg/m^2 IV), and prednisolone (2 mg/kg BID with tapering for 4 weeks). Written consent was also obtained from the owners.

Statistical Analysis: All statistical analyses were performed using Stata 12/MP4 and MedCalc Version 9.2.0.1. The variables were examined with the Shapiro-Wilk test and Levene test as parametric test assumptions. The differences in hematological parameters among the two groups were analyzed using the Student t-test when parametric test assumptions were met and the Mann-Whitney U test otherwise. ROC analysis was used to determine a predicted threshold for the identification of disease (Table 3). ROC curves for the detection of B-cell lymphoma were obtained for each hematological parameter. Sensitivity, specificity and area under the curve (AUC) were calculated for each variable. All data were presented as mean ± standard deviation (SD) and median. Differences with $P<0.05$ were considered statistically significant.

Results

Data were collected from 40 clint-owned dogs. The group of 14 dogs with B-cell DSLL consisted of mixed breed (n:5, 35.7 %), Husky (n:2, 14.4 %), Labrador Retriever (n:1, 7.1 %), Golden Retriever (n:4, 28.6 %), Rotweiler (n:1, 7.1 %) and Kangal (n:1, 7.1 %). The reference population also consisted of 26 healthy dogs including Labrador Retriever (n:2, 7.7 %), Golden Retriever (n:5, 19.2 %), mixed (n:3, 11.5 %), terrier types (n:8, 30.8 %), Akbas Shepperd Dog (n:2, 7.7 %), Pekingese (n:1, 3.8 %), Pointer (n:1, 3.8 %), Pug (n:1, 3.8 %), Cavalier King Charles (n:1, 3.8 %), English Setter (n:1, 3.8 %) and Cocker Spaniel (n:1, 3.8 %). The mean age, weight and gender distributions of dogs in groups were shown in Table 1. Hematology profiles in each groups were also presented in Table 2. The most common clinical signs were generalized lympadenopathy (92.85%), anorexia (57.14%), fever (14.28%) and weigth loss (57.14%). Most dogs presented with a combination of clinical sings but no dominant combination was apparent.

Table 1. Characteristics of Dog Population with Diffuse B-cell Small Lymphocytic Lymphoma.

Characteristics	Dogs with B-cell DSLL (n:14)	Healthy Dogs (n:26)
Age (year)	7.7 ± 2.88	7.9 ± 3.63
Weight (kg)	12.3 ± 2.46	14.9 ± 4.61
Gender (n,%)	14 (100)	26 (100)
Male	8 (57.1)	18 (69.2)
Female	6 (42.9)	8 (30.8)

DSLL: B-cell Diffuse Small Lymphocytic Lymphoma; Who Stage in B-cell DSLL: II, n:1 (7.1 %); III, n:7 (50 %); IV, n:6 (42.9 %); Who Substage: a, n:11 (78.6 %); b, n:3 (21.4 %).

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Table 2. Hematology profiles in groups.

Variables	Dogs with B-cell DSLL		Healthy Dogs		P
	mean ± SD	median	mean ± SD	median	
WBC *(10^9/L)*	14.36 ± 9.31	11.89	8.13 ± 1.96	7.40	**0.002**
RBC *(10^{12}/L)*	5.65 ± 0.97	5.89	6.65 ± 0.65	6.61	**0.003**
HGB *(g/dL)*	13.35 ± 2.72	13.50	16.23 ± 1.96	16.25	**0.002**
PCV *(%)*	37.86 ± 6.56	38	45.67 ± 4.48	44.65	**< 0.001**
MCV *(fl)*	68.26 ± 4.04	68.1	69.7 ± 2.76	68.5	0.435
MCH *(pg)*	23.17 ± 3.11	23.3	25 ± 1.39	24.95	0.06
MCHC *(g/dL)*	33.72 ± 4.56	35	36 ± 0.89	35.95	0.32
RDW *(%)*	17.55 ± 6.29	16	13.17 ± 0.65	13.1	**< 0.001**
PLT *(10^9/L)*	247.15 ± 119.9	210	292.38 ± 90.06	282	0.06
PCT *(%)*	0.24 ± 0.12	0.2	0.24 ± 0,07	0.23	0.67
NLR	13.14 ± 21.33	4.75	4.49 ± 2.75	3.88	0.69
LMR	6.91 ± 10.84	2.74	3.08 ± 0.92	3.10	0.87
PLR	251.90 ± 337.01	102	214.53 ± 111.68	189.08	0.15
PNR	36.86 ± 34.07	30.06	54.41 ± 21.15	49.4	**0.011**

WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; PCV: Packed Cell Volume; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Blood Cell Distribution Width; PLT: Platelet; PCT: Plateletcrit; NLR: Neutrophil-to-Lymphocyte Ratio; LMR: Lymphocyte-to-Monocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; PNR: Platelet-to-Neutrophil Ratio; DSLL: Diffuse Small Lymphoctytic Lymphoma.

Table 3. The ROC curves of the hematological parameters for the development of B-cell lymphoma.

Variables	Threshold	Sensitivity	%95 Cl for Se	Specificity	%95 Cl for Sp	AUC	P
WBC	>10.9	53.85	25.2 - 80.7	96.15	80.3 - 99.4	0.796	**< 0.001**
RBC	<=5.98	69.23	38.6 - 90.7	80.77	60.6 - 93.4	0.790	**< 0.001**
HGB	<=14.6	69.23	38.6 - 90.7	84.62	65.1 - 95.5	0.800	**< 0.001**
PCV	<=39.5	69.23	38.6 - 90.7	100	86.7 - 100.0	0.839	**< 0.001**
RDW%	>13.8	76.92	46.2 - 94.7	88.46	69.8 - 97.4	0.874	**< 0.001**
PLT	<=210	53.85	25.2 - 80.7	84.62	65.1 - 95.5	0.685	**0.034**
MPV	>8.8	76.92	46.2 - 94.7	69.23	48.2 - 85.6	0.768	**0.002**
NLR	>4.05	61.54	31.6 - 86.0	61.54	40.6 - 79.7	0.541	0.678
LMR	>5.29	38.46	14.0 - 68.4	100	86.7 - 100.0	0.482	0.858
PNR	<=30.09	61.54	31.6 - 86.0	92.31	74.8 - 98.8	0.749	**0.002**
PLR	<=110.61	61.54	31.6 - 86.0	88.46	69.8 - 97.4	0.645	0.112

Se: Sensitivity; Sp: Specificity; AUC: Area under the curve.

Discussion and Conclusion

B-cell lymphomas delineated as diffuse or nodular pattern sare the most common lymphoma histotype (60-70%) in dogs (24, 28). Several B-cell lymphoma subtypes such as marginal zone, mantle cell, follicular and small lymphocytic lymphoma have been defined previously (35). B-cell DSLL has also been reported to account for <1 % of all canine lymphomas (8, 24).

B-cell lymphocytic lymphoma and chronic lymphocytic leukemia (CLL) are considered the same disease in humans (31). In veterinary medicine,authors have attributed the rarer diagnosis of small lymphocytic lymphoma to less preference for lymph node biopsies in the diagnostic phase of CLL (13). There is also no consensus on the distinction between leukemia and the leukemic phase of canine lymphoma in veterinary literature (1). Therefore, inclusion criteria differed on B-cell chronic lymphocytic leukemia in previous studies (2, 5). The common opinion in these studies is that it is difficult to differentiate small cell lymphoma from chronic lymphocytic leukemia. The retrospective nature of all studies and the inadequacy of histopathological examination made it difficult to reach a consensus.

A study in dogs with B-cell DSLL suggested that the aggressive progression and mitotic count of B cell-DSLL are more similar to mantle cell lymphoma in humans than small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL) (13). In the same study, it was emphasized that flow cytometry was insufficient in the diagnosis of B-cell lymphoma, and histopathology is required for a definitive diagnosis. In thepresented study, while WHO classification of lymphoma was used in

staging the dogs clinically (20), histopathological classification was performed from a lymph node extirpated totally in each dog (34). Although CLL is also characterized by circulating small lymphocytes, we prefer to use the term of B-cell DSLL based on the histopathological classification and the dogs with lymphadenopathy, liver or spleen involvement.

In consistent with the studies considering that the incidence of lymphoma mostly affects medium and largebreed dogs (38), in our study, all dogs with lymphoma were also largebreed dogs. It is thought that the reason for this situation may be related to genetic susceptibility rather than growth hormone (38). Although no gender predisposition has been reported, lymphoma is less common in female dogs because of the protective effect of endogenous estrogens (36). In the study here, the majority of lymphoma dogs (57.1%) were male dogs compatible with the results previously described (9, 18, 23, 32).

In medicine, it is well-known the association of inflammation in lymphomagenesis and tumor progression (3, 12). Necrotic and infectious processes associated with neoplasia have also caused inflammation related to leukocytosis (18, 37). Therefore, the immune cell subsets from peripheral blood may directly reflect the inflammation and host-tumor interaction (7, 11, 18). In our study, remarkably increased leukocyte levels in dogs with lymphoma compared to healthy individuals were consistent with reports previously described (19, 22).

Few studies revealed the anemia rate in dogs with lymphoma as 57%, 48%, 41%, and 53%, respectively (9, 15, 19, 21). Although the anemia pathogenesis in dogs with lymphoma remains unknown, lots of processes including shortening of erythrocyte lifespan, auto-immune hemolysis, abnormal iron metabolism, decreased production of erythropoietin, interleukins and hepcidinplay an important role in the mechanism of anemia (11, 21). Anemia (defined as PCV<%39) was also remarkable in lymphoma dogs in the present study.

In human medicine, an increase in NLR has been reported as a negative prognostic indicator of prognosis in lymphoma patients (10, 17). Rejec et al. (2017), found a higher NLR value in dogs with oral tumors (26). In this study, it was reported that this value was higher in tumors with high malignancy. The increased levels of neutrophils in dogs with cancer may be caused by acute or chronic inflammation, tissue necrosis, and stres (4). Causes of the reduction in lymphocytes seen in dogs with cancer include decreased lymphocyte production or suppression of maturation, increased peripheral destruction, generalized lympholysis, or altered circulation patterns (18). In our study, no significant difference of NLR we determined in lymphoma dogs before chemotherapy compared to healthy ones. Mutz et al. (2015), obtained similar results in their research on dogs with lymphoma as well and the researchers recommended further investigation of the correlation of lower or higher NLR values associated with less or more aggressive biological behavior (18). Lymphocyte/monocyte ratio (LMR) hasprognostic importance in lymphoma patients in humans (29). LMR has previously been reported as prognostic significance for survival in canine multicentric centroblastic diffuse large B-cell lymphoma and canine cutaneous mast cell tumors (7, 30). In our study no significant changes of LMR we defined in two groups of dogs. We think that this is due to the difference in tumor type and the LMR value being affected by non-specific etiologies. Henriques et al. have reported the unrelated situation of PLR on prognosis (11). In the study here, although lower platelet level we defined in lymphoma dogs, no statistically significant differences of PLR were possible in consistent with the reports previously defined. Although the reason for the decrease in platelet count is not fully understood, it may be the result of upregulation of inflammatory markers, bone marrow involvement, autoimmune destruction and systemic inflammatory conditions, similar to humans. Contrary to our study, in a study including animals with oropharyngeal tumors and healthy ones, authors have observed higher PNR levels in dogs with tumors (26). We think this discrepancy is related to different tumor types and tumor stages. Henriques et al reported that dogs with large diffuse B-cell lymphoma with a PNR above a certain threshold tended to have earlier lymphoma progression. (11). The data obtained from the results of the current study have shown thatPNR can be used as a marker to distinguish between lymphoma dogs and healthy ones (Figure 1).

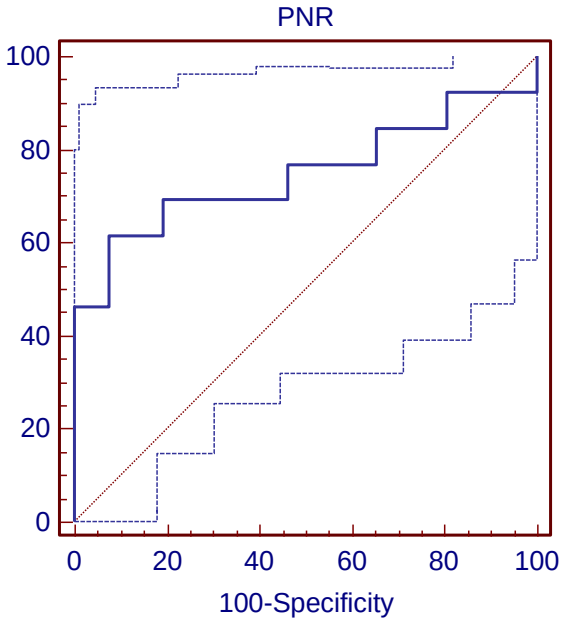

Figure 1. Display of ROC curve for threshold of PNR.

As a conclusion, in dogs with diffuse B-cell small lymphocytic lymphoma, total leukocyte count, hematocrit, and PNR values obtained from whole blood evaluation were different from those in healthy dogs. However, we also believe that more comprehensive studies are needed to understand the diagnostic and prognostic values of the mentioned parameters.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Effects of forskolin and PGE_2 on progesterone secretion by goat luteal cells at early and late stages of corpus luteum

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ABSTRACT

The aim of this research was to examine the effects of forskolin and PGE_2 on steroid synthesis in goat luteal cells, cultured at early and late corpus luteum. Therefore, the luteal cells removed from both stages of the corpus luteum were cultured with newborn calf serum for the first 18 h. Then the media was changed and different concentrations of forskolin (10, 100 ng/ml) or PGE_2 (10, 100 ng/ml) were added to the fresh media for another 96 h. The culture media was replaced every 48 h and the retrieval media was kept frozen at -20 °C, until hormone analysis. Luteal cells treated with forskolin produced between 1.87-13.17 times higher production of progesterone, in a dose-dependent manner, compared to the control at early and late stages of corpus luteum ($P<0.05$). Lower dose of PGE_2 increased the progesterone secretion between 2.19-3.28 times in luteal cells compared to the control groups at the late stage of corpus luteum ($P<0.05$), but not at early stage. The cells treated with a higher dose of PGE_2 had no significant effect ($P>0.05$) on progesterone synthesis at the early and late phases of goat corpus luteum, in comparison to control groups. As a result, this study in goat luteal cells shows that forskolin promotes progesterone synthesis at the early and late corpus luteum, but PGE_2 is only effective in cells treated with a low dose at the late stage.

Introduction

Female goats in temperate northern hemisphere parallel have repeated estrous cycles at 21-day intervals from mid-September to the beginning of February. The mating season begins when the day length decreases. Progesterone levels change during the estrous cycle. While plasma progesterone levels in the luteal phase are between 4 and 8 ng/ml, they decline to under 1 ng/ml throughout the estrous and seasonal anoestrus (10, 28).

In goats, as in other mammals, the corpus luteum (CL) has a key role in the control of the estrous cycle by synthesizing progesterone. Luteal tissue, is formed by steroidogenic and other cell types, such as endothelial cells, fibroblasts and blood cells (4, 22, 26). 3β-Hydroxysteroid dehydrogenase (3β-HSD) enzyme transforms pregnenolone to progesterone in the endoplasmic reticulum (18, 20, 24). Thus, steroidogenic cells were stained to identify 3β-HSD enzyme activity throughout the cell counting procedure in the luteal and granulosa cell studies (3, 6, 27).

Forskolin is a complex natural product derived from the *Indian Plectranthus barbatus* plant. Forskolin resensitizes cell receptors by activating adenylylcyclase and raising intracellular cyclic AMP (cAMP) levels (1). Cyclic AMP is a key signal carrier required for cells to have a proper response to hormones and other extracellular signals. It acts by activating protein kinase A, which has several functions, such as regulation of glycogen, sugar and lipid metabolism in the cells. Forskolin was widely used in the cell studies because of this steroidogenic pathways (1, 16). Previous studies have been shown that forskolin treatments may affect steroid synthesis in luteal cells cultured from bovine (19), human (29), pig (12) and rats (9).

Prostoglandins are involved in tissue remodeling, steroid synthesis, and the regulation of neovascularization of luteinized follicles. Prostoglandins, especially PGE_2, play a key role in the ovulatory process (11). Fitz et al. (14) observed that prostoglandin receptors in ovine are much more effective on large luteal cells than small luteal cells. Consistent with this data, they found that the large luteal cells treated with PGE_2 produced more progesterone than the small ones (13). This result is similiar to a study found in porcine (25). In other study, Wiesak et al. (31) showed that culture of mixed porcine luteal cells with PGE_2 enhanced progesteron secretion. None of these studies on forskolin and PGE_2 provide in-vitro effects on steroid production in luteal cells at the early and latest ages of goat corpus luteum.

The objective of this study was to investigate the effects of forskolin and PGE_2 on progesterone synthesis in goat luteal cells cultured from early (day 5) and late (day 15) stages of corpus luteum.

Materials and Methods

Animals: In this study, one male and eight female Angora goats were housed at Kırıkkale University following the animal experiments local ethics committee approval (2008/05). Female goats were placed into two groups. In addition, male goat was also housed separately in a cage next to females to induce estrous. Female goats were monitored for estrous activity twice a day by using the male goat. To prevent mating during this procedure, the male goat's waist was wrapped with a cloth. Following the estrous behaviour, the corpus luteum was surgically removed at an early (day 5) and a late stage (day 15) of the reproductive cycle.

Cell dissociation: Corpus luteum was transported to the laboratory immediately under cold chain and sterile conditions following the removal from the ovary. Firstly, the capsule of the CL was removed. Then luteal cells were separated by using the collagenase enzyme as outlined previously (3). Briefly, luteal tissues were chopped in HAM'S F-12 media including antibiotic mixture. Tissue pieces were taken into an Erlenmeyer flask and then added to HAM'S F-12 medium, which was aerated with O_2 for 3 min, containing 0.005% DNase I Type IV, 0.2% collagenase Type I, 0.5% bovine serum albumin (BSA) and 1% antibiotic/antimycotic mixture. Tissues in the flask were kept in a shaking machine (90 rpm, 37 °C) water bath for 1 h (Julabo, Labortechnik GmbH, Seelbach, Germany). The supernatant was taken into a 50 ml falcon tube after incubation. This dissociation process was repeated four times to completely detach the cells. Finally, the undigested tissue pieces were eliminated by filtering the extract collected from four cultures through a disposable strainer suitable for cells. All chemicals used in the cell dissociation were purchased from Sigma Chemical Company (Sigma-Aldrich, Co., Munich, Germany).

Steroidogenic cell identification: To identify the steroidogenic activity, luteal cells were stained for 3β-HSD enzyme according to the method reported by Arikan et al. (3). Shortly, luteal cells were fixed in 1% paraformaldehyde at 37 °C for 20 min. After that, cell suspension was centrifuged for 5 min at 400 g to remove the paraformaldehyde. Finally, the luteal cells were kept in staining solution (0.1 M phosphate buffered saline including 0.25 mM nitro blue tetrazolium, 0.1% BSA, 1.5 mM nikotinamide adenine dinucleotide hydrate, and 0.2 mM 5β-androstene-3β-ol-17 one) in the dark for 4 h at 37 °C.

Cell incubation: Luteal cells dispersed from corpus luteum were incubated as previously stated (2). Briefly, steroidogenic cells ($5x10^4$ live cells⁄well) having a positive 3β-HSD staining were incubated in culture plates with a six-well (Corning Life Sciences, Netherlands) in CO_2 incubator (Binder GmbH, CB150, Germany). Each well was filled with 2 ml medium (DMEM/F-12), including 10% newborn calf serum, 1% antibiotic/antimycotic mixture. The cells were firstly incubated for 18 h without treatment. After the first incubation the media was replaced with serum-free media, including ITS mixture (5.5 mg⁄ml transferring, 5 ng⁄ml sodium selenite, 1 mg⁄ml insulin), and plus the specific forskolin (10, 100 ng/ml) and PGE_2 (10, 100 ng/ml) concentrations for another 96 h. The doses of forskolin and PGE_2 used in the study were determined based on previous studies (1, 15). During treatment incubation, the media was replaced every 48 h and the retrieved media was stored at -20 °C until progesterone analysis. The same process was applied for the four independent cell cultures.

Monitoring cell growth: Apart from the treatment groups, an extra culture plate was incubated to check cell growth throughout the incubating period. Luteal cells on the culture dish were stained for 3β-HSD enzyme on days 3 and 5 of the culture as described before (5). After staining, culture dish was inspected by an inverted microscope (Olympus, Tokyo, Japan) to assess the attachment and growth of luteal cells incubated.

Progesterone assay and statistical analysis: The concentrations of progesterone in collected medium from all groups were analyzed by radioimmunoassay (RIA) using a kit specified for progesterone (Biosource Europe SA, Nivelles, Belgium). The manufacturer's instruction was followed. Coefficients of variation for the intra- and inter- assay were 4.2% and 8.5%, respectively. Assay sensitivity was 0.05 ng/ml. The recovery rate ranged from 92% to 103%.

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In this study, SPSS (version 14.0) was used for data analysis. The results were shown as mean ± standard error of the mean (± SEM) of four independent experiments and were considered significant at 5% ($P<0.05$). The progesterone levels produced by luteal cells were reported as ng⁄50.000 cells. The statistically significance of the differences among groups was investigated by using ANOVA followed by Duncan multiple range test.

Results

Cell staining: The cells having steroidogenic activity were stained before and after the incubation as blue/black dye following the reaction with 3β-HSD enzyme (Figure 1). The nucleus of cells and cell borders were monitored easily with this staining. Additionally, this staining procedure also allowed us to keep track of the cell attachment and the growth during the culture. The round shape of the cells was transformed into an elliptical shape, as cell membranes extended to the closest cells throughout the growth on the culture plate (Figure 2). In the case of any destruction or attachment problems of the cells during incubation could be monitored with this staining procedure.

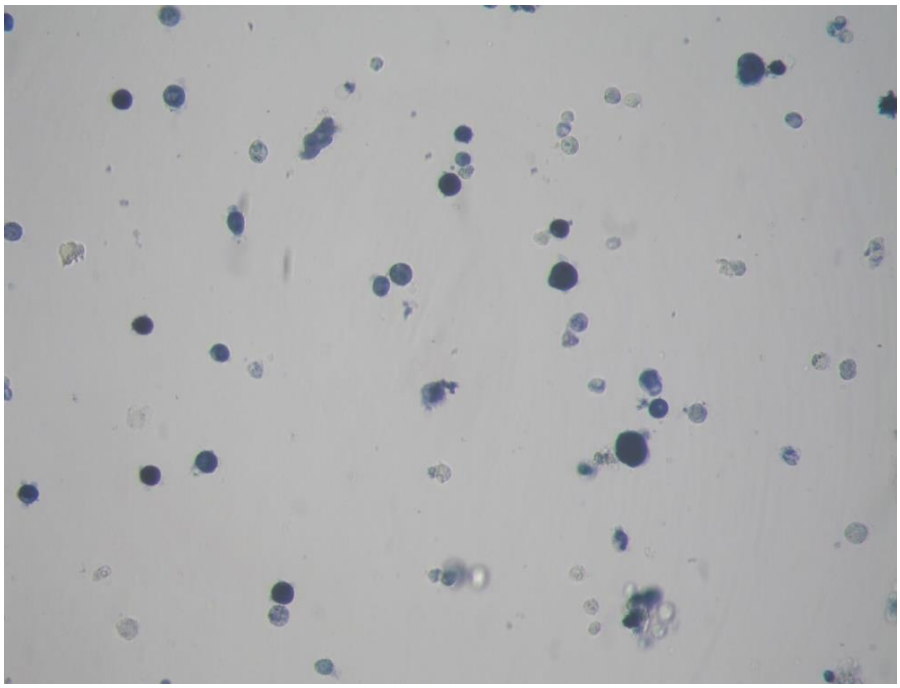

Figure 1. Image of cells stained for 3β-HSD activity in a cell suspension before incubation (magnification x200).

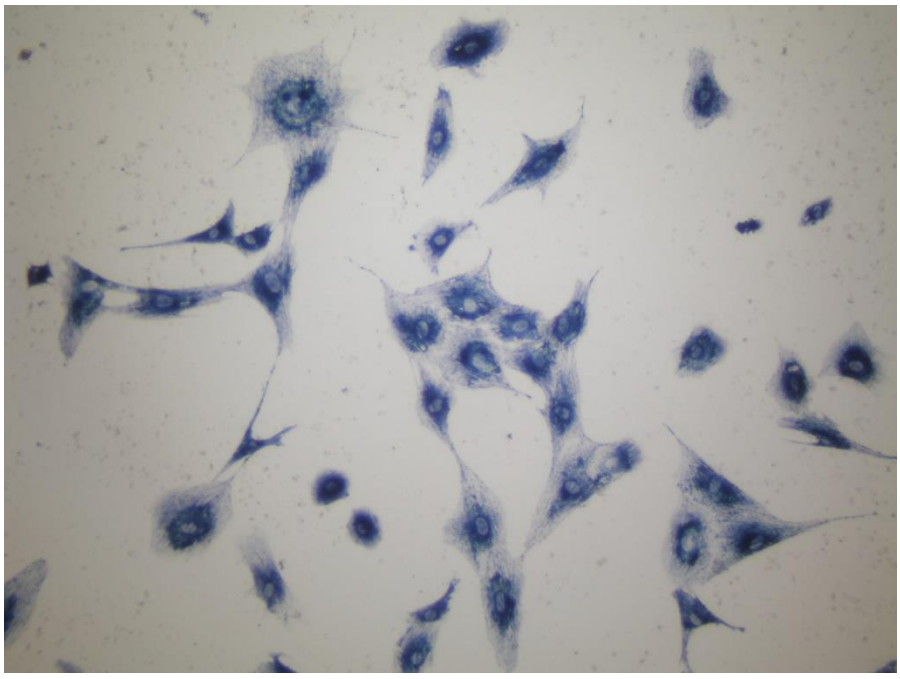

Figure 2. Image of cells stained for 3β-HSD activity on the bottom of the culture plate on day 5 of incubation (magnification x200).

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Cell treatments: The cells at early and late corpus luteum were cultured with low and high dose (10 and 100 ng/ml) of forskolin or PGE_2 for 4 days after the first incubation of 18 h. By day 5, the basal progesterone synthesis in untreated cells on days 5 and 15 of the reproductive cycle declined to 50% and 61% of the beginning value, respectively (Figure 3, 4).

The luteal cells incubated with different doses of forskolin resulted in the increase of progesterone synthesis ($P<0.05$) compared to the control on day 5 and 15 of the reproductive cycle (Figure 3, 4). This progesterone increase in cells treated with forskolin was between 1.87-4.54 and 3.26-13.17 fold on days 5 and 15 of estrous cycle, respectively. The cells incubated with high dose of forskolin produced significantly more progesterone than lower dose in both early and late stages of corpus luteum ($P<0.05$). This significant increase in cells treated with high dose forskolin was between 1.90-4.04 fold compared to the lower dose forskolin.

Lower dose of PGE_2 treatments resulted in 2.19-3.28 times more progesterone production compared to control groups at late stage of corpus luteum ($P<0.05$). The cells treated with higher dose of PGE_2 had no effect on progesterone synthesis at early or late phase of corpus luteum in comparison to the control groups (Figure 3, 4). Additionally, there was no significant difference between different doses of PGE_2 according to luteal steroid synthesis in the both groups of corpus luteum ($P>0.05$). When forskolin and PGE_2 treatments in luteal cells were compared, higher dose of forskolin had more progesterone secretion ($P<0.05$) than both doses of PGE_2 on day 5 and 15 of the reproductive cycle (Figure 3, 4).

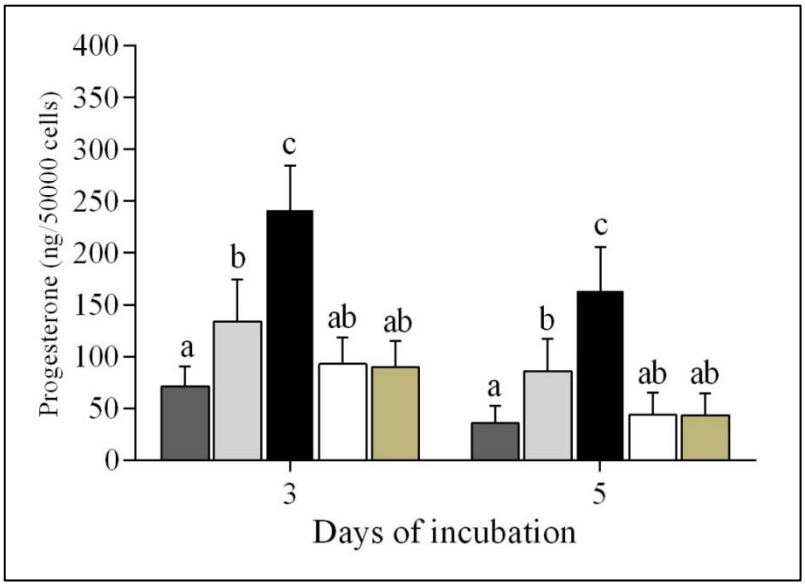

Figure 3. Effects of forskolin and PGE_2 on progesterone secretion by goat luteal cells collected at early stage of corpus luteum. Control (■), 10 ng/ml forskolin (■), 100 ng/ml forskolin (■), 10 ng/ml PGE_2 (□), 100 ng/ml PGE_2 (■). Letters indicate the difference significantly ($P<0.05$).

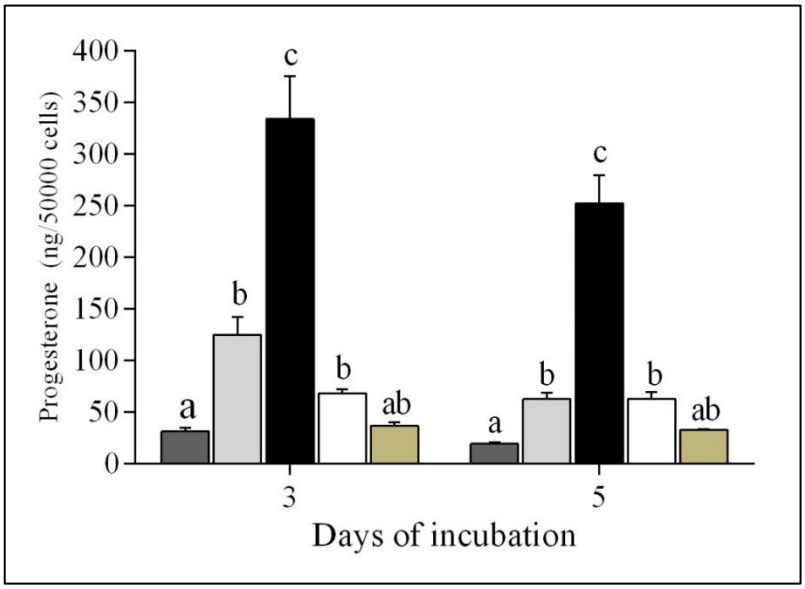

Figure 4. Effects of forskolin and PGE_2 on progesterone synthesis by goat luteal cells collected at late stage of corpus luteum. Control (■), 10 ng/ml forskolin (■), 100 ng/ml forskolin (■), 10 ng/ml PGE_2 (□), 100 ng/ml PGE_2 (■). Letters indicate the difference significantly ($P<0.05$).

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Discussion and Conclusion

This is the first report that focused on the effect of forskolin and PGE_2 on steroid production in early (day 5) and late (day 15) luteal stage of corpus luteum in goats. Although there are some studies in goat luteal cells, they are limited (3, 7, 8, 17, 23). None of them investigated the effects of forskolin and PGE_2 on progesterone synthesis in goats at the early and late luteal stages of the estrous cycle. Because of the paucity of literature, in this research, we examined the effect of forskolin and PGE_2 on progesterone synthesis in goat luteal cells on days 5 and 15 of reproductive cycle.

Steroid production in the cells isolated from goat corpus luteum was maintained for 5 days. However, basal progesterone secretion was decreased according to the incubation time in the control groups. Previous studies in luteal cells reported similar decrease in goats (17) and other species, such as bovine (2, 21) and cat (5). This decline might be due to the limited capacity in de novo cholesterol synthesis in goat luteal cells. Comparing the luteal stages of corpus luteum according to basal progesterone, in this work, luteal cells at early stage of corpus luteum produced more basal progesterone than the late stage, unlike our previous study in goats (17). This difference in our studies could be explained by incubating separately subpopulations of luteal cells in our previous study.

Forskolin is a natural product derived from the *Indian Plectranthus barbatus* plant. Alasbahi and Melzig (1) reported that forskolin resensitizes cell receptors by activating adenylylcyclase and increasing intracellular cAMP levels. Previous studies showed that forskolin induces the steroid production by using this steroidogenic pathway (1, 16). This effect was shown in luteal cells collected from human (29), pig (12), rats (9) and bovine (19). Similarly, in this study, forskolin treatments increased the progesterone synthesis between 1.87-13.17 fold compared to the control groups in the cells incubated from early and late corpus luteum. The cells treated with high dose of forskolin produced 1.90-4.04 times more progesterone than lower dose in both group of corpus luteum. These increases in steroid production could be explained that forskolin works as a cAMP pathway activator in luteal cells in goats like other species.

Prostoglandin E_2 play a key role in ovulation and work as a luteotrophic agent (11, 30). It has been shown in previous studies that PGE_2 induced more progesterone synthesis in large luteal cells than in small luteal cells from ovine (13) and pig (25). Additionally, Wiesak et al. (31) showed that incubation of mixed porcine luteal cells with PGE_2 enhanced progesterone secretion. Unlike these studies, in this research, lower dose of PGE_2 increased the progesterone synthesis in comparison to control group in the mixed luteal cells only at the late stage of goat corpus luteum. Prostoglandin E_2 had no effect on steroid production compared to untreated cells at the early phase of corpus luteum. Gregoraszczuk and Michas (15) have found similar results with our study in porcine luteal cells treated PGE_2 at early luteal stage. This could be explained that the late phase of corpus luteum includes more large luteal cells, which are rich with prostoglandin receptors, than small luteal cells.

In conclusion, this is the first time it has been shown by comparing the effects of forskolin and PGE_2 on progesterone production in early and late luteal stage of corpus luteum in goats. Culture of luteal cells with low and high doses of forskolin increased the progesterone production during five days incubation compared to the control groups at the both luteal stages. Incubation of cells with PGE_2 had no significant effect on steroid synthesis in comparison to untreated cells apart from lower dose PGE_2 treatments at the late stage of corpus luteum. These findings bring new insights to the understanding of the luteotropic effects of forskolin and PGE_2 in early and late stages of estrous cycle in goats.

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Conflicts of Interest

The authors declared that there is no conflict of interest.

Author Contributions

In general, all authors have partly contributed to all the aspects of research and analysis. Additionally, all authors provided critical feedback and assisted in the formation of this manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Decision number: 2008/05).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Determination of the effects of oxytocin and carazolol on uterine involution by pulsed-wave Doppler ultrasonography in Kıvırcık ewes

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ABSTRACT

The aim of this study was to determine the effects of oxytocin and carazolol on uterine involution in postpartum Kıvırcık ewes. Thirty primiparous suckling Kıvırcık ewes were divided into 3 groups: carazolol, oxytocin, and control. The ewes underwent transcutaneous B-mode USG in the 1st week after birth, and the examinations continued via transrectal USG until the 28th day of postpartum (pp). The pulsatility index (PI), resistive index (RI), and systolic/diastolic (S/D) velocity ratio were recorded for uterine artery by Doppler USG. On postpartum 21st day, gravid horn diameter reduction was best achieved in the carazolol group. The PI values were found to be statistically significant (P<0.001) on days 14 and 21 pp. The mean RI values of uterine artery were statistically higher (P<0.01) in the oxytocin group. The highest RI value was detected on the 14th day of pp. The carazolol group had statistically higher (P<0.05) mean S/D values when compared to other groups. In conclusion, the most effective drug used to accelerate involution was found to be carazolol. The drugs used in this study appeared to have a contraction effect on the uterine arteries.

Introduction

The postpartum period includes many progressive changes in the genital tract during the transition to the normal, pregravid state. The completion of uterine involution and regeneration of ovarian activity are essential for the resumption of the normal estrous cycles, allowing for pregnancy to occur again (27, 38, 39). Uterine involution is completed by day 28 in ewes. During this process, the width of the endometrium and myometrium decreases gradually to the minimum amount (16). The size of the uterus decreases rapidly from the 3rd to the 10th day postpartum (27). Degeneration of the caruncular and uterine surface occurs at the end of the 16th day pp (27). According to this degeneration, lochia, a thick, brown-colored discharge composed of fluids and cells originates directly in the uterus after parturition (32, 35). Doppler USG is a noninvasive technique that provides information about blood flow and vascular perfusion (15). Uterine blood flow changes have been evaluated in many species, including women (19, 26), cows (22), mares (25), dogs (10), sheep, and goats (14) to observe the progressive changes in the uterus during puerperium. Elmetwally et al. (13) used color Doppler USG to detect uterine blood flow throughout gestation in small ruminants transrectally.

They used the urinary bladder as a landmark during transrectal examinations and they activated the color Doppler function of the ultrasound device to detect and visualize the uterine arteries.

Carazolol 1's chemical structure is (carbazol-4-yloxy)-3-isopropilamino-2-propanolol; it is a beta-adrenoreceptor blocker drug and an adrenaline analogue of catecholamine. It has a blocking capacity of approximately 12 hours (2). Carazolol was reported used in many animal species to cure stress-induced circulatory disorders, especially in pigs; reduce fetal deaths caused by poor uterine contractions; shorten the parturation time that gives birth to multiple offsprings, thereby decreasing the loss of offspring; collect semen from men; artificially inseminate females; and, in combination with other drugs, treat endometritis in cattle and both prevent and treat retained placenta, which is formed due to dystocia (8, 29). Carazolol increases the uterine contractions when used via iv injection in sows (30). Januszewski (20) reported that the uterine contractions of cows treated with carazolol were more potent and the uterine involution was more rapid than those of the control cows. Delayed uterine involution in the pp period can impair the excretion of the lochia, which is a beneficial medium for microorganisms that may lead to the formation of various types of metritis. According to one study, beta-adrenergic receptor blockers used in the pp period of cows hastens the uterine involution and reduce the risk of placental retention and endometritis (34). Oxytocin is widely used in domestic animals to stimulate myometrial contractions in various conditions, including the expulsion of fetuses from the uterus, to enhance or accelerate uterine involution, or to reduce the incidence of uterine disorders (7, 23). The effects of these drugs seem to decrease with the progressive involution of the uterus with their local action in the uterine blood flow rather than systemic circulation.

The purpose of this study is to investigate the involution of the sheep uterus in the pp period following the use of carazolol and oxytocin by pulsed-wave Doppler USG. The previous studies on pp uterus were mostly performed by B-mode USG. According to the author's knowledge, this is the first report that investigates these findings.

Material and Methods

Animals and management: Thirty primiparous suckling Kıvırcık ewes (2-3 years old, gave birth to singletons) were used without any uterine infection or metabolic disease in the study. No dystocia was detected in any of the ewes included in the study. The ewes were housed in a barn with access to outside runs. The animals had ad libitum access to hay, a mineral supplement, and water. The ewes were fed with a standard ration prepared by the Department of Animal Breeding, Veterinary Faculty, University of İstanbul-Cerrahpaşa, according to their nutritional status. Ethical approval for the study was obtained from the Istanbul University Animal Research and Ethics Committee (2018/47, 31.05.2018).

Study design: The ewes were divided into 3 groups randomly. A dose of 0.5 mg/sheep carazolol (Simpanorm, Fatro Italy, 100ml) according to the drug prescription, 10 IU/ sheep oxytocin (Teknovet Oxytocin, Vetaş, Türkiye, 50 ml), and 1 ml/sheep saline solution were administered i.m. to carazolol group, oxytocin group, and the control group, respectively. All of the medications were given bid on the first 3 days pp and no injection were performed after the 3rd day of pp. The first day of the delivery was considered to be day 1 of the study.

B mode USG: Ultrasonography was performed on days 1, 2, 3, 7, 14, 21, and 28, once per day. The ultrasonographic examinations were performed 30 minutes after the carazolol injection in group C and 10 minutes after the oxytocin injection in group O. The ewes underwent transcutaneous B-mode USG (Esaote Pie Medical MyLab Five Vet, 5-8 MHz microconvex transducer, Esaote Pie Medical, Genoa, Italy) in the first week after birth to better visualize the enlarged uterus. For examination by transcutaneous technique, hair in the inguinal and caudal abdomen region was fully clipped, and a coupling gel was applied. The examinations were continued by transrectal USG (Esaote Pie Medical MyLab Five Vet, 10-MHz linear transducer, Esaote Pie Medical, Genoa, Italy) thereafter. The gravid and nongravid uterine horn diameter, the caruncular diameter were measured by B-mode ultrasonographic examination. The presence of fluid in the uterus and the character of the discharge were recorded. The uterine lumen diameter measurements were taken on the largest uterine horn without discrimination of gravid or nongravid uterine horn diameter in all animals. The completion of uterine involution was assigned to each ewe as the day when the transversal diameter of the uterus returned to its original nonpregnant size (as in the oestrus cycle), ≤ 2 cm, and the uterine cavity was empty.

Pulsed-wave Doppler examinations for uterine artery in postpartum period of ewe: The uterine artery in the pp ewe was evaluated transrectally at the standing position, as described by Elmetwally et al. (13). The feces were evacuated manually, and then 10-15 ml of ultrasound gel was applied intrarectally and a rod fixed transducer was introduced into the rectum. The color flow mode was activated on the device to identify the localization of the uterine artery. When the uterine artery was found cranio-lateral to the bladder and close to the external iliac artery, a spectral mode was activated on the device, and the insonation angle was set to ≤ 60 (Figure 1). Cardiac cycles

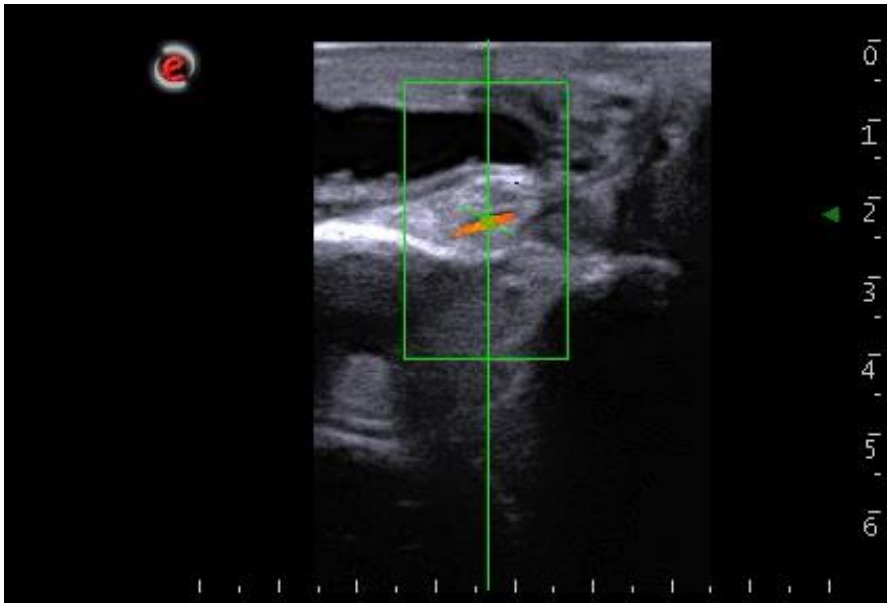

Figure 1. When the optimum color flow was achieved during ultrasonographic examinations, the pulsed-wave Doppler function was activated.

of the uterine artery have a characteristic diastolic notch. This notch is useful in demonstrating that the arteries of the sampling vessel belong to the arteria uterina (12). At least three consecutive cardiac cycles were taken in the same speed range. The hemodynamic parameters of the uterine artery, including the PI, RI, and S/D, were calculated automatically by the device.

Statistical analysis: Statistical analyses of the characteristics were conducted using the GLM repeated measures method between groups. The differences between the groups were compared with the Duncan method. The data were analyzed using an SPSS version 13.0 package program. For all statistical analyses performed, $P<0.05$ was accepted as significant.

Results

B mode USG results: The uterus could be visualised by transcutaneous USG during the first 3 days pp. However, the USG examinations were performed transrectally thereafter. The involution period was examined until the 28th day of pp in this study, and it was found to cease mostly on the 21st day, except in the 6 and 7 animals the oxytocin and control groups, respectively. In addition, involution ceased on the 14th day of pp in 1 ewe and 2 ewes from the oxytocin and carazolol groups, respectively. Thus, statistical analysis was conducted for all groups according to the 21st day of completion of the uterine involution in the study. The postpartum gravid horn diameter on the first day of pp was similar in all groups and was not statistically different between groups ($P> 0.05$) (Table 1). The gravid horn diameter decreased more rapidly in the oxytocin group than the carazolol group on day 2 pp and was statistically significant ($P<0.01$). The carazolol administration had a better effect on uterine involution on days 14 and 21 according to the pp gravid horn diameter and was found to be statistically significant on days 14 ($P<0.05$) and 21 ($P<0.001$) when compared to the other groups (Table 1). However, there were time-dependent significant differences ($P<0.001$) between all groups throughout the experiment days according to the pp gravid horn diameter results. The mean pp gravid horn diameter was 5.27±0.115 cm for all groups at the end of the 21st day of pp; gravid uterine horn diameter reduction was the best achieved (1.70±0.05 cm) in the carazolol group (Table 2). The diameter of the nongravid horn was lowest in the oxytocin-administered group compared to the other groups and was statistically significant on days 1, 2, and 3 ($P<0.01$, $P<0.001$, and $P<0.01$, respectively). But when the overall results were evaluated, carazolol was found to be more effective due to the decrease in the diameter of the pp nongravid horn on day 21 when compared to other groups and was statistically significant ($P<0.05$) (Table 1). All of the values tended to decrease throughout the experiment days, and there were time-dependent significant differences ($P<0.001$) between all groups. The thinnest mean diameter of the pp nongravid horn was that of the oxytocin-administered group compared with the other groups, and it was statistically significant ($P<0.001$). The mean pp nongravid horn diameter for all of the groups was 3.733±0.077 cm (Table 2). On day 2, the oxytocin group's pp caruncule diameters decreased more rapidly compared with the other groups, and it was statistically significant ($P<0.001$) (Table 1). The thinnest mean caruncule diameter was that of the oxytocin-administered group compared with the other groups, and it was statistically significant ($P<0.05$). All of the values tended to decrease throughout the experiment days, and there were time-dependent significant differences ($P<0.001$) between all groups (Table 2).

Table 1. The evaluation of the uterine measurements on experiment days according to the study groups.

Characteristics	PGHD (cm)			PNGHD (cm)			CD (cm)		
Groups	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**
Days	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err
1	8.78±0.24	8.34±0.18 NS	9.07±0.46	6.64±0.27^{a}	5.33±0.30^{b} **	6.96±0.48^{a}	2.25±0.09	2.07±0.12 NS	2.19±0.06
2	8.25±0.23^{a}	6.79±0.33^{b} *	7.68±0.57^{ab}	5.98±0.35^{a}	4.18±0.21^{b} ***	6.00±0.31^{a}	1.94±0.06^{a}	1.59±0.04^{b} ***	1.94±0.08^{a}
3	6.58±0.24	5.57±0.26 NS	6.13±0.42	4.64±0.39^{a}	3.28±0.16^{b} **	4.53±0.24^{a}	1.51±0.08	1.31±0.04 NS	1.48±0.10
7	3.94±0.18	4.17±0.17 NS	4.60±0.34	2.73±0.05	2.78±0.08 NS	2.96±0.14	1.04±0.04	1.07±0.02 NS	1.15±0.07
14	2.51±0.20^{b}	2.94±0.22^{ab} *	3.40±0.22^{a}	1.88±0.12	2.18±0.10 NS	2.29±0.13			
21	1.70±0.05^{b}	2.12±0.10^{a} ***	2.37±0.12^{a}	1.48±0.03^{b}	1.57±0.11^{b} *	1.80±0.14^{a}			

PGHD:Postpartum gravid horn diameter, PNGHD: Postpartum nongravid horn diameter, CD: Caruncule diameters.
NS: P>0.05, * : P<0.05, **: P<0.01, ***: P<0.001
$^{a, b, c:}$ the difference between the characteristcs indicated by different letters in the same line is significant.

Table 2. The results of the study and statistical analysis of the groups vs time.

Characteristics		PGHD (cm) Mean±Std. Error	PNGHD (cm) Mean±Std. Error	CD (cm) Mean±Std. Error	PI Mean±Std. Error	RI Mean±Std. Error	S/D Mean±Std. Error
Groups	Carazolol	5.296±0.199	3.891±0.134^{a}	1.686±0.052^{a}	2.170±0.078	0.814±0.08^{a}	12.705±0.215^{a}
	Oxytocin	4.990±0.199	3.221±0.134^{b}	1.511±0.052^{b}	2.052±0.078	0.815±0.08^{a}	11.516±0.215^{b}
	Control	5.541±0.199	4.088±0.134^{a}	1.690±0.052^{a}	1.944±0.078	0.779±0.08^{b}	9.943±0.215^{c}
		NS	***	*	N.S.	**	*
Time	1	8.730±0.182^{a}	6.311±0.209^{a}	2.171±0.054^{a}	1.824±0.079^{c}	0.798±0.007^{c}	5.833±0.156^{e}
	2	7.575±0.231^{b}	5.387±0.171^{b}	1.826±0.036^{b}	1.828±0.081^{c}	0.791±0.010^{bc}	9.443±0.250^{d}
	3	6.094±0.184^{c}	4.146±0.161^{c}	1.432±0.046^{c}	1.724±0.049^{c}	0.761±0.008^{d}	11.584±0.325^{c}
	7	4.236±0.141^{d}	2,820±0,057d	1.088±0.027^{d}	2.081±0.058^{b}	0.811±0.009^{bc}	12.688±0.288^{b}
	14	2.953±0.125^{e}	2.116±0.068^{e}		2.459±0.083^{a}	0.843±0.010^{a}	15.692±0.216^{a}
	21	2.064±0.053^{f}	1.619±0.060^{f}		2.414±0.088^{a}	0.812±0.005^{b}	13.085±0.193^{b}
		***	***	***	***	***	***
Group*Time		***	***	*	NS	NS	***
Overall Average		5.275±0.115	3.733±0.077	1.629±0.030	2.055±0.045	0.803±0.008	11.388±0.124

PGHD: Postpartum gravid horn diameter, PNGHD: Postpartum nongravid horn diameter, CD: Caruncule diameters.
NS: Non-significant, P>0.05, * P<0.05,**P<0.01, *** P<0.001
^{a,b,c,d,e,f}: The significance between the groups in the columns is shown in different letters

Pulsed-wave Doppler USG results for uterine artery in postpartum period of ewe: According to the Doppler ultrasonographic examinations, the PI values of the uterine artery were not statistically significant (P>0.05) between groups throughout the pp period (Table 3). In fact, the PI values fluctuated throughout the study period. The mean PI value for all groups was 2.055±0.045. The PI values on pp days 14 and 21 were statistically significant (P<0.001) when compared with the other examination days (Table 2). The mean RI value of the uterine artery was higher (0.815±0.08) in the oxytocin group and was statistically significant (P<0.01) when compared with the other groups. The highest RI value was detected on the 14th day of pp and was statistically significant (P<0.001).

The RI values fluctuated throughout the study period (Table 2). Although there was a fluctuation in the S/D values throughout the pp period in all groups, the carazolol group, compared to the oxytocin and control groups, had a higher mean of S/D values and was statistically significant (P<0.05) (Table 2). Specifically, the carazolol group had a higher mean S/D value and was statistically significant (P<0.001) between all groups on pp days 2., 3. and 21 (Table 3). Meanwhile, on pp days 7 and 14, the oxytocin group had a higher mean S/D, which was statistically significant (P<0.05 and P<0.001, respectively) between all groups (Table 3). The mean S/D of all the groups was detected as 11.388±0.124. The S/D values were statistically significant (P<0.001) on pp day 14 when compared to the other examination days (Table 2). There were significant time-dependent changes in all parameters (PI, RI and S/D) of the uterine artery throughout the study (P<0.001), (Table 2). The findings of uterine fluid accumulation and vaginal discharge throughout the study period are shown in Table 4.

Table 3. The evaluation of the hemodynamic changes of the uterine artery on experiment days according to the study groups.

Characteristics	**PI**			**RI**			**S/D**		
Groups	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**
Days	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err
1	1.98±0.14	1.82±0.178	1.67±0.057	0.82±0.009^{a}	0.81±0.016^{a}	0,77± 0,008^{b}	6.08±0.26	5.80±0.26	5.62±0.29
		NS			*			NS	
2	2.09±0.19	1.73±0.14	1.66±0.14	0.83±0.02^{a}	0.79±0.01^{ab}	0.75±0.007^{b}	12.34±0.54^{a}	8.04±0.36^{b}	7.94±0.38^{b}
		NS			**			***	
3	1.83±0.05	1.700±0.13	1.63±0.011	0.76±0.01	0.77±0.02	0.75±0.01	13.61±0.68^{a}	11.61±0.47^{b}	9.54±0.52^{c}
		NS			NS			***	
7	2.23±0.14	2.03±0.83	1.97±0.48	0.81±0.002	0.83±0.001	0.79±0.008	13.17±0.38^{a}	13.46±0.56^{a}	11.43±0.53^{b}
		NS			NS			*	
14	2.30±0.23	2.63±0.08	2.45±0.006	0.83±0.02	0.88±0.01	0.82±0.02	16.78±0.32^{a}	16.85±0.46^{a}	13.45±0.32^{b}
		NS			NS			***	
21	2.57±0.24	2.39±0.09	2.28±0.06	0.83±0.009^{a}	0.82±0.01^{ab}	0.79±0.005^{b}	14.25±0.44^{a}	13.33±0.19^{a}	11.67±0.33^{b}
		NS			*			***	

NS: P>0.05, * : P<0.05, **: P<0.01, ***: P<0.001

$^{a, b, c:}$ the difference between the characteristcs indicated by different letters in the same line is significant.

Table 4. The presence of uterine fluid during ultrasonographic examinations and determination of bloody vaginal discharge throughout the involution period.

	Carazolol Group										**Oxytocin Group**									
	Bloody vaginal discharge										**Presence of Uterine fluid**									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 3	+	+	-	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Day 7	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Oxytocin Group										**Oxytocin Group**									
	Bloody vaginal discharge										**Presence of Uterine fluid**									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 3	+	+	-	+	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+
Day 7	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. The presence of uterine fluid during ultrasonographic examinations and determination of bloody vaginal discharge throughout the involution period (continued).

	Control Group																			
	Bloody vaginal discharge										Presence of Uterine fluid									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
Day 2	+	+	-	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+	-	+
Day 3	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-
Day 7	-	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Discussion and Conclusion

There are very few reports on postpartum uterine involution in small ruminants, and most of them focus on postmortem evaluations (11, 17, 22); moreover, the previous studies on the postpartum uterus were mostly performed only by B-mode USG (1, 3, 18, 38). This study was designed to investigate, by pulsed-wave Doppler sonography, the involution of the sheep uterus in the postpartum period following the delivery of medications that contract the uterus. In a study, the effect of prostaglandins (PGF2α) and oxytocin on postpartum uterine involution of Awassi ewes were evaluated (3) which the postpartum gravid horn diameter was found as 6.50±0.45 and 6.45±0.25 in the PGF2α and oxytocin groups, respectively, on the 7th day after parturition. On the 21st day of pp, Ahmed et al. (3) found the diameter to be 2.92±0.43 and 3.45±0.13 in the PGF2α and oxytocin groups, respectively. The pp gravid horn diameter decreased more rapidly in the current study than in Ahmed et al.'s (3) study. In addition, carazolol (3.94±0.18 cm) was found to have a more effective reduction than oxytocin (4.17±0.17 cm) on the 7th day of pp. The involution period was completed on the 21st pp day in this study; on the other hand, Ahmet et al. (3) reported the involution period as ending on the 28th day pp. This difference could be attributed to the different medications used in the studies. The mean pp gravid horn diameter (1.70±0.05 cm) decreased the best to the lowest diameter in the carazolol group on pp day 21, compared with 2.12±0.10 cm and 2.37±0.12 cm for the oxytocin and control groups, respectively, in this study. The gravid horn diameter was found to be smaller on the first day of pp in Elmetwally and Bollwein's (14) study than in this one. However, this parameter ended up being much smaller at the end of the current study compared with their study. Although the drugs used in this study cause the uterus to contract, these differences may have resulted from the greater gravid horn diameter measurement at the begining and the different breed (German Merino vs. Kıvırcık) of ewes used in both of the studies. Bademkiran and Horoz (6) compared the effect of carazolol and cloprostenol on the involution of cows and found that carazolol had shortened the completion of the involution compared with cloprostenol, but the difference in the involution time between these groups was not statistically significant. Hauser and Bostedt (18) measured a linear reduction of the caruncule size throughout the involution period in ewes, with an initial value of 2.02±0.16 cm on day 1 pp. They found a 1.24±0.17 cm caruncule diameter on day 8 pp and stated that ultrasonographic differentiation and accurate measurements of the caruncules were almost impossible to be carried out after day 8 pp. In the current study, similar results were achieved. On day 1 pp in unmedicated; control group, a caruncule diameter of 2.19±0.06 cm was recorded, and the caruncules were undetectable on the ultrasound screen after day 7 pp. The mean caruncule diameter was 1.15±0.07 cm on the last examination on day 7 pp, and no other measurements could be achieved for caruncule diameters on pp days 14, 21, and 28 of this study, ultrasonographically. Krajnicakova et al. (21), on the other hand, detected relatively measurable caruncles on the endometrium on day 17 pp, and the caruncles were barely visible on day 25 in their study of the microscopic examination of tissues obtained from the animals postmortem. The difference in the study plans may explain the different time points at which the caruncles disappeared. In a study that investigated the vascular changes of the uterine artery during pregnancy and the postpartum period, uterine artery S/D, PI, and RI were found to increase significantly after parturition (pp day 1). The maximum levels detected at PI and RI on the 15th day pp can be associated with the morphological regression of the uterus in the ewes (37). The onset of uterine involution is evident by a decrease in uterine blood supply. The cessation of circulating hormone concentrations due to the expulsion of fetal

membranes, vasodilation effect on uterine artery was found to be disappeared indeed decreasing of local blood perfusion leads to placental detachment (28). An acute reduction of uterine vasculature during the pp period is essential for uterine involution and endometrial repair (37). In this study, the same results were achieved for PI and RI values (2.459±0.083 and 0.843±0.010, respectively) for the uterine artery 14 days after lambing. The increase in PI and RI observed in this study is compatible with the decreased perfusion and decreased blood supply to the tissue. Elmetwally and Bollwein (14) reported constantly increasing PI values throughout the involution period in ewes, especially on day 6 pp, and their study's PI values ranged between approximately 0.5 (min) and 1.5 (max) during the 21 days of pp. In this study, the PI values ranged between 1.63 (min) and 2.63 (max) in different groups on different days of the study throughout the involution period. This difference may be a result of the decreased blood flow volume caused by medications used in this study that led to the contraction of the uterus. Birth was inducted with a progesterone antagonist 41 hours prior to the estimated date of lambing in Veiga et al.'s (37) study; the mean RI was ≅ 0.55, and the mean PI was ≅ 0.80 on pp day 1. In this study, the mean RI was 0.82±0.09 in the carazolol group, 0.81±0.016 in the oxytocin group, and 0.77±0.08 in the control group, respectively on pp day 1. The mean PI was 1.98±0.14 in the carazolol group, 1.82±0.178 in the oxytocin group, and 1.67±0.057 in the control group, respectively on pp day 1. The mean values of PI and RI were clearly higher than those of Veiga et al.'s (37) study. This difference may be due to the different protocols and the administration of different hormones in the studies. The RI values found in the present study were higher than Veiga et al.'s (37) results. The higher resistance detected in this study can be an indication of the decreased uterine artery's diameter and lower blood flow. In this study, when we investigated the average RI values of all the groups, we found that the highest mean RI value belonged to the oxytocin group, and the highest value was 0.843±0.010 on the 14th day of pp, so the blood flow may have decreased due to oxytocin use. This finding supports the finding of oxytocin receptors in vascular smooth muscle by Chen et al. (9) and Miller et al. (24). Furthermore, Vedernikov et al. (36) found that oxytocin can contract the uterine artery via its own receptors. Additionally, in this study, the carazolol results for this parameter (mean RI values) were very close to the oxytocin results, both of which were statistically significant ($P<0.01$) compared to the control group's results. Veiga et al. (37) detected an increased PI and RI and a peak S/D ratio 15 days after lambing, and they reported that this increase may be attributed to the decrease in the diameter and blood flow volume of the uterine artery, which was associated with the morphological regression of the uterus; this ratio tended to decrease on the 30th pp day in their study. Our study results regarding the S/D ratio are in line with Veiga et al.'s (37) study, and the same ratio tended to decrease in this study on day 21 pp. The highest mean S/D ratio was detected in the carazolol-administered group, and it was statistically significant ($P<0.05$) when compared with the other groups. The highest S/D ratio was 15.692±0.216 on day 14 pp. Elmetwally and Bollwein (14) reported the completion of the clearance of lochia occurring between days 12 and 15 pp. In the current study, we determined that lochia was not seen in most animals past the 7th day. This difference can be attributed to the medications used in this study. Ahmed et al. (3) observed uterine fluid accumulation on pp days 4–7 days in Awassi ewes. Ababnef and Degefa (1) observed the accumulation of fluid and tissue debris during the first 4 days pp in Balady goats. Badawi et al. (5) reported uterine fluid accumulation during the first week pp in Nubian goats. In this study, bloody vaginal discharge and uterine fluid accumulation had disappeared on the 7th day pp, except for two animals that received carazolol. In the oxytocin-administered ewes, as well, bloody discharge had disappeared on the 7th day of pp, except for two ewes, and fluid accumulation ended on day 7 pp in the oxytocin group, except for 3 ewes. Bloody vaginal discharge and uterine fluid accumulation disappeared on the 7th day pp, except for 3 animals, in the control group. The results of all these studies were similar to our study, but the differences between the studies might depend on the differences in animal breed and the species used in the experiments. Marnet et al. (23) reported that a lower dose of oxytocin (2 IU in total dose) was more effective for increasing rhythmic uterine contractions than higher doses. Tian and Noakes (33) and Sheldon et al. (31) reported that none of the hormonal treatments, including prostaglandin $F_{2\alpha}$, ostradiol-17 β, or an oxytocin analogue that was used shortly after lambing, had any effect on the increasing rate of uterine involution in sheep. Assali et al. (4) measured uterine blood flow with an electromagnetic flowmeter and suggested that the nonpregnant uterus was more responsive to the vasopressor hormone than to the oxytocic fraction. However, a contrary result exists for oxytocin in the pregnant or early pp uterus. They found that the decrease in the uterine blood flow induced by oxytocic drugs was due to its local action on uterine vessels. This action could be a result of direct vasoconstriction or a mechanical constriction of the vessels produced by contracting uterine musculature.

In conclusion, the most effective drug used to accelerate involution was found to be carazolol, according to the pp gravid horn diameter regression in this study. The

increased RI and PI values may reflect the decrease in blood flow volume and uterine artery diameter. In this study, carazolol and oxytocin administration led to an increase in PI and RI values, respectively; while carazolol was found to be more effective in increasing the S/D ratio, which leads to a decrease in vessel blood flow volume. Hence, Doppler ultrasound imaging can be used successfully to evaluate hemodynamic changes in the uterine vasculature during the pp period and suggests that the drugs used in this study had a contraction effect on the uterine artery. It is thought that involution can be accelerated depending on the use of drugs that increase uterine contractions in ewes whose postpartum involution is delayed.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

SÖE, GED, AS conceived and planned the experiments. SÖE, GED, ACÇ, AS, KB carried out the experiments. GED, ACÇ, KB contributed to sample preparation. AS, GED, ACÇ contributed to the interpretation of the results. SÖE took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

Ethical approval for the study was obtained from the Istanbul University Animal Research and Ethical Committee (2018/47, 31.05.2018).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Clinical-haemostasis assessment of anaesthesia regimens in dogs with the somatic type of pain response

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ABSTRACT

The article investigates into the influence of somatic pain syndrome during osteosynthesis on dogs' clinical parameters and haemostasis. It was found, that the best variant for osteosynthesis operations in dogs is acepromazine-butorphanol-propofol-ketamine anaesthesia. This regimen has provided complete analgesia in half the time recovery of dogs without significant changes in heart rate (HR), respiratory rate (RR), blood pressure (BP), and haemoglobin saturation (SpO_2) during surgery. Acepromazine-ketamine-thiopental anaesthesia has showed pronounced analgesia with a decrease in HR and BP. Xylazine-ketamine-thiopental anaesthesia, under apparent analgesia, led to hypotension (decreased HR, BP) and hypoxia (decreased RR, SpO_2). The hypercoagulable syndrome was recorded in dogs of all experimental groups before surgery. It indicates the urgent need for its correction in the postoperative period. The data obtained will optimize the selection of drugs' combinations for dogs' anaesthesia, taking into account the type of pain response.

Introduction

Among dogs, of the total number of surgical pathologies, injuries account for about 50% (1, 29). The most common consequences of trauma are bone fractures (6, 34), accompanied by a predominantly somatic pain response (22, 24).

One of the fundamental differences in the regulation of somatic and visceral pain is their anatomical and functional organization (9, 21). Somatic pain is characterized by a clear somatotopy, the proportionality of the nociceptive stimulus' intensity feeling, and the adequate behaviour's formation and protective-adaptive reactions (14).

There is a misconception about the "universal injection" among some animal owners and veterinarians. In their opinion, it will be effective, efficient, safe, and provide the appropriate anaesthetic effect for surgery. At the same time, the problem of anesthesiological support remains unresolved (25, 41). Modern scientific research and practical experience convincingly prove the absence of such universal drugs and methods (31).

Local anaesthesia is the simplest, most accessible and safest. However, it cannot claim the role of a universal method (19, 35). Ensuring the correct level of anaesthesia should include three main components: sleep (depression of consciousness), removal of muscle tone (muscle relaxation), and general anaesthesia (analgesia) (27, 38).

It is impossible to estimate the intensity of the pain response in animals based only on clinical indicators – manifestations of consciousness and muscle tone (12, 36,

39, 40). That is since some anaesthesia regimens suppress the dynamic function of the muscles well and cause sleep while not providing enough analgesic effect (26).

Pain is an evolutionarily formed defence reaction that occurs due to the action of pain (nociceptive) factors and the weakening of analgesic (antinociceptive) mechanisms (28, 42). Both somatic and visceral pain systems are components of the overall system of pain excitation, which determines the formation of various behavioural and autonomic manifestations of pain. It's considerable for choosing an adequate anaesthesia regimen depending on the anatomical and topographic data (15). Morphological and functional connections of nociceptive systems make it possible to link two fundamentally different types of pain perception: primary rapid and localized associated with direct effects on nerve endings and secondary (inflammatory) diffuse with negative vegetative-motor manifestations, often combined with the trauma of internal organs. Its' degree of manifestation, as well as the strength of the pain response, are directly related to the plasma systems' activity and their interaction (13, 16, 17).

Surgeons often use reduced doses of the basic anaesthetic agent in combination with various neurotropic drugs to reduce the toxicity of anaesthesia drugs. This way, doctors are also trying to prevent adverse effects and maintain a sufficiently high level of analgesia. But almost no attention is paid to the rational selection of drugs' optimal combinations taking into account the visceral and somatic components of pain (5, 10).

The recent studies' analysis indicates that currently in the issues of dogs' quality anaesthesia, there are unresolved gaps in terms of the species' sensitivity to pain and reactivity of the haemostasis system in choosing an adequate anaesthesia scheme. It became the basis for our work. The study aimed to determine the clinical and experimental justification of anaesthesia regimens in dogs with the somatic type of pain response.

Materials and Methods

The work was performed at the Department of Surgery and Diseases of Small Domestic Animals of Bila Tserkva National Agrarian University (Kyiv region, Ukraine) during 2019-2021. The research protocol of the present study was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Approval number: 23.10.2018 / №2, conclusion 5). The material for the study was clinically healthy and sick dogs admitted to the Clinic of Small Animal Diseases of the University.

Animals and study design: Studies of the somatic type of pain response were performed in dogs with bone fractures during their surgical treatment (osteosynthesis). Animals were selected by the method of analogues. When forming groups conducted a general clinical study of basic vital signs, and studies of morphological and biochemical parameters of the blood. If necessary, instrumental examinations were performed (radiography and ultrasound examination). Dogs with fractures of the femur or humerus aged 1 to 10 years (n = 45), depending on the scheme of anaesthesia, were divided into three groups (n = 15). In group 1, 15 min before the injection of the main anaesthetic, intramuscularly 1% solution of acepromazine (0.5 mg/kg) was injected in combination with 5% ketamine (8 mg/kg) for premedication and anaesthesia. A 5% sodium thiopental solution (5 mg/kg) was used immediately before osteosynthesis intravenously slowly, and 2.5 mg/kg was used to prolong anaesthesia. In group 2, 2% xylazine (2 mg/kg) combined with 5% ketamine (8 mg/kg) was administered 15 min before the main anaesthetic injection intramuscularly for premedication and anaesthesia. Immediately before surgery, a 5% sodium thiopental (5 mg/kg) was used intravenously slowly, and 2.5 mg/kg was used to prolong anaesthesia. After premedication with acepromazine (0.5 mg/kg) immediately before surgery, animals of group 3 were intravenously administered 0.3 ml/kg of anaesthetic mixture. One ml of this mixture contained 7.5 mg of propofol and 12.5 mg of ketamine. For deepening or prolongation of anaesthesia, a mixture (1 ml of 5% ketamine solution + 3 ml of 1% propofol solution) was injected at a dose of 0.15 ml/kg.

Clinical study: The clinical study of anaesthetized animals was performed according to the following scheme. The stages of anaesthesia were determined: the beginning of anaesthesia; the stage of surgical tolerance; recovery after anaesthesia. The main criteria for their evaluation were dilation or narrowing of the pupils, age, palpebral, anal reflexes, and chewing muscle tone. Tissue perfusion was assessed by pulse oximetry or by pressing a finger on the gums, recording the time of filling the capillaries with blood (19). Clinical indicators were registered at the following stages: before, during, after anaesthesia, and in the most traumatic surgery moments. Heart rate and were determined by palpation of the heart and auscultation with a stethoscope. The frequency and depth of respiration were controlled by observing the movements of the chest and abdominal wall.

Indicators of hemodynamics and tissue perfusion were determined using a resuscitation and surgical monitor UM-300R (Yutas, Ukraine, Kyiv). Indicators such as heart rate (HR), respiratory rate (RR), blood pressure (BP), electrocardiogram (ECG), and arterial blood haemoglobin saturation with oxygen (SpO_2) were monitored.

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Study of the haemostasis system: Blood samples from animals were taken before anaesthesia and during 1 hour after surgery. The functional state of the haemostasis system was determined using the coagulometer HumaClot DUO Plus (Human GmbH, Germany) on the following indicators: fibrinogen concentration, fibrin stabilizing factor activity (FXIII), soluble fibrin concentration, prothrombin time.

Statistical analysis: Statistical processing of the results was carried out using Statistica 13.3 IT Application. Multiple variance comparisons were performed using the Fisher distribution (ANOVA). Variance analysis (ANOVA) was used for determining a statistically significant effect on the factors researched. The reliability of the data was evaluated using the Fisher F-test with a $P<0.05$ confidence level.

Results

The proposed general anaesthesia regimens were accompanied by central nervous system depression, loss of consciousness, skeletal muscle relaxation, and analgesia. In dogs with bone fractures, the onset of anaesthesia in the 1st and 2nd groups did not exceed 1 min, while in the 3rd, it was 1.38 min (Table 1). During a study of hemodynamic parameters (Table 2) in animals it was found that HR is at the upper limit of the physiological norm, decreasing during anaesthesia. The RR in the groups ranged from 20.9 to 22.0 breaths/min, which was at the upper limit of the physiological norm. During anaesthesia, it decreased in dogs of the 1st and 2nd groups by 17.2% and 35.9% ($P<0.05$), respectively. Instead, in dogs of the 3rd group that indicator did not change significantly. Monitoring of the arterial blood haemoglobin saturation level with oxygen showed the hypoxic state development in dogs of the 2nd group during anaesthesia.

Table 1. Clinical characteristics of intravenous anaesthesia different schemes in dogs during osteosynthesis surgeries, n=15.

Indicators	Animal groups, anaesthesia schemes		
	1st, acepromazine-ketamine-sodium thiopental	2nd, xylazine-ketamine-sodium thiopental	3rd, acepromazine-butorphanol-propofol-ketamine
Beginning of anaesthesia, min	0.50±0.04	0.46±0.07	1.38±0.06*
Duration of anaesthesia, min	29.0±0.66	32.3±0.68*	30.2±0.59
Analgesic effect	++	++	+++
Respiration effect	↑↓	↓↓	↑
Cardiovascular effect	↑↓	↓↓	↑
Recovery after anaesthesia, min	60.7±4.2	68.1±4.1	30.1±3.5*

Note: 1. Effect: ++ – expressive; +++ – absolute; 2. Impact: ↑ – increased, ↑↓ – short-term increase followed by decrease, ↓↓ – significant decrease; 3. The value of the indicator P: * – $P<0.05$, compared with the 1st group.

Table 2. Haemodynamic and respiratory parameters in dogs for osteosynthesis and various anaesthesia regimens, n=15.

Animal groups, anaesthesia schemes	Period	HR, beat/min	BP systolic, mm Hg	BP diastolic, mm Hg	BP mean, mm Hg	RR, breath/min	SpO_2, %
1st, acepro-mazine-ketamine-sodium thiopental	I	113.2±4.1	134.1±4.0	80.2±2.9	98.2±3.4	21.5±1.0	96.8±0.8
	II	98.4±4.2*0	117.6±3.8*0	69.0±3.0*0	85.2±3.2*0	17.8±0.9*0	95.3±0.7
	III	101.7±4.3	115.3±3.7*	68.9±3.1*	84.4±3.3*	18.1±1.0*	95.8±0.7
	IV	111.8±4.00	120.1±3.8*	77.4±3.1	91.6±3.2	19.4±1.1	97.5±0.6
2nd, xylazine-ketamine-sodium thiopental	I	109.7±3.9	128.4±4.2	80.4±3.3	96.4±3.7	20.9±1.1	98.1±0.7
	II	71.5±3.8*0	105.7±3.0*0	60.3±2.2*0	75.4±2.9*0	13.4±0.7*0	88.4±0.6*0
	III	74.8±3.7*	102.5±2.9*	60.1±2.2*	74.2±2.9*	13.9±0.7*	89.3±0.7*
	IV	89.3±4.0*0	107.1±3.2*	67.2±2.5*0	80.5±3.1*	16.2±0.8*0	94.9±0.8*0
3rd, acepro-mazine-butorphanol-propofol-ketamine	I	115.1±4.2	129.4±4.2	78.9±3.1	95.7±3.4	22.0±1.0	97.2±0.7
	II	122.4±4.6	130.7±4.1	75.8±3.0	94.1±3.3	20.4±0.9	97.0±0.7
	III	120.1±4.3	128.1±4.0	75.1±3.0	92.8±3.3	20.8±0.9	96.8±0.8
	IV	112.2±4.1	127.3±3.9	74.4±3.1	92.0±3.2	21.2±1.0	98.1±0.8

Note: 1. Periods: I – pre-anaesthesia, II – during anaesthesia, III – the most traumatic moments of the surgery, IV – after the surgery; 2. The value of the indicator p: * – $P<0.05$, compared with pre-anaesthesia; 0 – $P<0.05$, compared with the previous indicator for the group.

Table 3. The state of the blood clotting system in dogs during osteosynthesis surgeries.

Indicators	Fibrinogen, g / L	Soluble fibrin, mg%	FXIII, %	Prothrombin time, sec
Clinically healthy (n=15)	2.53±0.13	0	99.2±2.4	16.1±0.3
Pre-anaesthesia (n=45)	2.03±0.10^{0}	45.2±2.1^{0}	69.4±3.1^{0}	21.7±0.4^{0}
After the surgery, n=15				
1st, acepromazine-ketamine-sodium thiopental	3.12±0.17*	47.7±3.2^{0}	62.3±4.3^{0}	22.1±0.3^{0}
2nd, xylazine-ketamine-sodium thiopental	2.80±0.19*^{0}	50.1±3.4^{0}	60.1±4.1^{0}	22.4±0.4^{0}
3rd, acepromazine-butorphanol-propofol-ketamine	3.05±0.21*	49.8±3.6^{0}	64.5±4.0^{0}	22.3±0.3^{0}

Note: * – P<0.05, compared with pre-anaesthesia; ^{0} – P<0.05, compared with clinically healthy dogs.

Haemostasis system's activation with the formation of the hypercoagulable syndrome was detected in dogs with long tubular bones' fractures (Table 3). It was expressed in a decrease of the fibrinogen concentration by 19.8% (P<0.05) as well as in the appearance of its' metabolite (soluble fibrin) in blood plasma at a quite high concentration. In addition, the prothrombin time was prolonged by 34.8% (P<0.05), and the activity of FXIII decreased by 30.0% (P<0.05). Thus, according to haemostasiological signs, the consumption coagulopathy condition was registered in dogs with bone fractures.

Discussion and Conclusion

The somatic type of pain reaction is stipulated by injury to bones, muscles, skin, joints, tendons, and ligaments. This type of pain reaction is characterized by acute, more intense than visceral, nociceptive impulse (9, 14). Clinical-experimental substantiation of dogs anaesthesia with the somatic type pain response surgical interventions was performed on the animals with fractures of the femur or humerus. Osteosynthesis method the animals underwent depended on the anatomical and topographic location and the nature of the fracture. Particular attention in the perfomance of somatic pain anaesthesia was paid to the nociceptive protection of animals.. After all, it is for this type of pain response that adequate analgesia is important because the intensity of pain stimulation increases significantly.

In present studies, traditional and new anaesthesia regimens have been used for somatic pain in dogs with bone fractures (8). To improve the anaesthetic properties of the acepromazine-butorphanol-ketamine regimen and reduce the dose of the latter, we have proposed its combination with propofol. Propofol has rapid and smooth induction, pronounced hypnotic and amnestic effects, rapid recovery after anaesthesia, and rare side effects. At the same time propofol anaesthesia may be accompanied by hypotension, bradycardia and direct myocardial depression, impaired baroreflex mechanisms, nervous system depression, suppression of the nervous system (38). In contrast to propofol ketamine has a moderate hypertensive and a pronounced analgesic effect. It stimulates the sympathetic nervous system and its general analgesic effect is 3-4 times longer than propofol (2, 4). Thus, by combining these two anaesthetics into one anaesthesia regimen, we eliminated the negative effects and complemented the positive ones (32).

Effective control of anaesthesia is important, which is currently achieved only by using inhalation anaesthetics due to their ultrashort action. But, according to the results of current studies, the combined use of short-acting (ketamine) and ultra-short-acting (propofol) anaesthetics also has made it possible to achieve adequate anaesthesia control. With the introduction of the propofol, the action of which lasts an average of 4-6 min, conditions are created to achieve good control of anaesthesia without inhalation anaesthetics.

An equally important effect achieved was the rapid recovery of dogs after anaesthesia. Other authors also testify to good anaesthesia control at the use of propofol in other drug combinations (30, 38, 43). In particular, the researchers point out that propofol ranks second after inhaled desflurane, ahead of isoflurane and sevoflurane in the rate of patients' recovery after anaesthesia.

According to the results of current studies, various combinations of neuroleptics (acepromazine, xylazine) with general anaesthetics (ketamine) provided a rapid introduction of animals into anaesthesia (0.46-1.38 minutes) with a duration of 29-32 minutes. At the same time, the successful anaesthesia of surgical care is a multifactorial process, so the duration of anaesthesia cannot be decisive.

It is known (7, 18), that anaesthetics can affect the cardiovascular system in different ways. Some of them, such as ketamine, stimulate the increase of BP in dogs, others (xylazine, acepromazine, propofol) on the contrary, cause hypotension. For the surgery process, it is important how these drugs affect the cardiovascular system when they are used in anaesthesia regimens that combine antihypertensive and hypertensive preparations. According to the results of the current research, the peculiarities of their interaction were established. Using acepromazine with ketamine, the latter neutralizes the

hypotensive effect of acepromazine. In the case of a combination of xylazine with ketamine, the hypotensive properties of the former outweigh the hypertensive ability of the latter. Therefore, dogs with impaired or cardiovascular insufficiency should be closely monitored: the use of xylazine-ketamine in dogs during surgery led to the deprinsion of the respiratory system and could cause it to stop.

Nociceptive stimulation with insufficient analgesia during the most traumatic moments of the operation briefly accelerates RR, while with its' sufficient adequacy it remains stable.

A significant decrease in HR and BP in dogs after anaesthesia showed that xylazine-ketamine-thiopental anaesthesia creates a risk of haemodynamic disorders. That is especially important in animals with cardiovascular failure and significant blood loss. In contrast, acepromazine-butorphanol-propofol-ketamine anaesthesia has no significant adverse effect on dogs' haemodynamics during osteosynthesis.

Under acepromazine-ketamine-thiopental anaesthesia, ketamine counteracts the hypotensive effect of acepromazine and sodium thiopental and is therefore accompanied by a moderate effect on haemodynamics.

We found that xylazine-ketamine-thiopental anaesthesia developed a hypoxic state during osteosynthesis that can lead to respiratory arrest. Instead, acepromazine-butorphanol-propofol-ketamine or acepromazine- ketamine-thiopental anaesthesia had no significant effect on the respiratory system.

Only a few studies are devoted to the research of the anaesthetics effect on the haemostasis system (3, 23). In these studies, acepromazine or xylazine-thiopental anaesthesia without surgeryhad no haemocoagulation changes. Abdominal pathology in dogs causes the development of a hypercoagulation state (11, 20, 33, 37). Comprehensive data on the state of haemostasis in dogs during osteosynthesis have not been published. According to the present study results of the haemostasis system, repeated bone injury, caused by osteosynthesis, with adequate analgesia provokes moderate hyperfibrinogenemia and thrombinemia with a deficiency of plasma coagulation factors, deepens the hypercoagulable state without significant effect on its components.

Summing up, the study results showed that the neurohumoral mechanisms of pain regulation and modern schemes of anaesthesia are justified. The combination of acepromazine-butorphanol-propofol-ketamine provides complete analgesia with twice faster recovery of dogs from anaesthesia without significant changes in HR, RR, BP, and SpO_2 during surgery. Acepromazine-ketamine-thiopental anaesthesia is accompanied by pronounced analgesia with a decrease in HR and BP. At the same time, xylazine-ketamine-thiopental anaesthesia in severe analgesia leads to hypotensive and hypoxic conditions. Further research could usefully explore the effect of different anaesthesia regimens on the clinical-haemostasis condition of dogs in abdominal surgery, with a visceral type of pain response. Taken together, the optimal option for osteosynthesis in dogs is a combination of acepromazine-butorphanol-propofol-ketamine. However, like others, this anaesthesia regimenrequires correction of the hypercoagulative syndrome in the postoperative period.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

SR, MR, AY and TB-K conceived and planned the experiments. SR and MR carried out the experiments. SR, MR, and AY contributed to sample preparation. SR, AY and TB-K contributed to the interpretation of the results. TB-K took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Approval number: 23.10.2018 / №2, conclusion 5).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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The quantitative evaluation of cardiac structures and major thoracic vessels dimensions by means of lateral contrast radiography in Wistar albino rats (*Rattus norvegicus*)

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ABSTRACT

The aim of this study was to define reference values for vertebral heart score (VHS) and modified left atrium (LA)-VHS, cardiac structures, and major thoracic vessels measurements and ratios obtained from thoracic contrast radiography Wistar albino rats. VHS, modified LA-VHS, left (LV) and right (RV) ventricles, interventricular septum (IVS), aorta (Ao), caudal vena cava (CaVC), and fourth thoracic vertebrae (v) length (T4) were measured from left lateral thoracic contrast radiographs of 50 young, healthy adult male Wistar albino rats. LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, and CaVC/ Ao ratios were calculated from these values. Measurements were performed by two observers unaware of the signalment data for interobserver repeatability analysis. Median values and references ranges were 8.2v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.1.37) for CaVC/Ao. Further studies are now needed to determine whether measurement values obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be used as a reference baseline for both pet and laboratory rats.

Introduction

Cardiac diseases are seen in both pet (12) and laboratory (36) rats. Spontaneous cardiomyopathy is reported to be relatively common in elderly rats, and the presence of left atrial and ventricular thrombosis is associated with this cardiac disease (30). Rat models are frequently used in cardiovascular research. These models include coronary ligation-induced cardiac hypertrophy, aortic banding (overload-induced cardiac hypertrophy), diabetic cardiomyopathy, the desoxycorticosterone acetate/salt model of hypertension, renal ischemia via renal artery stenosis, and aortocaval fistula (3, 10). Imaging methods are important for the diagnosis and follow-up of the progression of cardiac diseases in both pet rats and experimental cardiovascular rat models. Although echocardiography is the primary diagnostic method in cardiac diseases, it is not routinely used in rats. The size and shape of the heart and thoracic vessels can be evaluated using thoracic radiography (21), which is easier to perform and less expensive than echocardiography.

Since 1995, a quantitative assessment of the size of the cardiac silhouette from thoracic radiography has been performed via the vertebral heart score (VHS) system, which is simple, objective, and repeatable (8). Using this system, cardiac axes measured from thoracic radiographic views are compared with the length of specific thoracic vertebrae, thus minimizing the error associated with subjective interpretations and interpatient variability (37).

Normal VHS values have been established for Sprague-Dawley and Wistar albino rats (13, 15), as well as for various other animal species (5, 11, 14, 16, 17, 26, 32, 37, 38).

Radiographic indices that permit evaluation of the left atrium (LA) at lateral thoracic radiography are available. Vertebral left atrial size (29) and radiographic left atrial dimension (34) are widely recognized indices for dogs. LA-VHS, modified from VHS, was defined by Schober et al. (35) for cats. However, these indices have not been reported for rats.

Obtaining thoracic radiography at the peak of inspiration in rats is problematic due to their high respiration rate (13). The cranial border of the cardiac silhouette on lateral radiographs is also insufficiently clear due to soft tissue opacity of the cranial and ventral mediastinum. The apex of the heart may be indistinct on radiographs obtained during expiration as it may be located beyond the diaphragm. These factors can all adversely affect VHS measurements in rats. Our study hypothesis was that the sizes of the heart and LA can be directly evaluated with their distinct borders and that the widths of the left (LV) and right (RV) ventricles, the thickness of the interventricular septum (IVS), the widths of the aorta (Ao) and caudal vena cava (CaVC) can be measured using contrast radiography, independently of the cardiac silhouette.

The aim of this study was to define normal values for radiographic indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, fourth thoracic vertebrae length (T4)), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/ Ao) obtained from left lateral thoracic contrast radiography of 50 healthy, adult male Wistar albino rats.

Materials and Methods

Animals: The experimental protocol was approved by the Akdeniz University animal care ethics committee on the use of animals, Türkiye (No: B.30.2.AKD.0.05.07.00/27). Fifty healthy, young adult, intact male rats (*Rattus Norvegicus*, Albinus, Wistar) were housed in the Akdeniz University Experimental Research and Application Center (Türkiye), in groups of four to six animals (of the same sex) at 50-60% humidity and 20-21°C in a 12-h dark/light cycle. Standard rat chow and free access to water were provided.

Rats underwent daily physical examinations during the study. Neither cardiovascular nor pulmonary abnormalities (murmur, arrhythmia, abnormal respiratory sounds, etc.) were detected in any of the rats included in the study, and their hydration status was normal.

Anesthesia protocol: Radiographs were obtained from all rats under general anesthesia. The anesthesia protocol was applied as previously described by Dias et al. (13). Briefly, the rats were placed in an induction chamber, and anesthesia was induced using 5% isoflurane (Aerrane Volatil®, Eczacıbası-Baxter, Istanbul, Türkiye) plus oxygen (2 L/min). Anesthesia was maintained using 1–3% isoflurane delivered in oxygen at 1 L/min, by means of a small face mask and non-rebreathing circuit. All animals maintained spontaneous breathing throughout the entire procedure, except for the exposure time. Intravenous access was established in the tail vein of each animal under anesthesia for the administration of contrast medium.

Thoracic contrast radiography: Each animal was imaged in the left lateral projection (X-ray tube: ORIX-65, Ardet®, Istanbul, Türkiye. Parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance). The animal was placed lying on its left side on the X-ray cassette. The thoracic legs were then pulled cranially, and the beam was centered at the level of the thorax between the scapulohumeral joint and the last rib. Contrast radiography was performed after plain radiography (Figure 1A). However, radiographic measurements were performed only from contrast radiography. Contrast radiographs were obtained by bolus injection of 0.75 ml of non-ionic opaque contrast agent (300 mg I/ml, Iohexol, Omnipaque®, Opakim, Türkiye) from the tail vein. Exposure was performed as soon as the contrast agent injection was completed (Figure 2B). A positive pressure breath-hold was performed at the time of radiographic exposure in order to obtain radiographic images at the inspiration peak. While positive pressure breath-hold was being employed, care was taken not to cause pulmonary hyperinflation, to preserve the opacity of the CaVC, and not to cause excessive caudal displacement of the diaphragm. Otherwise, all animals continued breathing spontaneously throughout the entire procedure.

Radiographs with substantial symmetry/superposition of the ribs and scapulae and widely accessible cardiothoracic structures with adequate contrast and pulmonary inflation were considered to be of sufficient diagnostic quality.

Radiographic images obtained using a computed radiography reader (FCR Prima T2, FujiFilm®, Tokyo, Japan) were stored. These radiographic images were anonymized and randomized and then evaluated by two observers using commercially available computer software (Image Intelligence™, FujiFilm®, Tokyo, Japan). The observers were blinded to the age and body weight of each rat. They were able to manipulate the images as needed, including by changing the window width, window level, and magnification, and measurements were made of the radiographic cardiac indices and ratios. Corresponding mean radiographic cardiac indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, T4), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/Ao) were calculated for each observer, these being used for statistical purposes.

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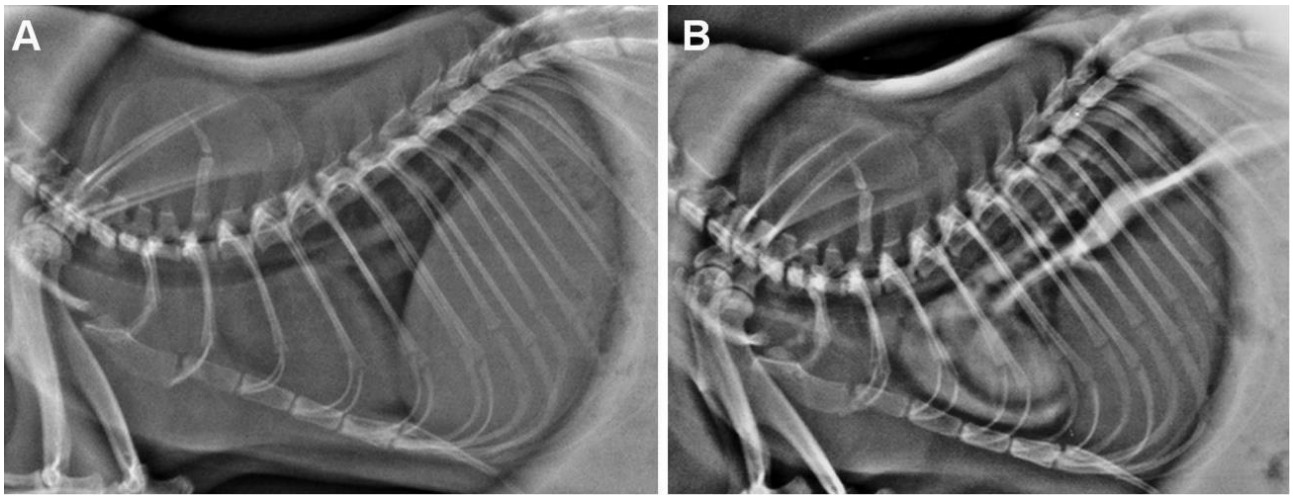

Figure 1. Plain (**A**) and contrast (**B**) radiographic studies of the thorax in the left lateral recumbency (parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance).

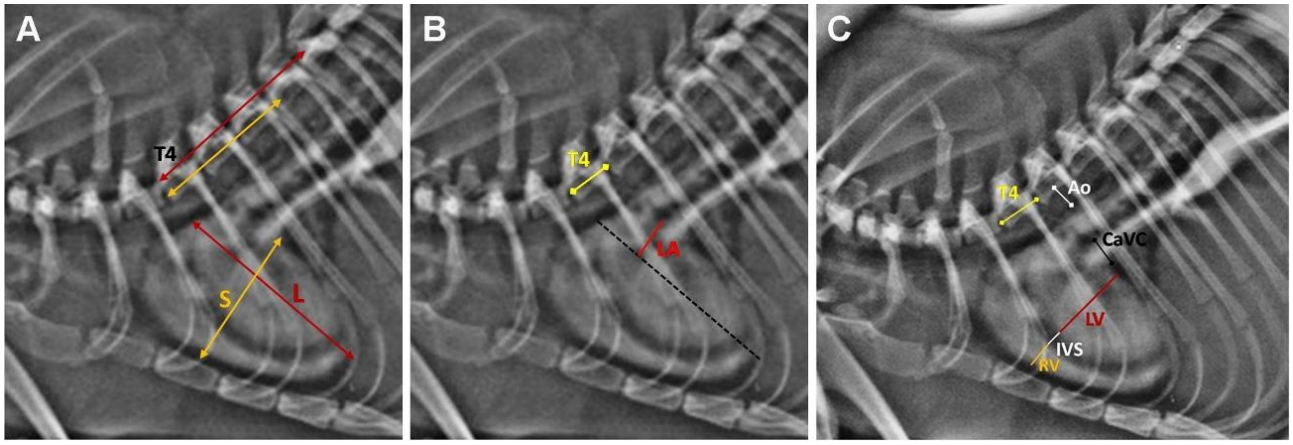

Figure 2. Examples of VHS (**A**), modified LA-VHS (**B**), aorta (Ao), caudal vena cava (CaCV), left ventricle (LV), interventricular septum (IVS), right ventricle (RV), fourth thoracic vertebrae (T4) (**C**) measurements on left lateral contrast views. L: long axis of VHS, S: short axis of VHS, LA: left atrium.

Measurements of radiographic cardiac indices and structures and major thoracic vessel: The heart was assessed by measuring the VHS, as described by Buchanan (9). On left lateral contrast rat radiographs, the cardiac long axis (L) was measured from the ventral border of the left mainstem bronchus (carina) to the cardiac apex. The cardiac short axis (S) was determined by measuring the widest distance between the cranial and caudal borders of the heart. Commercially available computer software was used to apply a 90-degree rotation between L and S. These two axes were then repositioned over the thoracic vertebrae from the cranial edge of T4, parallel to the vertebral column, and each length was then expressed in terms of the number of thoracic vertebrae (v) to the nearest 0.1v. The sum of the two values was used as VHS (Figure 2A).

The LA-VHS method previously described by Schober et al. (35) was subjected to some modification. A line was drawn between the carina and cardiac apex. The distance between this line and the intersection of the dorsal wall of the CaVC and the caudal border of the heart was measured perpendicular to this line. This measurement was divided by the T4 and used as modified LA-VHS (Figure 2B).

The widest parts of the LV and RV were measured perpendicular to IVS (Fig 2C). IVS thickness was measured from the same level. The widths of the Ao and CaVC were obtained by measuring the distance between the dorsal and ventral borders of each vessel. The Ao was measured caudally to the carina and dorsocaudally to the base of the heart. CaVC was measured immediately caudally to the intersection of the caudal border of the heart with this vessel. T4 was obtained by measuring the distance between the cranial and caudal edges of the vertebral body (21, 22) (Figure 2C). The measurements were performed digitally using the software. Except for VHS and modified LA-VHS, all the data obtained were expressed in mm. LV/T4, RV/T4, IVS/T4, Ao/T4,

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CaVC/T4, and CaVC/Ao ratios were calculated from these measurements.

Statistical analysis: All statistical analyses were performed on Statistical Package for the Social Sciences software version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were generated. The normality of data distribution was assessed using the Shapiro-Wilk test. None of the variables were normally distributed, and results were reported as the median and interquartile range (IQR). Correlation between radiographic cardiac indices, measurements, and ratios was calculated using Spearman's rank-order correlation coefficient. Interobserver variabilities were assessed for radiographic cardiac indices, measurements, and ratios via intraclass correlation coefficient (ICC) estimates and their 95% confidence intervals (CI) based on a single rater, absolute agreement, and a two-way random (interobserver) effect. An ICC value >0.9 was considered excellent, 0.75 to 0.9 good, 0.5 to 0.75 moderate, and <0.5 poor (23). *P* values < 0.05 were considered significant for all analyses.

Results

The study population consisted of 50 healthy, male Wistar albino rats with median age and weight of 12 weeks (10-16) and 370 g (275-460), respectively.

No anesthesia or contrast agent-related complications or death occurred in any animals. Measurements of radiographic cardiac indices and structures and major thoracic vessels were performed uneventfully from all contrast radiographs.

The radiographic cardiac indices and measurements and ratios for cardiac structures, major thoracic vessels, and T4 reported by the observers are summarized in Table 1-3.

Median values and references ranges were 8.2v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.1.37) for CaVC/Ao.

Table 1. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for vertebral heart score (VHS) and modified left atrium-vertebral heart score (LA-VHS) recorded by two observers in healthy male Wistar albino rats.

Observer		**Radiographic Cardiac Indices**	
		VHS	**Modified LA-VHS**
Observer1	Mean±SD	8.21±0.36	1.15±0.12
	95% CI	8.12-8.32	1.11-1.18
Observer2	Mean±SD	8.25±0.4	1.17±0.13
	95% CI	8.14-8.36	1.13-1.21

Table 2. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for measurements of radiographic cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in healthy male Wistar albino rats.

Observer		**Measurements (mm)**					
		LV	RV	IVS	Ao	CaVC	T4
Observer1	Mean±SD	7.69±0.69	3.51±0.39	2.09±0.29	2.13±0.29	2.19±0.26	3.97±0.24
	95% CI	7.49-7.88	3.39-3.62	2.0-2.17	2.05-2.21	2.05-2.21	3.89-4.03
Observer2	Mean±SD	7.88±0.72	3.48±0.43	2.15±0.26	2.13±0.21	2.26±0.26	3.95±0.21
	95% CI	7.68-8.09	3.35-3.60	2.08-2.23	2.1-2.22	2.18-2.34	3.9-4.04

LV: Left ventricle, RV: Right ventricle, IVS: Interventricular septum, Ao: Aorta, CaVC: Caudal vena cava, T4: Fourth thoracic vertebra length.

Table 3. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for ratios of cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in male Wistar albino rats.

Observer		**Ratios**					
		LV/T4	RV/T4	IVS/T4	Ao/T4	CaVC/T4	CaVC/Ao
Observer1	Mean±SD	1.94±0.20	0.89±0.12	0.53±0.08	0.54±0.08	0.55±0.06	1.04±0.13
	95% CI	1.89-2.0	0.85-0.92	0.50-0.55	0.52-0.56	0.54-0.57	1.00-1.08
Observer2	Mean±SD	1.99±0.21	0.88±0.13	0.55±0.07	0.55±0.06	0.56±0.07	1.05±0.12
	95% CI	1.93-2.05	0.84-0.92	0.52-0.57	0.53-0.56	0.54-0.57	1.01-1.09

LV: Left ventricle, RV: Right ventricle, IVS: Interventricular septum, Ao: Aorta, CaVC: Caudal vena cava, T4: Fourth thoracic vertebra length.

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Positive correlation was observed between VHS and modified LA-VHS (r= 0.327, P< 0.021), between Ao and CaCV (r= 0.471, P< 0.001), between LV/T4 and RV/T4 (r= 0.505, P< 0.000), between RV/T4 and IVS/T4 (r= 0.321, P< 0.023) between Ao/T4 and CaCV/T4 (r= 0.472, P< 0.001), and between CaCV/Ao and CaCV/T4 (r= 0.307, P< 0.03). Negative correlation was observed between CaCV/Ao and Ao/T4 (r= -0.681, P< 0.000).

ICC evaluation revealed an agreement of 0.91 (95% CI: 0.85-0.95) for VHS, 0.76 (95% CI: 0.66-0.89) for modified LA-VHS, 0.93 (95% CI: 0.89–0.96) for LV, of 0.78 (95% CI: 0.64-0.87) for RV, 0.68 (95% CI: 0.49-0.80) for IVS, 0.78 (95% CI: 0.64-0.87) for Ao, 0.77 (95% CI: 0.63-0.89) for CaVC, 0.92 (95% CI: 0.88-0.97) for T4, 0.95 (95% CI: 0.92-0.97) for LV/T4, 0.86 (95% CI: 0.77-0.92) for RV/T4, 0.75 (95% CI: 0.60-0.85) for IVS/T4, 0.81 (95% CI: 0.69-0.89) for Ao/T4, 0.71 (95% CI: 0.54-0.83) for CaVC/T4, and 0.53 (95% CI: 0.29-0.70) for CaVC/Ao. Agreement between the observers may be therefore considered excellent for VHS, LV, T4, and LV/T4, good for modified LA-VHS, RV, Ao, CaCV, RV/T4, IVS/T4, and Ao/T4, and moderate for IVS, CaVC/T4, and CaVC/Ao.

Discussion and Conclusion

VHS, derived from measurements of the cardiac silhouette from thoracic radiographs, is based on a good correlation between silhouette size and the length of the thoracic vertebral body (34). This radiographic cardiac index is affected by respiratory phases and the cardiac cycle (7, 31). While the effect of the cardiac cycle on this index cannot be eliminated, the effect of respiration can be minimized by radiographs obtained at the inspiratory peak. Rats have a high respiration rate and cardiac cycle. Unfortunately, it is not possible to obtain thoracic radiographs in the inspiratory phase in non-intubated rats because intubation is both difficult and not routinely performed (13). In the present study, respiratory movement-related motion artifact was minimized with the radiographs obtained by positive pressure breath-hold at the time of radiographic exposure. The soft tissue opacity of the rat cranial ventral mediastinum due to soft tissues such as the thymus and intrathoracic fat often causes insufficient prominence of the cranial border of the heart (Figure 1A), especially if pulmonary inflation is poor. False VHS values may therefore be obtained from plain radiographs. Moreover, since only the cardiac silhouette is evaluated via this index, high VHS values can be obtained in the presence of pericardial effusion or pericardial fat. VHS is a reliable index for evaluating cardiac enlargement associated with eccentric hypertrophic cardiomyopathy (HCM) in patients with suspected heart disease, particularly due to volume overload (8, 9). However, the cardiac silhouette may be normal in size in concentric HCM, and VHS values can thus be obtained within the reference range. Contrast radiography made it possible to define all the cardiac borders, LV, RV, IVS, Ao, CaCV and the heart was also evaluated directly rather than using the cardiac silhouette in this study (Figure 2A).

VHS values obtained from healthy rats have been reported in previous studies (13, 15). The VHS values presented in the present study were higher than those obtained elsewhere from Sprague-Dawley rats (13). The difference between our values and those reported by Dias et al. (13) can be attributed to VHS being breed-specific in rats as well as dogs (4, 6, 33). Although Dogan et al. (15) also measured VHS in Wistar albino rats, the difference between the VHS values may be due to the fact that they used a different VHS measurement method (27) and obtained VHS values from elderly rats (10 months of age). Further studies are now needed to evaluate whether breed-associated variations and age would affect VHS in rats.

Quantitative evaluation of LA size is important in both pet and laboratory rats with suspected heart disease, similar to cats (19, 35) and dogs (29, 34). LA was subsequently further quantified by applying a VHS system for radiographic measurement of the LA, known as LA-VHS, in cats (35). Rats are comparable to cats due to their relatively standard thoracic conformation and size. The present study, therefore, evaluated the left atrium using the modified LA-VHS. Previous studies involving healthy cats have reported 1.0v (range: 0.72-1.30) (35) and 0.87±0.21v (19) values for LA-VHS. Schober et al. (35) explained that the portion of LA measured on thoracic radiographs is small, that it is difficult to divide a thoracic vertebra into 10 equal segments in cats, and that this is a potential cause of the error. We, therefore, modified the LA-VHS using the LA/T4 ratio. The LA can be evaluated more objectively in rats with this modification.

The cardiac cycle is a factor that directly affects ventricle and IVS measurement values. However, these measurement data may be useful as baseline values in the radiographic differential diagnosis of different forms of cardiomyopathy. Further studies are now needed to determine whether the differential diagnosis of cardiomyopathies (dilated *vs* hypertrophic, and eccentric *vs* concentric hypertrophic cardiomyopathy) can be established with these measurement values.

CaVC is visible on lateral thoracic radiographs as it runs from the diaphragm to the caudal border of the cardiac silhouette. Right heart function can be assessed by measuring the CaVC (2). In dogs and cats, dilatation of the CaVC is often used as an indicator of right-sided congestive heart failure (18, 24). In addition, dilatation of these vessels is also useful as a radiographic manifestation of heartworm disease (1, 25, 28), pericardial disease (18), pulmonic stenosis (20), tricuspid valve regurgitation, and dilated cardiomyopathy (24). The ratio of CaVC to other

anatomic structures may permit quantitative analysis of the CaVC. Quantitative evaluation is therefore performed to compare the average diameter of the CaVC with the diameter of the Ao, and the length of the T4 (20, 24). The CaVC/Ao ratio is sensitive in cats with right heart failure compared to healthy animals (39). The CaVC/T4 ratio is less than 1 in healthy dogs and cats (24, 39). In the present study, the median CaVC/T4 ratio was 0.53. Similar to our results, the CaVC/Ao ratio is approximately equal to 1 in dogs (21).

This study has several limitations. The cardiac cycle, phase of respiration (despite our efforts to obtain radiographs at the inspiratory peak), hydration status, and intra-thoracic pressure may have interfered in the radiographic cardiac indices, measurements, and ratios obtained from this study.

The radiographs generated in this study were diagnostic, permitting accurate radiographic cardiac indices, measurements, and ratios in all animals.

In conclusion, this study established reference values for VHS, modified LA-VHS, and ventricle, IVS, Ao, and CaVC measurements on left lateral contrast thoracic radiographs from healthy, male Wistar albino rats. The results show that lateral thoracic contrast radiography is a simple, uncomplicated, and effective imaging technique for the quantitative evaluation of cardiac size and chambers and major thoracic vessels in healthy rats. Further studies are now needed to determine whether radiographic indices and ratios obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be useful as a reference baseline for both pet and laboratory rats.

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Conflict of Interest

We declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Author Contributions

MAÇ and MK conceived and planned the experiments. MK and MAÇ carried out the experiments. DB helped with experimental protocols. MK and MAÇ contributed to the interpretation of the results. MK and MAÇ wrote the original draft and contributed to reviewing and editing.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Animal Care Ethics Committee of Akdeniz University (no: B.30.2.AKD.0.05. 07.00/27).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Evaluation of distal femur fractures in cats by hybrid external fixator

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ABSTRACT

In this study, clinical and radiological findings obtained from the treatment of distal femur fractures in cats with hybrid external fixator were evaluated. A total of 10 cats of different ages, breeds and genders with clinically diagnosed femur fractures were used as research material. In the study, hybrid external fixators consisting of circular and linear fixators were used as osteosynthesis material. Closed reduction and external fixation methods were used in 2 cases diagnosed with closed fractures, while limited open reduction and external fixation methods were used in 8 cases diagnosed with open fractures or excessive dislocations. In the radiological examination findings, it was determined that fracture consolidation started on the post-operative 7th day in 9 cases and on the 10th day in 1 case, respectively. Fracture healing was completed in 4 weeks in 2 cases, in 5 weeks in 2 cases, and in 6 weeks in 6 cases (osseous callus was detected). Fixators were removed one week after healing was completed in all patients. In the study, soft tissue complications such as edema in the extremities in 3 cases, mild pin infection in 3 cases and open wounds in 4 cases were determined. However, it was observed that these complications did not adversely affect the recovery time. As a result, with the data obtained from the study, it was concluded that the distal femur fractures in cats of the age and weight scales examined in the study can be successfully treated with the hybrid external fixator system.

Introduction

It is known that femoral fractures of cats are caused by falls from heights, traffic accidents, firearm injuries, fight with other stray animals and human blows (13, 31) Femoral fractures may occur as open, closed, comminuted or segmental. Long-bone fractures constitute approximately 50% of the fracture cases observed in cats, as well as 60% of these fractures are comprised of femoral fractures (13). In addition to this distal femoral fractures comprise 20-30% of all femoral fractures in cats (40).

The treatment of fractures should essentially aims to regain previous anatomic shape of the bone, to improve the functions of traumatized soft tissues and to enable the animal to walk. It has been also emphasized that selected fixation technique should be minimally invasive and easy-applicable (24). In this direction, orthopedic operations should be applied in the treatment of distal femur fractures in order to ensure the normal movement of the joint and to maintain the development of the physis (1, 31, 38).

Single and multiple intramedullary pins, modified Rush pins, cross pins and stiffening wires are used to achieve stability of the fracture fragment (3, 40). Furthermore, small contoured plates, cancellous bone screws and external fixators are the other choices of fixation methods (1, 29, 31). However, the rate of major complications is extremely high in the application of non-rigid stabilization methods such as intramedullary pins (41). In addition, the small fragments in distal femur fractures limit the use of pins or plates, and immobilization of the fracture line can not be achieved adequately (28, 33). Significant complications such as prevention of skin closure, early implant failure, loss of joint range of motion and new bone fractures are encountered in plaque applications (19).

The stabilization obtained using external fixators provides a resistant fixation against shearing, nudging, distraction, compression as well as rotational and torsional forces (11). External fixators are the effective systems that are easily applied in the treatment of various fracture types detected in the patients with different alive weights and sizes (12). Also, Infected wounds with regional material losses, comminuted fractures, open infected fractures preclude achievement of reduction and desired stability and such cases can be treated utilizing external fixator systems (11, 21, 33). These systems are classified in 4 groups as linear external fixators, circular external fixators, hybrid external fixators and computed-assisted external fixators (30).

Biomechanically, circular external fixators have advantages over linear external fixators in terms of stability and rigidity (14, 17, 32). However, the anatomical structure of the femur in cats is not suitable for the application of circular external fixator. For this reason, hybrid external fixator systems consisting of a combination of linear and circular systems are preferred in pet animals, especially in distal femur fractures (17). Thus, circular fixators can be fixed with tensioned k-wires and thus allow micro-movement to shorten the healing time of the fracture (30), while linear fixators can be applied to circular external fixators, one-way to the bone with simple assembly (17).

In this study, it was aimed to evaluate the clinical and radiological findings of treatment of distal femoral fractures by using hybrid external fixators that consisted of circular and linear external fixators in cats.

Materials and Methods

Inclusion Criteria: This study included 10 cats with different breeds, ages and genders and the complaint of severe lameness diagnosed with femoral fracture according to clinical and radiographical examinations without any other systemic disease.

Preoperative Management: The general examination was performed preoperatively and blood parameters were tested (Mindray Bc-2800 Vet, Hasvet, Antalya, Türkiye) in the patients. The patients were administered prophylactic parenteral antibiotic [20 mg/kg ceftriaxone (Unacefin® 0.5 g, Yavuz ILAC, Istanbul, Türkiye)] until the operation. The patients were kept under cage resting until the operation. All the patients were taken to operation within 1-4 days.

In the study, hybrid external fixators designed by Tasarım-Med® Company as the osteosynthesis materials were used. The circular fixator of the hybrid system is composed of half and 5/8 rings in diameter of 50-70 mm made of carbon-fiber alloy with varying number of holes. Linear fixator part of the hybrid system was also constituted by the finger fixator with a capacity of sending at least 4 Schanz pins allowing unilateral pin fixation. Beside these, screws (6 mm) and nuts (6 mm) were used together with 1-1.5 mm Kirschner wire, 2.5 mm Schanz pin, pin tensioner, projections, electric drill, wrenches, soft tissue and orthopaedic sets.

The locations and shapes of the fractures were determined according to preoperative radiographic images of the cases included in the study. Accordingly, hybrid fixator system (frame) was established by determining ring levels and pin-delivery sites.

Retrieved Data: Study data involves fracture etiology, fracture configuration, time from trauma to surgical intervention, findings of physical examination (including neurological examination), surgical technique (including used implants), postoperative complications, time elapsed to fracture healing and time elapsed to removal of the implant.

Surgical Technique: After shaving and disinfection of the fracture site, anaesthetic induction of the patient was achieved by administration of 2 mg/kg intramuscular xylazine HCL (Alfazyne® %2, Egevet, Türkiye) and 10 mg/kg ketamine HCL (Alfamine® %10, Egevet, Türkiye). Maintenance of anaesthesia was performed by administration of 2% sevoflurane (Sevorane®, Abbott, Italy) using closed-circuit anaesthesia device (SMS 2000 Klasik Vent-V, SMS Medical Device, Limited Corporation, Ankara, Türkiye).

The patients were placed in the lateral recumbency on the operating table to place the related extremity on the top. Depending on the status of the fractures; two different techniques were applied as closed reduction external fixation (Cases 1 and 8) and limited incision open reduction external fixation (Cases 2-7, 9 and 10) (Table 1).

Closed Reduction and External Fixation Methods: This method was applied in the cases (Cases 1 and 8) that were diagnosed with closed fracture and that had no excessive dislocation. Traction method was applied to the related extremities of the patients. After achievement of reduction, hybrid system was placed on the related extremity. Kirschner pin was inserted from the closest point to the knee joint on the distal fragment toward caudomedial direction through craniolateral aspect operating the lowest speed of drill. The pin was fixated to the ring using pin-holders and tensioned. Following, linear part of the system was fixated to the bone with 1 piece of Schanz pin at the closest point to coxa-femoral joint and reduction was controlled by radiography. Then, one more Kirschner pin was inserted to the ring from cranio-medial aspect toward cauda-lateral direction by making at least 60° angle with the initially inserted Kirschner pin. The linear

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Table 1. Operation methods applied in fracture cases.

Apenndix table 1 Case No	Operation Technique	Surgery Time (h)	Configuration ring number/ diameter	Fixation Elements on ring
1	CREF	2.30	70 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
2	LİOREF	1.35	70 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
3	LİOREF	2.00	50 mm	2x1.0 mm Kirschner wire 6x2.5 mm Schanz wire
4	LİOREF	1.05	50 mm	1x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
5	LİOREF	1.15	50 mm	1x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
6	LİOREF	1.35	70 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
7	LİOREF	1.50	50 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
8	CREF	1.35	50 mm	1x1.0 mm Kirschner wire 6x2.5 mm Schanz wire
9	LİOREF	1.30	50 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire
10	LİOREF	1.30	70 mm	2x1.0 mm Kirschner wire 5x2.5 mm Schanz wire

LİOREF: limited incision Open Reduction External Fixation,
CREF: Closed Reduction External Fixation

part of the system was fixated using 3 more Schanz pins and the operation was finalized. The cortical perforation procedure by all Kirschner pins was initiated after pushing the pin until the bone using hands. To prevent necrosis and releasing that may occur during perforation procedure; the pin was supported by holding it with a gauze bandage soaked with mixture solution of alcohol and antibiotic.

Limited Open Reduction and External Fixation Methods: This method was applied in the cases (Cases 2-7, 9 and 10) that were diagnosed with open fracture or those with excessive dislocation despite closed state of the fracture. The fragments of the fracture were reached with a limited incision (approximately 3 cm) in the lateral region of the femur and just over the fracture line. The reduction of the fracture fragments was performed using a thin intramedullary pin (a K pin with a diameter of 1-2 mm) that covers one third of medullary canal via retrograde route. Intramedullary pin was kept in the medullary canal until the hybrid system was inserted into the bone. Intramedullary pin was removed after insertion of the hybrid system into the extremity as described for the closed reduction system. The operation was finalized after the appropriate corrections were made.

Postoperative Care: Detailed clinical examination was performed to control whether vascular and muscular structures were active. The patients were administered broad spectrum antibiotics [20 mg/kg ceftriaxone (Unacefin® 0.5 g, Yavuz ILAC, Istanbul, Türkiye), 15mg/kg metronidazole (Flagly 500 mg/100 ml, Sanofi Aventis, Istanbul, Türkiye), 20 mg/kg 5% enrofloxacin (Baytril, 100 mg, Bayer, Istanbul, Türkiye)] alone and in combination regarding postoperative blood count (leukocyte count). Besides, 0.4 mg/kg tolfenamic acid (Tolfine, Novakim, Gebze, Kocaeli, Türkiye) was administered to reduce pain and inflammation. In the cases, pin bases were cleansed using 0.1% rivanol antiseptic solution twice a day for the first 5 days. After 5th day, pin bases were cleansed once daily using 10% povidone iodine (Povidone®, Kimpa, Istanbul, Türkiye). On the other side, pin-base care was performed twice daily in the cases detected with pin-base infection. All the cases were hospitalized for proper postoperative care until completion of fracture healing and removal of fixator.

In the postoperative period; the first radiological examination was carried out on the postoperative 1st day in all the cases. Thereafter, radiological examinations were routinely repeated every week until removal of the fixators. CR Fuji roentgen system (Portable X-Ray Epx-3200, Fujifilm FCR Prima T2, Hasvet, Antalya, Türkiye) was used for radiological examination. One more week was waited in the cases with completed fracture consolidation to loose pin-holders under sedation and fixator was removed by cutting pins.

Clinical outcome assessment: The radiological examination of the patients was performed with an interval of 7 days in the postoperative period and the patients were evaluated regarding continuity of anatomical position, development of a new fracture at the levels of pin insertion and level of fracture healing. The criteria such as use of extremity, the presence of pain and edema, joint functionality and the presence of regional muscular and tendon contractures were reviewed in the clinical control examinations. The findings of the cases were graded according to functional and esthetic grading described by Rovesti (2007) (Table 2).

Results

Of the cases included in the study, 8 were hybrid breeds and 2 were Turkish Van cats, whereas the ages of the cats ranged between 3-5 years (2.7 years) and alive weights were also measured to be between 2-6 kg (4.17 kg). In 10 cases involved in the study, fracture occurred due to traffic accident, trauma and attack by a stray dog in 5, 3 and 2 cases, respectively, and femoral fractures were localized at the distal diaphysis in all the cases. It was determined considering infection and necrotic state in the soft tissue that 6 cases had closed fractures while open and non-infected fractures were found in 4 cases (Table 3).

Table 2. Functional and esthetic grading of the cases in the postoperative period (Rovesti, 2007).

Grade	Lameness Status	Appearance of Extremity
Excellent	Gait is normal, no lameness or pain	Normal appearance
Good	Gait is normal, mild lameness in the extremity	Normal appearance
Moderate	Mild or moderate lameness	Appearance is not excellent
Weak	Extremity is occasionally used, permanent lameness	Abnormal appearance

Table 3. Signalment, Aetiology, Tissue Condition, Fracture Location, Complicatio and outcome veterinary assessment of case.

Case	Signalment* (age, sex, bodyweight)	Aetiology	Tissue Condition	Fracture Location	Complications	first time to use the limb	Completion of Consolidation (day)	Time to fixation removal (day)	Outcome veterinary assessment
1	2-years old Female crossbred 5.3 kg	Traffic Accident	Closed	L-Distal 1/3 diaphyseal transversal	Edema	2	42	49	Excellent
2	5-years old Male crossbred 5.8 kg	Traffic Accident	Closed	L- Distal diaphyseal transversal	soft tissue infections	1	28	35	Good
3	3-years old Male van 5.5 kg	Trauma	Open	R- Distal diaphyseal Oblique	soft tissue infections/ Recurrent Fracture	2	42	42	Good
4	1-years old Female crossbred 2.6 kg	Dog attack	Closed	L- Distal diaphyseal transversal	NO	1	28	35	Excellent
5	3-years old Female crossbred 4.6 kg	Traffic Accident	Closed	L- Distal diaphyseal Oblique	Reduction Deterioration	1	35	42	Excellent
6	3-years old Male crossbred 5.4 kg	Dog attack	Open	R- Distal diaphyseal transversal	Edema	2	42	49	Good
7	2-years old Female crossbred 3.6 kg	Traffic Accident	Closed	L- Distal diaphyseal Oblique	soft tissue infection	2	35	49	Excellent
8	3-years old Female crossbred 4.1 kg	Trauma	Open	R-ant Distal diaphyseal transversal	NO	2	42	49	Excellent
9	2-years old Male van 2 kg	Trauma	Open	L-mt Distal diaphyseal transversal	NO	2	42	49	Good
10	3-years old Female crossbred 2.8 kg	Traffic Accident	Closed	R- Distal diaphyseal transversal	NO	2	42	49	Excellent

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The radiological examination performed at the postoperative 1st week revealed a slight shift on the fracture line in 1 case (Case 5). No procedure was performed in that mentioned case since the fracture fragments contacted to each other by about 90%. However, formation of a new fracture was detected just beneath the former fracture line due to the excessive mobility of the patient and these cases were re-operated. No complication related with reduction was encountered in the other 8 cases according to the radiological results.

Fracture consolidation started on the postoperative 7th day in most of the cases, whereas it observed in 1 case (Case 3) on the 10th day. The radiological examination in the second week revealed that impairing reduction encountered in the 5th case on the first week did not progress. Refracture developing in the 3rd case was operated again and reduction procedure was carried out. The complication was resolved by addition of 1 piece of Schanz pin to the ring in the distal fragment in the operation. It was monitored that healing process of the patient was not negatively affected by this complication and that radiological improvement was similar with the other cases. Besides, no periosteal reaction was radiologically detected on the entrance points of the pin although mild pin-base infections developed in 3 cases (Cases 2, 3 and 6) after the first week (Table 3).

It was determined that fracture line almost disappeared after the third week in most of the cases and that consolidation was nearly completed. It was determined that consolidations were completed within a period ranging between 4-6 weeks (37.8 day) in the fracture line. It was found that secondary fracture recovery occurred in all the cases. The fixators in all cases were removed 1 week after completion of the consolidation of fixator (Figs. 1-4) (Table 3).

Postoperative daily clinical examinations revealed that all the patients tolerated fixators very well. Edema was found in only 3 cases (Cases 1, 6 and 7) on the postoperative 1st day. This condition was detected to be regressed in the 2nd-3rd days. It was determined that all the cases could apply their weight on the related extremities on the postoperative 1st -2nd days (Fig. 5). Lameness degree was observed to be mild and moderate in 6 (Cases 1, 4, 5, 7, 8 and 10) and 4 (Cases 2, 3, 6 and 9) cases, respectively. It was noted that the signs of lameness disappeared in almost all the cases beginning from the postoperative 7th day and the patients could use their extremities functionally. Pin-base infection and subsequent soft tissue infection developed in 3 cases (Cases 2, 3 and 6). However, the infection was eliminated by increasing the number of daily pin-base care. (Pin bases were cleansed using 0.1% rivanol antiseptic solution forth times in a day) Open wound developed in 4 cases (Cases 1, 2, 3 and 6) at the level of femoral lateral condyle after the operation. These cases were applied dressing with rivanol solution three times a day along 3 days. In the following days, dressing was continued with mixture of *Centella asiatica* (Madecassol®, Bayer, Topkapı, Istanbul, Türkiye), nitrofurazone (Furacin®, Zentiva, Inc., Prague, Czech Republic) and rifamycin sodium (Rif®, Koçak Farma, Üsküdar, Istanbul, Türkiye). The wounds were found to be recovered within a period ranging between 13-21 days.

During the study, no complication such as broken ring, broken pin or loosened nut was determined. A slight inclination was detected in Schanz pin in 1 case (Case 3). However, no intervention was carried out since no impairment developed in the reduction. No abnormal looseness was identified in the clinical examination after removal of the fixator in the cases and this evidence was confirmed also with radiological examination.

The patients were hospitalized for one more week after removal of the pins for treatment of the developing lesions and clinical follow-up. This follow-up process indicated that general condition of the patients was good and that none of them has any finding related with lameness (Fig. 6).

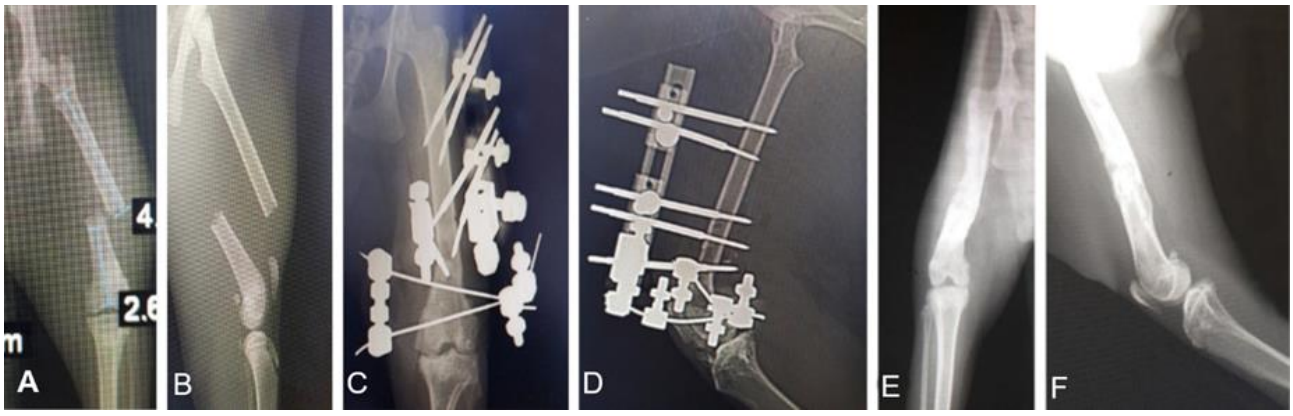

Figure 1. Case no. 2: X-ray images: (A) pre-operative A/P, (B) pre-operative M/L, (C) post-operative day 1 A/P, D) post-operative day 1 M/L (E) after the removal of fixator A/P, (F) after the removal of fixator M/L.

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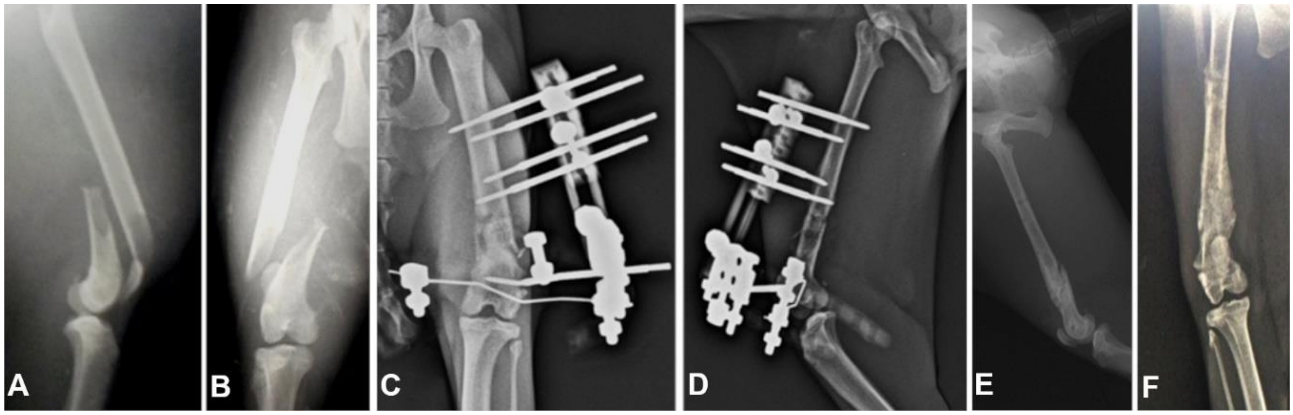

Figure 2. Case no. 5: X-ray images: (A) pre-operative M/L, (B) pre-operative A/P, (C) post-operative day 1 A/P, D) post-operative day 1 M/L (E) after the removal of fixator M/L, (F) after the removal of fixator A/P.

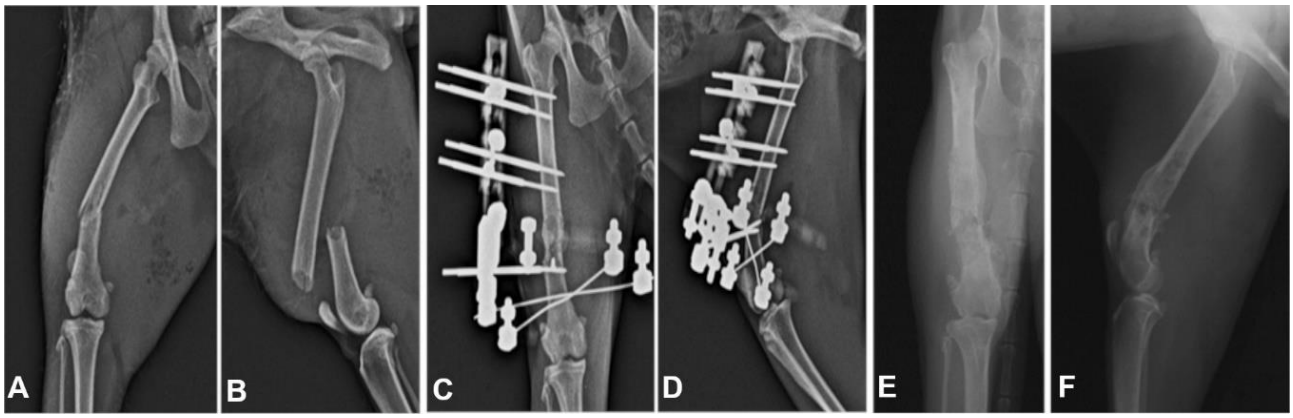

Figure 3. Case no. 6: X-ray images: (A) pre-operative A/P, (B) pre-operative M/L, (C) post-operative day 1 A/P, D) post-operative day 1 M/L (E) after the removal of fixator A/P, (F) after the removal of fixator M/L.

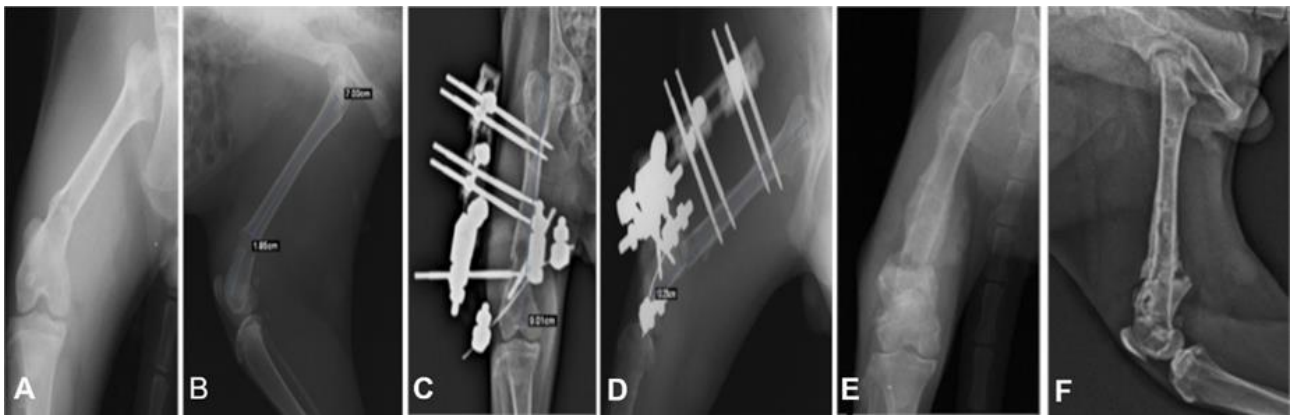

Figure 4. Case no. 8: X-ray images: (A) pre-operative A/P, (B) pre-operative M/L, (C) post-operative day 1 A/P, D) post-operative day 1 M/L (E) after the removal of fixator A/P, (F) after the removal of fixator M/L.

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Figure 5. Postoperative day 1, images of cats stepping on the ground, pertaining to (A) Case no. 1, (B) Case no. 2, (C) Case no. 5, (D) Case no.7.

Figure 6. Images for stepping on the ground after removing the fixator for (A) Case no. 1, (B) Case no. 2, (C) Case no. 5, (D) Case no.7.

Discussion and Conclusion

Classical cage resting (24), bandage, cerclage, screw, plate ve intramedullary pin (4, 23, 24) ve external fixation procedures (22, 23) are used for treatment of the fractures. It has been overemphasized that treatment method preferred in fractures should be minimally traumatic and easy-applicable as well as providing well-fixation (24). The treatment methods mentioned are routinely applied, but when intramedullary pin applications are evaluated, 6% more major complications are experienced compared to plate and external fixator applications (20). When plaque applications are considered, many major complications occur, especially in distal fractures (19). In addition, several factors such as open infecting wounds with regional material loss and formation of open infecting fractures due to perforation of skin caused by the fracture fragment preclude achievement of the desired stability and such circumstances direct the physicians to apply external fixator in the treatment of the fractures (11, 33).

When linear external fixators and circular external fixators are compared, both have advantages over each other in different subjects. Linear external fixators; While it can be applied in many fractures, provides simple assembly and disassembly, it has been determined that circular external fixators have superior biomechanical properties. However, application difficulties, requiring more intensive post-operative care and low tolerance for the patient are important disadvantages (17, 32). Hybrid external fixators are formed by connecting linear external fixators to circular external fixators. These fixators can be applied in deformity corrections, and they are versatile in fracture repair and can be applied in different fracture types with configurations (17, 37). Hybrid external fixators are applied more easily and in a short time compared to circular external fixators, are better tolerated by the patient and allow postoperative frame adjustments (28, 30). Hybrid external fixators have important advantages over linear external fixators in fracture repair because they have some positive features of circular external fixators. These advantages are; These are important criteria such as fixing the fragments or small bone fragments with stretched thin krishner wires, resisting bending forces, preventing torsional displacements that are negative for the bone, and accelerating bone healing by allowing the formation of axial micro-movement (17). In the present study, hybrid external fixator system was used in the treatment because of fracture line was localized at the distal femur in 10 cases constituting the study population and 4 cases had open fractures. Thereby, it was targeted to minimize complications as well as potential treatment-limiting factors in the cases. Additionally, advantages of the system such as early onset of joint motions, concurrent treatment of soft and bone tissue, allowance to perform closed reduction and achievement of biological biosynthesis were used (6). In the present study, 2 and 8 cases were operated using closed reduction and limited open reduction techniques, respectively. During the study it was monitored that the related extremities can be used in all cases after the postoperative 1^{st}-2^{nd} days. Daily wound care was carried out without complication in the cases with open wound. Thanks to these advantages, hybrid method was evaluated to be an applicable and useful technique in small pets.

The diameter of the ring used in the hybrid external fixators is one of the most essential criteria that determines the biomechanical compatibility of the fixator (9, 18). Increased ring diameter leads to increased length of the wire placed on the ring and decreased stability. It is recommended as a general rule that diameter of the ring should not exceed 1.5-2 fold of the extremity diameter (12). Accordingly, the present study has aimed to use the smallest ring as possible as and use of the rings with a diameter of 50-70 mm was found to be more considerable. On the other side, finger fixator that sends at least 4 Schanz pins and facilitates distraction and compression by allowing unilateral application of pins was used in the linear fixator part of the hybrid external fixator system.

One of the most important factors that affect the stability of the hybrid system is diameter of the wire. It has been essentially reported that increasing diameter of the wire increases the stability of the system (25). However, some authors have advocated that application of large-diameter wire may cause osteoporosis and reduced bone stability, therefore the diameter of the pin or wire should be meticulously selected. Nevertheless, it has been reported that diameter of the wire should not exceed 20% of the diameter of the bone that the system will be applied (2). Ferretti (8) reported that the use of wires with a diameter of 1.0-1.6 mm on cats and dogs in his study give positive outcomes. In the present study, these data has been taken into consideration and are used 1-1.5 mm Kirschner wire and 2.5 mm Schanz pin. No complication such as breaking of the wire or ruptur occured in the cases is not observed during the study. In addition, radiological examinations indicated no finding of complication in the bone tissue. It has been concluded that diameter of the wire used in the study is appropriate for bone tissue and patient weight.

In the present research, it has been determined that operation duration ranged between 65-150 min depending on the state of the fracture. The operations were performed using hybrid external fixation system and applying two different techniques as closed reduction external fixation and limited open reduction external fixation. It has been emphasized in the studies (5, 11). that experience is gained as the number of the practices increased and consequently

duration of the operation shortens. In this study, it has also been observed that the essential determinant factor for operation duration is the success of the intraoperative intervention. It has been monitored that repeats of the practice dramatically shortened the operation duration and this fact has been accepted as an important advantage of the technique.

It has been noted in a study on the treatment of humerus fractures in cats using hybrid external fixator that administration of ceftriaxone sodium (22 mg/kg) or the combination of amoxicillin clavulanic acid (20 mg/kg) eliminated the formation of infection in the postoperative period (38). It has been reported in another study that pin-base infection was monitored in some cases after twice daily administration of cefotaxime (10 mg/kg) along postoperative 7 days, however, this condition caused no complication in the cases (34). In the present study, leukocyte count was tested weekly during postoperative two weeks and broad spectrum antibiotics were administered alone or in combination according to the results. Beside to this, tolfenamic acid (0.4 mg/kg) was used for 5 days to relieve the pain and inflammation in the postoperative period. Mild pin-base infection was identified in 3 cases whereas no complication of infection was observed in the other cases.

In the external fixator applications, use of 10% povidone iodine, 2% hydrogen peroxide or 0.05% chlorhexidine is also recommended for postoperative pin-base care (10, 34). Differently, Singh et al. (39) have reported that 0.9% NaCl can be used combined with antibiotic and povidone iodine for the same procedure. Bilgili et al. (**7**) have reported that a rifamycin-nitrofurazone impregnated tamponade is placed to the pin-bases and additionally the whole system is protected from the external environment by bandaging with compression bandage completely. In the present study, pin-base cleaning was performed twice daily using 0.1% rivanol (rivanolum 1gr) antiseptic solution within the first postoperative 5 days. In the following days, pin-bases were cleansed using 10% povidone iodine (Povidone®, Kimpa, Istanbul, Türkiye) every day. The whole system was protected from the external environment by bandaging completely using compression bandage. All the cases were hospitalized in the clinic for postoperative care during fracture healing (formation of bone callus) and removal of the fixator.

External fixator applications is providing significant advantage by allowing concurrent treatment of the fracture and soft tissue (15, 26, 39). By the hybrid external fixation system applied in the present study, the cases with open fractures were treated with open wound care. For this purpose; initially antiseptic rivanol solution within the first 3 days and subsequently wound bandage containing the mixture of Centella asiatica (Madecassol®), nitrofurazone (Furacin®) and rifamycin sodium (Rif®) was applied. Consequently, improvement was achieved in open wounds of the cases within 13-21 days. External fixator applications allow mobility of the related extremity without loss of position in the fracture fragments and weight-bearing. Thereby, functional impairments in the joints, muscles and bones as well as muscular atrophies that occur due to application of other treatment methods can be minimized (15, 26, 39). In a study on the treatment of fractures using intramedullary pin, it has been reported that the time elapsed to bear weight on the related extremity may range between 1-2 weeks (26). Contrarily, Silva et al. (38) investigated the treatment of humerus fractures using hybrid external fixator in cats and reported that bearing weight on the related extremity occurs within postoperative 1-3 days. In a similar study carried out using circular external fixator, it has been stated that the related extremity can be used within postoperative 1-3 days (35).

It has been emphasized in another study which applied hybrid external fixator for femoral fractures in dogs that time elapsed to use the related extremity varied between 3-6 days (35). On the other hand, in a study (30) conducted on 49 dogs it has been stated that time elapsed to bear weight on the extremity varied between 1-38 days (averagely 8 days), while in another study (22) carried out on 30 dogs it has been reported that bearing weight started on the postoperative 1st, 7th- days in 8, 13 and 9 cases, respectively. In the present study, in all cases it could be beared weight on the related extremities within the postoperative 1-2 days. The findings of mild and moderate lameness were identified in 6 (Cases 1, 4, 5, 7, 8 and 10) and 4 (Cases 2, 3, 6 and 9) of the cases, respectively.

It has been reported that the decrease in the sharpness of the fracture edges, the disappearance of the fracture line and the callus structure are taken into account in the radiological evaluation of fracture healing (16, 36). Although, it is known that fracture edges usually become indistinct within the postoperative 5-7 days and bone callus formation become visible within 10-12 days, Piermattei et al. (27) have stated that fracture edges are remarkable in the first week whereas this markedness decreases in the 2nd week. Rao et al. (30) have noted in their study that consolidation starts by the 15th day and formation of bone callus becomes visible after 21th day. The researchers have detected that corticomedullary continuity returns in the postoperative 45-60 days in all the cases and reported that time elapsed to remove fixators averagely ranges between 30-60 days. Sailaja (34) has reported in his study conducted on 6 dogs that callus formation started on the 3rd week in all the cases and fixators were removed after completion of fracture healing on the postoperative 5th and 7th weeks in 3 and 3 cases, respectively. Sancak et al. (35) in their study in which treated tibial fractures in cats using circular external

fixator, reported that healing time and time elapsed for fixator removal were 35-55 days, respectively. In the present study, the time of onset of callus at the fracture line; It was determined as the post-operative 7th day in 9 of the cases and the 10th day in 1 case. Consolidation was completed in 4 weeks in 2 cases, in 5 weeks in 2 cases, and in 6 weeks in 6 cases. It was determined that secondary fracture healing occurred in all cases. Fixators were removed 1 week after the consolidation was completed. These results show that, as in similar studies, HEF provides a tight fixation and relatively reduced fracture healing times thanks to its biomechanical advantages in our cases. External fixators are associated with several complications such as wound formation in soft tissues, pin-base infection, pin loosening, pin inclination and pin fraction (11, 12). Rao et al. (30) have reported complications such as mild pin-base infection, loosening of K-wire, wound formation and mild radius/ulna deformity in their study. However, it has been emphasized that complications experienced in the research did not impair the stability of the fixator and that excellent improvement was achieved in all the fractures. Silva et al. (38) have noted in their study that it has been detected pin loosening as a complication in only 1 case on cats. Mutlu and Özsoy (22) have expressed in their study that it is encountered pin-base infection in 10 of 30 dogs and that this complication occurred due to the neglection of the pet owners. In the present study, all the cases were hospitalized in the clinic to perform their postoperative care, nevertheless, edema was monitored in 3 cases (Cases 1, 6 and 7) on the postoperative 1st day. However, edema that emerged in the extremities on the postoperative 2-3 days was found regressed by medical treatment. Pin-base infection and secondary soft tissue infection developed in totally 3 cases (Cases 2, 3 and 6). However, complication was eliminated by increasing the number of daily pin-base cleansing procedures. A mild inclination was detected in the Schanz pin applied to the distal fragment in 1 (Case 5) of the cases, but no intervention was performed since no impairment occurred in the reduction. The formation of a new fracture was discovered in Case 3 on the postoperative 4th day in the related extremity due to the excessive mobility, and the patient was re-operated and reduction was renewed. The complication was eliminated by adding 1 piece of Schanz pin to the ring applied on the distal fragment. Despite complications, healing time of the patients were found not to be affected negatively similarly with the other studies (22, 36).

In the present study, treatment of distal femoral fractures was achieved using hybrid external fixators in the cats with various breeds, age and sizes. The results revealed that application of hybrid external fixator system was well-tolerated by the cats, system allowed micromobility between the fracture fragments and consequently healing was achieved in a shorter time period. In addition, it was observed that postoperative care also contributed to the positive outcome of the treatment success and that healing time was similar with previous studies in the literature (36, 38). It has been monitored that this system provided many options to perform various interventions on the fragments including rotation in the postoperative period. Besides, it has been discovered that system allowed closed reduction and thereby decreased the infection risk significantly.

Considering the data obtained, it was predicted that hybrid external fixation system provides contribution to biological osteosynthesis within a shorter period compared with the other techniques. As a result, it has been concluded that carrying out comprehensive studies applying hybrid fixator system at the fractures in the appropriate sites would be beneficial in discovering ideal combinations of the system and expanding its application field by developing novel economic alternatives.

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Conflict of Interests

The authors declared that there is no conflict of interests.

Author Contributions

AG and İA designed the study. AG did the preoperative and post operative care of the patients AG applied the operation technique. İA evaluated the results. AG and İA wrote the manuscript. İA provided technical and supervisory support.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was carried out after the animal experiment was approved by Siirt University Local Ethics Committee (Decision number: 2018/16).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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Ethylene glycol toxicity in two calves

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ABSTRACT

Burdur Mehmet Akif University Veterinary Faculty Animal Hospital was brought from livestock to 3 calves aged between 15 and 20 days with complaints of weakness, dehydration, inability to get up and nervous findings. Two of them failed the treatment and were brought to the pathology department after being euthanized. Both animals were macroscopically dehydrated, cachectic, and had hair loss in some parts of the body. There was 1-2 liters of white light-colored transparent fluid in the abdominal cavity. Many organs and serous membranes in the abdominal cavity were hyperemic. Histopathological examination revealed bleeding, edema and degenerative changes in the heart, lungs and kidneys. Calcium oxalate crystals were found in many tubuler lumens, especially the proximal convoluted tubules in the kidneys. In the light of clinical, macroscopic, and histopathological findings, the case was diagnosed with ethylene glycol toxicity. Ethylene glycol toxicity is often seen in cats and dogs living near industrial or auto car repair shops, and in animals that accidentally drink antifreeze waste. This case report is the first case of ethylene glycol toxicity reported in livestock calves in Türkiye.

Ethylene glycol (EG) is an automotive product and can be toxic when exposed to human and animals. EG usually causes irreversible kidney damage. However, it may also affect many systems (5). EG is often drunk accidentally since it is sweet (7). Affected animals are mostly cats and dogs, but ruminants may also rarely be affected rarely (1, 8). Clinical signs of affected animals include dyspnea, hypersalivation, ataxia, paraparesis, depression, and death (3). Toxicity is related to the in vivo conversion of the main compound into toxic metabolites. It does not have a direct toxic effect. Metabolites such as oxalic acid and calcium chelates may lead to the formation of calcium oxalate complexes to become difficult-to-soluble. The accumulation of these metobolites into renal tubules causes acute tubuler damage and renal failure (10). The crystals are light yellow in color and have rosettes or prismatic formations (11).

In this case, renal oxalosis was encountered due to ethylene glycol poisoning. Renal oxalosis, which is characterized by the appearance of oxalate crystals in the kidneys, can also be seen when the autosomal recessive gene is dominant genetically, the mothers consume oxalate-containing plants during pregnancy, and the patient directly consumes oxalate-containing plants. In this case, the onset of the disease in 10 animals at a time eliminates the first cause. Since it is known that the mothers do not leave the farm and are fed with roughage and concentrated feed during pregnancy, they are eliminated for the second reason. The fact that the leak in the machine used to feed the calves on the farm was noticed and when it was repaired, no other cases were seen, also indicates that the diagnosis was correct (6).

Three of 13 calves, aged between 15 and 20 days, were brought to Burdur Mehmet Akif University

Veterinary Faculty Animal Hospital for treatment with complaints of weakness, dehydration, inability to get up and nervous symptoms. Two animals were sent to the Pathology Department for autopsy after they died during the treatment. At autopsy, approximately 2 liters of yellowish-white transparent fluid was found in the abdominal cavity of one of the calves (No: 2). The kidneys were swollen and pale in appearance. The lung was swollen, dark red in color, and contained areas of petechiae scattered over the lobes. Petechial hemorrhages were observed on the epicardium and endocardium, especially in the m papillaris and areas under the valves. In the other calf (No: 1), no significant macroscopic finding was observed except severe hyperemia in the serosa of the organs in the abdominal cavity and pulmonary edema. Samples taken from lesioned tissues were fixed in 10% buffered formaldehyde and embedded in paraffin blocks after routine tissue follow-up. Sections of 5 µm thickness were taken from the blocks and stained with routine hematoxylin & eosin. Histopathological examination revealed subpleural hemorrhage and alveolar edema in both calves (Figure 1A). One of the calves (No: 1) had subepicardial hemorrhages in the heart sections (Figure 1B). Epithelial desquamation, edema in the propria and mononuclear cell infiltrations were detected in the intestines of both calves. In one of the calves (No: 2), the Virchow-Robin spaces were enlarged due to edema in the brain (Figure 1C). Calcium oxalate crystal deposits were found in both calves with marked enlargement of Bowman's spaces (Figure 1D) and in the lumens of many proximal convolutes and distal tubules.

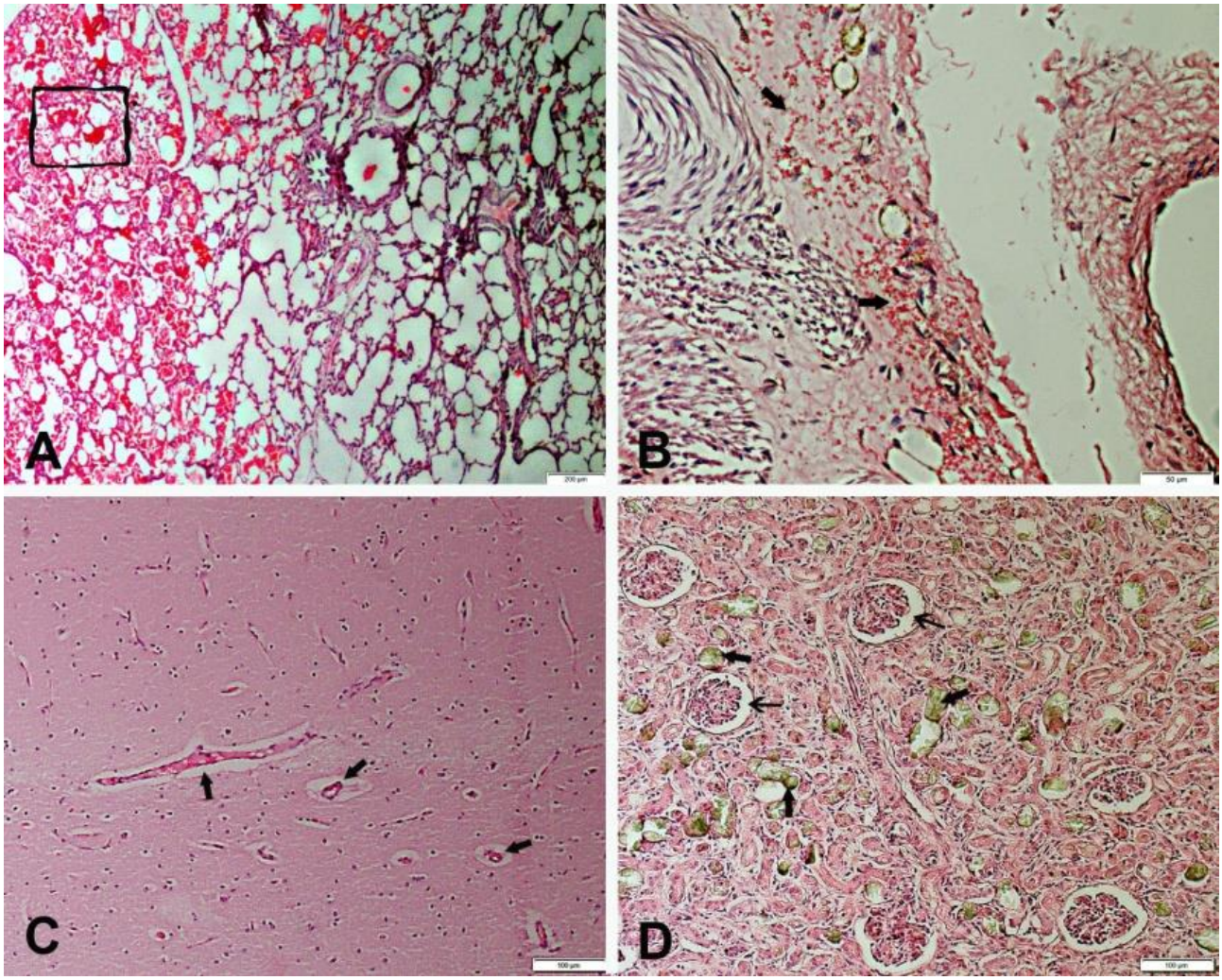

Figure 1. A. Calf, lung, subpleural hemorrhage, (rectangular), HE X40, Bar=200 µm. **B.** Heart, subepicardial hemorrhages (arrows), HE X100, Bar=100 µm. **C.** Brain, enlargement of Virchow-Robin spaces due to edema (arrows) HE X40, Bar= 200 µm. **D.** Kidney, calcium oxalate crystals in many tubuler lumens (thick arrows), Bowman spaces enlargement (thin arrows), HE X200, Bar= 50 µm.

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Although EG toxicity is rarely reported in farm animals, it is seen frequently in cats and dogs that drink EG due to its sweetness. On the other hand, poisonal exposure in calves may be rare and it is usually by accident or food contamination. EG has no use in food preparation units other than its accidental intake. However, it is a matter of curiosity how food contamination can occur. Today, automatic machines are used to feed calves on modern farms. EG is used to maintain the temperature of the food in these machines. In this case report, EG leakage from an automatic machine used in calf feeding is mixed with calf food. The cause of the calcium-oxalate crystals seen in histopathological examinations was not understood at first, but it was revealed as a result of deep anemnesis investigation.

Previous studies report that laboratory results such as serum urea, creatinine, phosphate, total protein, and the amount of white blood cells increase but hemoglobin decrases (11). The laboratory findings of this study showed that despite increasing serum creatinine and lactate levels, hemogram levels were surprisingly normal.

At the necropsy, the marked lesions were gastrointestinal hyperemia and pulmonary edema. Petechial or echymotic hemorrhages were observed in the supcapsular surface of the kidneys, and the heart was enlarged (2). The macroscopic lesions of this study were similar to the previous reports. In addition, petechial hemorrhages were found near the valves.

The renal toxicity of ethylene glycol is generally described as renal tubuler damage caused by the accumulation of calcium oxalate crystals (4, 10). Additionally, focal interstitial lymphocytic infiltration and mild degenerative-necrotic tubuler changes may be seen in some cases (1). In this case, characteristic renal lesions such as Bowman's spaces and dilatation and calcium oxalate deposits, especially in the distal tubules, and tubular epithelial eruptions in some areas were consistent with the findings of previous reports.

Glycolic acid, which is one of the ethylene glycol metabolites, may result in coma due to encephalopathy or brain edema (9, 12). In this case, enlargement of Virchow-Robin spaces in the neuropile confirms that there was brain edema due to glycolic acid but no damage to neurons.

In this case, clinical, macroscopic, and microscopic findings of ethylene glycol poisoning due to food contamination in fattening calves were reported. This extremely unusual case demonstrated how important the synthesis of anemnesis and pathological findings is in diagnosis.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

ZÖ, ÖÖ and RY conceived and planned the experiments. İYA, GO and MA carried out the experiments. ZÖ, ÖÖ and RY planned and carried out the simulations. İYA, GO and MA contributed to sample preparation. ZÖ, ÖÖ and RY contributed to the interpretation of the results. ZÖ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Ethical Statement

This study does not present any ethical concerns.

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First genetically confirmed case of Lethal Acrodermatitis in a Bull Terrier in Türkiye

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ABSTRACT

Lethal acrodermatitis (LAD) is a rare disease affecting bull terriers and miniature bull terriers characterized by poor growth, progressive skin lesions, and immunodeficiency. A mutation in the Muskelin 1 (*MKLN1*) gene was determined as the causative mutation for LAD, and a genetic testing method for affected dogs has been established. A bull terrier representing symptoms similar to those of LAD was submitted to clinics, and a blood sample was taken for genetic testing. DNA was extracted, and direct mutation screening confirmed the causative mutation MKLN1:c.400+3A>C. Due to the severe progression of the disease and lack of available treatment, we have been informed that the patient was euthanised. This is the first reported case of LAD from Turkiye, and based on our findings, we strongly suggest that owners and breeders implement genetic testing before breeding to reduce and eventually eradicate this mutation from the population.

The Bull Terrier is a breed that originated from crossing Bulldogs and the now extinct White English Terrier back in 1835 by James Hinks in Britain. To develop breed characteristics and improve the overall quality of the breed, the Bull Terriers were outcrossed with Spanish Pointers, Dalmatians, and Greyhounds. The breed was initially bred for pit fights; thus, they have been famous for their agility, tenacity, and constitution (3). However, in time, probably due to changing demands and improvements in animal rights, breeders have added companionship to breed personality. Currently, the Bull Terriers are categorised according to their colour (white, coloured) and size (standard, miniature) (2). Even though there is a lot of information on Bull Terriers and their traits, there seems to be no publicly available information on their breeding records.

Lethal acrodermatitis (LAD) is a rare disease affecting approximately 12% of the bull terrier population as either carriers or affected patients (3). LAD is characterised by poor growth, skin lesions, and immune deficiency (1, 5-8). The disease was first recognised at the University of Pennsylvania genetics clinic veterinary school in 1982 (11). Because the disease was reported only in the Bull Terrier, a genetic background was suggested (5). In the following years, it was generally agreed that LAD must have a genetic background, no study was conducted to determine the causative mutation until 2018. A splicing defect on the canine MKLN1 gene, located on the 14th chromosome spanning across 5574374-5904923 (CanFam3.1), was associated with LAD in Bull Terriers (OMIA 002146-9615). The causative mutation is monogenic, transmitted in an autosomally recessive manner in bull terriers and miniature bull terriers (1).

The reported phenotypic representation of LAD is growth retardation, progressive skin lesions, paronychia, diarrhoea, abnormal behaviour, bronchopneumonia, and death by around one and a half years old. When affected puppies reach 8 weeks of age, they are noticeably smaller

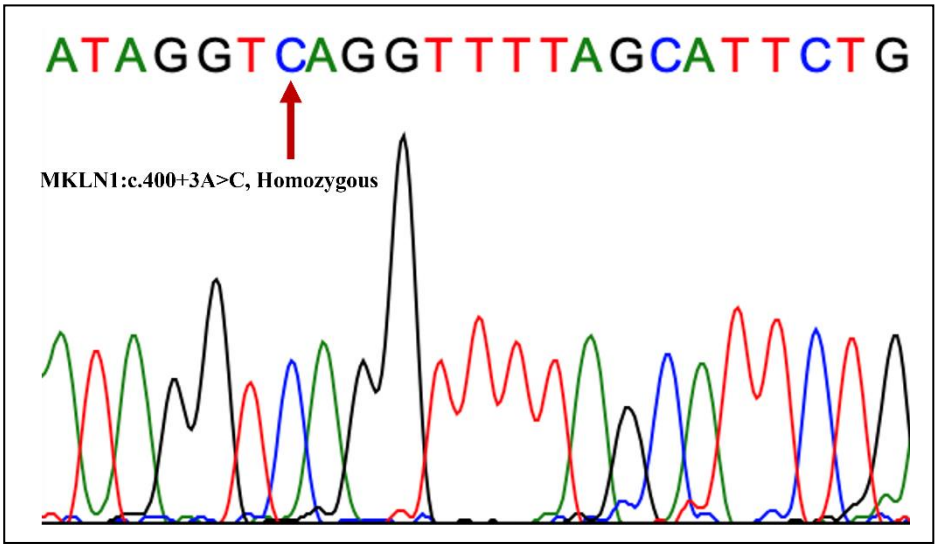

Figure 1. Sanger sequencing chromatography revealing the causative homozygous mutation MKLN1:c.400+3A>C (Arrow).

than their littermates. Some of the puppies affected by LAD show a lighter pigmentation that becomes pronounced with age compared to their normal/healthy littermates. Usually, characteristic skin lesions appear on the feet and face by the time the puppy reaches 6-8 weeks of age. Prominent symptoms begin in the extremities, consisting of splayed digits, erythema, interdigital pyoderma, and paronychia, followed by hyperkeratotic footpads. Affected dogs have difficulty eating solid foods and lodge the food into their abnormally arched hard palate. Many affected dogs also show symptoms such as diarrhoea, lethargy, and decreased responsiveness as the disease progresses. When affected puppies reach one year of age, they have half the body size and weight of a healthy littermate (5, 6, 8, 9). Due to their immunodeficient state and reduced IgA levels, they are susceptible to infections and suffer from skin (Malassezia, Candida) and respiratory tract infections. Bronchopneumonia is identified as a common cause of death (7, 9).

A blood sample from a bull terrier with LAD symptoms was submitted to Ankara University Faculty of Veterinary Medicine, Department of Genetics for mutation screening. The DNA was extracted from the whole blood with a commercial DNA extraction kit (GF-1 Blood DNA Extraction Kit, Vivantis, Malesia) following the manufacturer's instructions. DNA quality and quantity were measured spectrophotometrically (Nanodrop 2000, Thermofischer, USA), and DNA integrity was visualised via agarose gel electrophoresis (Kodak, Logic 200 imaging system, USA). Direct mutation screening for the candidate variant MKLN1:c.400+3A>C was performed by PCR and Sanger sequencing. To amplify the region harbouring the mutation, PCR was done using primers Dog: MKLN1F CCATGCACTGTAGCCACATC and Dog: MKLN1R TGGAAAAGGTTCCACTTGAAAT.

PCR was set up containing 80 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, 1 X PCR Buffer, and 1 U/µl of Taq DNA polymerase (Fermentas, Thermo Fischer Scientific), and added ddH_2O to a final volume 25 µl. Thermal cycling was carried out using the Mastercycler thermocycler (Eppendorf, USA) with an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec extension at 72 °C for 30 sec and final extension at 72 °C for 5 minutes. PCR products were purified and sequenced in both directions. PCR products were visualised safely by dyed agarose gel electrophoresis and scanned (Gel Logic 200 Imaging System, Kodak). A 796 bp long PCR band was obtained, and then the PCR products were purified (GeneJet PCR purification Kit, ThermoScientific) following the manufacturer's instructions. Purified PCR products were used as templates in the BigDye Terminator v3.1 cycle sequencing reaction, bi-directionally, using the same primers. The sequencing reaction products were purified (DNA Sequence Purification Kit, Zymo, USA), and products were sequenced using an ABI310 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Electroforegrams and chromatographs were analysed with Bioedit Sequence Alignment Editor (4), and aligned to the reference gene sequence (ENSCAFG00000001406), confirming the presence of the MKLN1:c.400+3A>C splicing defect mutation (Figure 1).

LAD has similarities to zinc-responsive dermatosis of canines, acrodermatitis enteropathica (AE) of humans, and lethal trait A46 of Black Pied Danish cattle. These diseases are associated with zinc absorption and/or metabolism. Although the skin lesions are similar in these diseases, oral or intravenous zinc supplementation does not show any curative signs in LAD patients (5, 8, 13). According to a study, LAD patients showed considerable

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improvements in skin lesions when zinc supplements was supported with vitamin complexes, omegas, and copper. Unfortunately, the outcome remained the same, which is either death caused by LAD or euthanasia (10). In our case, after the confirmation of the mutation, we have been informed, that with the owner's consent, the patient was euthanised.

Moreover, abnormal behaviour is considered one of the symptoms caused by LAD; in our case, abnormal behaviour was not reported. Also, in a study analysing behaviour differences in 28 dogs, none of the dogs showed any abnormal behaviour according to their owners (8). However, since behaviour is a subjective topic, it should not be considered a good indicator (12).

Although there are no publicly available records for Bull terrier breeding in Turkiye, it is public knowledge that it continues to this day. The breeders should acknowledge the LAD presence in the Bull terrier population. There is no known cure currently available for dogs affected by LAD. Early genetic diagnosis is crucial for eliminating this mutation from the population. To help the survival of the following generations of bull terriers, breeders should implement genetic testing when choosing the sires and dams to decrease and eventually eliminate LAD mutations from the population.

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Conflict of Interest

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Author Contributions

NB conceived and planned the experiments. FK carried out the experiments. FK took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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This study does not present any ethical concerns.

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The use of plastinated specimens for hybrid education of Veterinary Anatomy

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ABSTRACT

With the official announcement of the new type of coronavirus-induced COVID-19 outbreak as a global pandemic, an extraordinary situation that no one has ever encountered has started. Just as life was about to return to normal in Türkiye, two devastating earthquakes, centered in Kahramanmaraş, affected ten different cities. Many global and national developments in various fields, which are expected to happen in the distant future, were completed within 3 years. One of these fields was undoubtedly education. Hybrid learning is seen as a trending educational approach combining face-to-face and online learning. Plastinated specimens come to the forefront for hybrid education with various advantageous features. They are not only non-toxic, dry, odorless materials, but also can simulate the natural anatomic appearance in detailed manner. With the help of new-generation acrylic paints and dyes which can penetrate into tissues, plastinates offer a unique natural look rather impressive than any other techniques. Due to the features mentioned above, plastinates are also convenient materials for handling, transportation or storage. These issues will be discussed in our article in terms of compatibility with hybrid learning. The aim of this article is to give ideas and make suggestions about how plastinates, which have been used efficiently in anatomy practices and professional training, can be used in hybrid veterinary anatomy education.

Introduction

On January 30, 2020, with the World Health Organization's (World Health Organization / WHO) official announcement of the new type of coronavirus-induced COVID-19 outbreak and then declaring it as a global pandemic, an extraordinary situation that no one has ever encountered in the world before has started. And subsequent measures-restrictions quickly entered people's lives (12, 16, 21, 31). While countries were searching for solutions to this pandemic by using their own scientific and administrative resources, various regulations and protocols were put into effect in a short time (16, 17).

Improvements and updates that are expected to happen in the next 10 years in many fields such as technology, communication, economy, health and so on have been completed in the last 3 years. One of these fields was undoubtedly education. On March 11, 2020, face-to-face education was completely stopped in Türkiye. At the first months of Covid-19 outbreak, in order to ensure the continuity of education in the current education systems in the world and in Türkiye, emergency remote education implementations had been started quickly and imperatively before the distance education. This happened because of the inexperience of the whole world in this extraordinary situation (4, 8, 10). Afterwards, all schools, including universities, continued to interact with their students through distance education methods, digital content and e-learning materials for more than a year. After the total lockdown, educational institutions started to reopen gradually. On-line and in-class (face-to-face) education with reduced student groups were initially implemented together. Finally, the classrooms were meeting with the students again (4, 8, 10, 20, 37). While daily life in our country would start to return to the pre-

pandemic period, two devastating earthquakes occurred nine hours apart and caused severe destruction in 10 different cities. Shortly after the pandemic, unfortunately, the state of emergency regulations and restrictions started again in Türkiye (41).

Although the COVID-19 infection is no longer a deadly pandemic, do you think the danger has passed completely? Unfortunately, the Critical Disease Control Center (CDC), the World Health Organization (WHO) and many other global health organizations are discussing the same issue on these days: When will be the next pandemic? Many health authorities believe that a new pandemic will, unfortunately, threaten humanity again in the near future. This threat can originate from biological terrorism, or it can start with a simple virus hiding in a jungle and ready to mutate. If an unexpected global pandemic occurs, is the world ready for this war in all fields (5, 19)?

During the total lockdown, the efficiency of the education offered to undergraduates at various universities decreased undeniably. Veterinary faculties were among the institutions most affected by the negative effects of distance education, as they included intensive practical courses and clinical training. Because no one was experienced enough in this matter. Neither trainers nor learners… This unfortunate global pandemic triggered improvements in educational methods, fortunately. Although these alternative education methods have been around for some time now, they have gained traction in the last 3 years.

Even though the pandemic seems to reach a plateau and efforts to reduce the negative effects of the earthquake are at a good stage, learners as well as educators gained lots of experience on distance education and some of these new techniques may be preferred whether there is a need or not in the future (20, 26, 27, 37).

Hybrid learning is a trending educational approach that combines face-to-face and on-line learning. Some of the learners attend the class in person, while others join virtually and remotely through hybrid learning. Trainers and lecturers utilize various tools such as video conferencing or electronic learning materials for the optimum education of both of these student groups in hybrid education. Hybrid education can entail some online exercises, e-learning materials, videos, and other materials that will also support in-person classes. Not only for the official lectures but also to create extracurricular learning activities, hybrid education seems to be a convenient alternative for the teaching of veterinary anatomy (15, 34). However, for hybrid education to be useful and effective, appropriate learning tools, facilities and materials must be used.

Can plastinated materials be efficient tools to implement these updated learning methods for veterinary anatomy education? The purpose of this review is to give ideas and make suggestions about how plastinates, which have been used efficiently in anatomy practices and professional training, can be used in on-line courses or hybrid education.

Although the plastination was first discovered by Dr. Gunter von Hagens in the early 1970s, it is still one of the most up-to-date anatomical techniques with new modifications. In 1986, the pioneers in plastination established the International Society for Plastination and only after one year the Journal of the International Society for Plastination has published the first issue. On the other hand, von Hagens followed a bit fancy way to improve and get the plastination around the world. He established The Institute for Plastination, an alternative organization at Heidelberg in 1993. And immediately after that, von Hagens designed the most extraordinary exhibition, Body Worlds, in 1995 (18, 30). Thus, this idea, which was initially put forward to support anatomy students who are trying to complete their doctoral thesis, has turned into a tool used in anatomical and clinical education and a method to prepare popular and impressive exhibitions visited all over the world in recent years. Although this technique is still a matter of ethical and moral debate for various scientific groups, and even though some articles go even further and call the exhibitions "freak show", plastination has become an integral part of anatomy education (9, 33, 38).

Plastination Techniques Applied Worldwide

Briefly, plastination is a technique performed by replacing body fluids in tissues and organs with a reactive curable polymer at appropriate pressure and temperature. Essential but hazardous hydrophilic intermediary solvents, mostly acetone or alcohol, are used while the tissue fluids are replaced with the polymer. Three standardized plastination techniques have generally been used in plastination laboratories around the world (Figure 1) (13, 14, 18).

Silicone Plastination: It is the most widely used and basic method worldwide, in which the whole body, body parts, organs or tissues are plastinated through a reactive silicone polymer. Silicone plastination is an ideal method for beginners and newly established laboratories due to its relatively easy application compared to other plastination techniques (13, 35).

Epoxy Plastination: Epoxy plastination produces 2-8 mm thickness of semi-transparent plastinates by using epoxy resins. In this technique, all tissue fluids and a significant amount of adipose tissue are replaced with a curable epoxy resin mixture. In these cross-sections, anatomical structures can be examined with naked eye in a commendable quality down to a submacroscopic level (35, 36).

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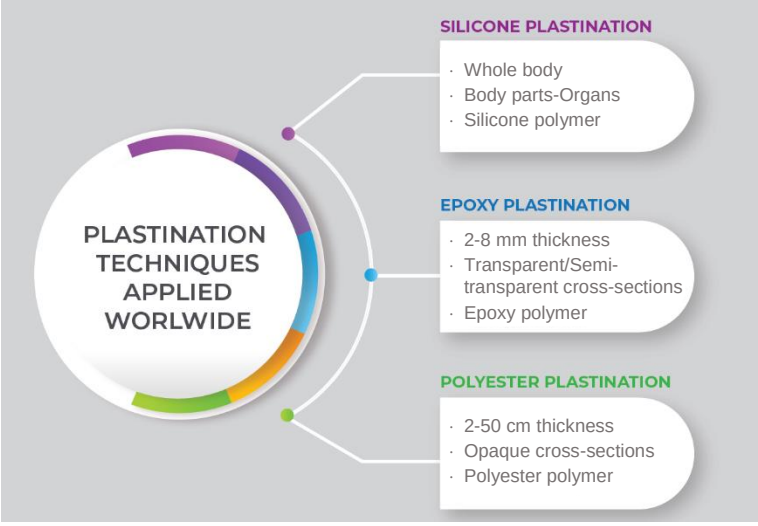

Figure 1. Standardized plastination techniques that have been used in plastination laboratories around the world.

Figure 2. Five consecutive steps that are used for silicone, epoxy and polyester plastination procedures.

Polyester Plastination: Polyester plastination produces, body or organ slices, especially brain cross-sections, with 2 mm to 5 cm thickness. The tissue fluids are removed and replaced with a curable polyester resin in this method. While epoxy plastination produces transparent sections, specimens prepared with polyester plastination are opaque plastinates. Because, a considerable amount of adipose tissue is removed by acetone or methylene chloride (1, 2, 39).

Steps of Plastination Procedure

Five common steps can be described for the general procedure of plastination: Specimen preparation and fixation, dehydration, defatting, forced impregnation, polymerization and hardening (Figure 2).

Specimen Preparation and Fixation: Early preparation of the biological tissues by minor or gross dissections has been the most important part of the plastination process, because the final appearance of the plastinates are formed by the meticulous application of this very first step (11, 18).

Dehydration: Dehydration (or freeze substitution) is the main stage that tissue fluids are replaced with an organic solvent. This solvent must be hydrophilic and volatile enough as an intermediary chemical which is the key for the next step. The most convenient solvent is acetone for this step. Prepared and fixated specimens should be kept in pure acetone baths in order to eliminate all unnecessary fluids (7).

Defatting (Degreasing): Excess lipids in the tissue or extraordinary fatty specimens can affect the final quality of plastinates and should be removed if possible. Defatting is the removal of excess fat from the specimen. Although the procedure is quite similar to those in the dehydration stage, the main difference is the acetone bath should be performed at room temperature in order to eliminate excess fat (7).

Forced (Polymer) Impregnation: This stage is the replacement of volatile organic solvent in the specimen with curable silicone, epoxy or polyester polymer at a specified temperature and negative pressure. These polymers are quite viscous to come to equilibrium with the intermediary solvent. Thus, a negative pressure (vacuum) is needed to get the polymer inside the specimen. This special aplication gives the name "forced impregnation" to this step (7, 13).

Polymerization and Hardening: Polymerization and finally hardening of the specimen is the last stage for a specimen fully loaded with polymer. Chain extension and cross-linkage of polymer molecules to provide longer chains and to form a firm 3D meshwork respectively is the basic principle for this stage. This polymerization activity can be formed by exposing the specimen to a curable gaseous hardening chemical or an ultraviolet-A light with a specified wavelength or regulated temperature (7, 23, 36).

Advantages & Disadvantages of Plastination for Anatomy Education

All organic specimens, even macerated bones, have an estimated useful life. Although you preserve your samples in the best manner, decomposition eventually occurs on the tissues and the specimen becomes out of use and discarded in a proper way. When compared with the alternative anatomic specimens, plastinates are distinguished with their very durable structures. Besides, plastinates are easy to keep and use for various courses and practices. Once you manage to plastinate and preserve your specimen in a very optimum way, it can be everlasting. This word may come as "exaggerated" to the readers. However, the first samples which had been prepared by von Hagens in early 70's are still in use (13, 33).

Different kinds of toxic or hazardous chemicals are used for the various stages of plastination. Formalin is almost an essential solution for the proper preparation of specimens in the first step. On the other hand, removing all formalin from the specimen is also essential for the next step. Again acetone, a toxic and very explosive chemical, is an irreplaceable chemical for the dehydration stage of plastination. However, this intermediary volatile solvent is totally removed in the next step. Therefore, specimens transform into biosafe and non-toxic plastinates at the end of the plastination procedure. And this makes plastination privileged among the various anatomic techniques (11, 40).

Final products are not only non-toxic, dry, odorless materials. A well-prepared plastinate can simulate the natural anatomic appearance in a detailed manner. With the help of new-generation acrylic paints and water-based dyes which can penetrate into tissues, plastinates offer a unique natural look rather impressive than any other techniques. Due to the features mentioned above, plastinates are also convenient materials for handling, transportation or storage (30, 40).

Beside the remarkable advantages of plastination, some disadvantages should be considered. The leading problem that should be mentioned is the financial cost. The initial investment cost for a plastination lab can be rather high. However, the maintenance of the lab and the plastination procedures will be much more manageable after setting up the lab. The unit cost of the plastinates will decrease day by day (40).

At that point, the most important situation that should be noticed by the staff is the laboratory safety rules. Some of the essential chemicals which are used during the plastination procedure can be corrosive or explosive. And the laboratory personnel should know the regulations before taking the first step to the plastination lab (18, 29, 40).

As mentioned at the beginning of the article, ethical concerns are still a serious matter of debate, especially for the human-origin specimens (9, 38) . However, it seems to be a surmountable issue for the veterinary medicine field. With the proper ethical legislations and professional regulations, which have been in service in European countries, the plastination technique has been applied in veterinary institutes for many years.

Plastination for Hybrid Education of Veterinary Anatomy

At this point, plastinated specimens distinguish by their advantageous features. Various techniques can be recommended for hybrid learning of veterinary anatomy through plastinated specimens (Figure 3).

The first stage of hybrid learning in anatomy is undoubtedly face-to-face or in-person activities. Plastinated specimens have proved their effectiveness in regular face-to-face education in recent years. Due to their simulation capacity of anatomic structures as in their natural appearance, plastinates became essential learning materials for anatomy practices. In order to reduce the negative effects of fixated cadavers for the learners in practice, plastinates come to the front with non-hazardous features (24, 40).

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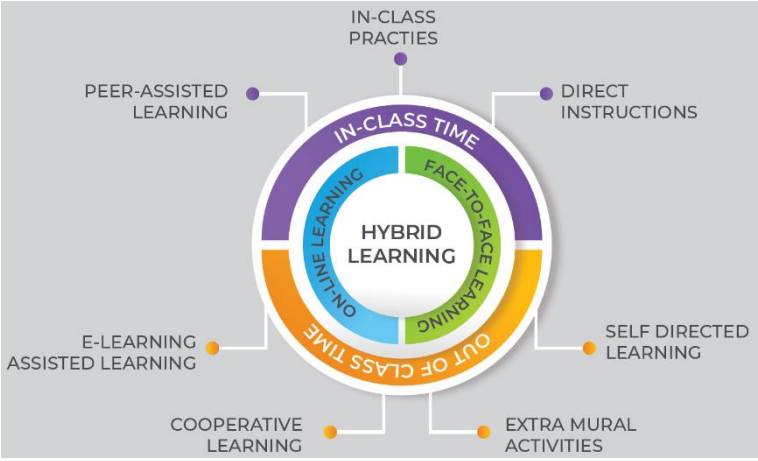

Figure 3. Convenient techniques for hybrid learning of veterinary anatomy by using plastinated specimens.

Figure 4. While trainers are performing on-line practical sessions or e-learning videos on the cadavers or plastinates, on-line learners can practice simultaneously on their plastinated specimens.

Fresh organs or cadavers are always a gold standard for anatomy education. However, fresh specimen means using the body or body part of an individual that will be sacrificed somehow for this purpose. Besides, the short-time use necessity of fresh cadavers can be a struggling limitation for the trainers in terms of fast decomposition or microbiological activity (40). However, the primary desire of the instructors is to be able to use the convenient learning materials for a longer period of time in order to perform various in-class or hybrid education techniques (32). Therefore, fresh specimens may be the first choice instead of plastinated specimens for such kind of long-term practical trainings (40). The increased student groups mean extra learning materials for practice (5). If you have even a basic silicone plastination lab working properly, providing various plastinated specimens for student groups will be an effective way to support the face-to-face part of hybrid learning (24).

The second method of hybrid learning, as mentioned above, is to combine in-person and on-line training for the learners (15). Thanks to durable, non-toxic and non-hazardous properties, plastinates can easily be transferred, handled or shared with the learners. While trainers are performing practical lectures on the cadavers or plastinates, on-line learners can practice simultaneously on their plastinated specimens which were provided previously (Figure 4).

In instructor-centered processes, learners cannot gain enough self-confidence and autonomy. Therefore, they cannot develop self-directed learning skills (4). However, self-directed learning and peer-assisted learning are another effective tools not only for in-class activities, but also for hybrid education (6, 25). Learners can acquire skills such as greater autonomy, self-sufficiency and developing a critical perspective with a well-designed hybrid learning processes. At this point, it is important to

use abstract and concrete techniques together in the use of educational technologies (4).While studying through pre-recorded videos or similar e-learning materials, the presence of anatomical practice materials for the learners increases the efficiency of self-directed or peer-assisted learning even in the class or out of class learning times. However, this type of learning, without supervision, would be risky in terms of biological safety or lab security regulations when classical fixated cadavers or fresh specimens are used. Plastinated specimens can overcome this problem with the specified features mentioned above (40). Learners can make use of plastinates either in the labs or any related extramural training facilities (Figure 5).

Today's students are expected to be lifelong learners throughout their student and professional lives. Therefore, university trainings should also be designed to self-directed learning in real-life working areas beyond formal education. This can be achieved by hybrid learning configurations where working and learning are integrated together (6). Within this scope, extramural learning can also be used for the hybrid education. Extramural assignments are an essential part of practical learning as they provide a unique opportunity for learners to gain professional experience and practical skills acquired during clinical anatomy education (3, 22, 28). Production of goal-directed plastinates and transferring the specimens to the related facilities may provide an effective extramural learning period not only for the anatomy students, but also for the clinical anatomy trainees who are willing to take professional skills from various establishments (Figure 6).

Figure 5. Self-directed or peer-assisted learning, without supervision, would be risky in terms of biological safety or lab security regulations when classical fixated cadavers or fresh specimens are used. Plastinated specimens can overcome this problem with their unique properties.

Figure 6. Production of goal-directed plastinates will provide an effective extramural learning period not only for the anatomy students, but also for the clinical anatomy trainees who are willing to take professional skills.

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Mahdy and Ewaida mentioned that the need of technological tools such as internet connection and electronic devices could be a problem for on-line learners during pandemic period. Unstable network connection in different regions, problems to join on-line learning, interrupted electricity connection, imperative social isolation, ocular problems because of screen use, and lack of appropriate learning environment at home were the major deficiencies (26). Karadağ and Yücel (20) reported that only 63% of the university students have an internet connection at home, 66% have a computer or tablet in Türkiye. And 23% of the learners stated that they could not continue their distance education (20). Therefore, the necessity of active use of plastinates and similar anatomical education materials not only in on-line education but also in hybrid learning or in-class practices should be taken into consideration.

Routh et al. mentioned that institutional cultural resistance can be occured in various establishments while transition to on-line education in extraordinary situations like outbreaks (34). Despite all these negative approaches, the use of plastinates in hybrid learning can be an alternative to gradually reducing resistance in trainers who believe in traditional education methods.

Conclusion

The experiences in the last 3 years have proved us that nothing will ever be the same again in many different fields, especially in education. If instructors want to bring much more competent colleagues to veterinary profession, they have to adapt to these changing-developing processes. In this review, we wanted to convey to the readers the usage areas of plastination in hybrid learning, which can be an effective learning tool for changing veterinary education. The opinions and suggestions expressed in this article will be carried to much more useful points with scientific studies on the subject and different techniques to be applied to target groups.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Author Contributions

OE conceived and planned the concept and content of the review. OE, CB, BB and ST contributed to the retrospective analyses of the current techniques. OE and CB contributed to the interpretation of the data. OE took the lead in writing the manuscript.

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