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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

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The effect of mating method and mating season on the number of puppies and some reproductive parameters in Pomeranian dogs

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ABSTRACT

In the present study, the 3-year breeding behavior of the parent dogs in a Pomeranian farm was followed, and the effects of season and breeding method (mating or artificial insemination) on their reproductive performance were investigated. The gestation period was 62.4 days, the age at which sexual maturity is reached was 404.4 days and the average period between two estruses was found as 205.2 days. The mean number of offspring, and the mean number of male and female puppies were found as 3.4, 2.15 and 1.4 respectively. The stillbirth rate was found as 1.4% and death rate up to 1 year 16.7%. The pregnancy rate, the average number of female puppies, the number of stillbirths and the total number of offspring were found to be similar in naturally and artificially inseminated females (P>0.05). While 47.6% of estruses were seen in the spring -summer months, 52.4% were seen in the autumn-winter months (P>0.05). The mating season did not affect the number of female offspring, the stillbirth rate or the total number of offspring (P>0.05). However, when compared to the spring-summer months, the mortality rate of puppies within 1 year of their birth was found to be significantly higher in the females showing estrus in the autumn-winter season (P<0.01).

Keywords: artificial insemination, mating season, Pomeranian dogs, reproductive parameter

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Introduction

The interest in owning dogs, which started as a hobby for people, has become even more popular with the COVID - 19 pandemic. The decrease of socializing and the increase in the time spent at home during the pandemic have resulted in changes in human psychology and behavior. It has been determined that keeping pets has a protective effect against the psychological effects of the pandemic (Akalın et al., 2020). The increasing demand for dog ownership has increased the number of dog producing enterprises both in the world and in our country. The large numbers of offspring obtained in one birth and the low cost of feeding and care compared to farm animals make it desirable to breed these animals. Due to the increasing demand for high-priced purebred dogs as pets, dog owners today often consult veterinarians for solutions for fertility problems (Fontbonne, 2011; Zubair, 2014). The increasing dog population and developing canine medicine reveal the need for detailed knowledge of the reproductive physiology and endocrinology of dog breeds. Detailed elucidation of the reproduction of both male and female dogs will contribute positively to dog breeding (Ülgen and Soylu, 2000). Many studies have been conducted on the topic

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of reproduction in numerous animal species that have economic benefits. As a result of these studies, some methods have been developed to solve the reproductive problems of these animals. However, studies on the reproductions of animals kept as pets, which are looked after for different purposes, are not at the desired level compared to other animal species. Knowing the reproductive characteristics of dogs, which constitute an important number of pet animals, would be useful in solving reproductive problems that may occur in these animals (Ülgen and Soylu, 2000). Genetic changes in dogs, which occur throughout many years, have caused structural and reproductive differences as well as phenotypic (height, weight, figure, etc.) changes among dog breeds. Although many studies have been conducted on topics related to reproduction in dogs, some areas have not been fully clarified (Sundqvist et al., 2006).

The breed known as Pomeranians, Pomeranian dog or boo is a Spitz type dog breed named after the historical Pomeranian Region of Central Europe (at present, the part of Northern Poland and East Germany). When we look at the physical characteristics of this breed, which has no distinctions between males and females in regards to weight and height measurements, we see that their heights vary between 18 and 30 cm and that their weights vary between 1.4 and 3.2 kg. Depending on the conditions they live in, Pomeranians can live 12 to 16 years. They are among the 25 most popular dog breeds in the USA (AKC, 2022). The popularity of Pomeranian dogs has increased after 2009, especially with the aid of social media (Hutchinson, 2014). It is necessary to learn the reproductive characteristics of these dogs and to carry out breeding studies in the light of the obtained information. This information is very important for commercial businesses that breed pure-bred dogs as well as animal owners who keep these dogs at home and veterinarians. To the authors' knowledge, there are a few information on reproductive properties of Pomeranian breed in literature, however, there is no comprehensive study similar to the present study. This study was performed to investigate the effects of the age of the first use of Pomeranian breed female dogs in mating, the length of gestation periods, the length of periods between two estruses, the average number of offspring, the average number of female and male puppies, stillbirth rates and death rate up to 1 year as well as breeding season and insemination method on certain reproductive characteristics.

Material and methods

Experimental protocols and animal care were approved by Dicle University Health Sciences Research and Application Center Ethics Committee. Document number: 2021/103303.

The present study was conducted at a farm in the city of Diyarbakır in south eastern of Turkey. This region is situated at 37°55'01"N latitude, and 40°16' 46"E longitude, and at an altitude of 660 m. The age of the first use of the dogs (n: 21) in mating, the gestation periods, the periods between two estruses, the average number of offspring, the average number of female and male offspring, the stillbirth rate and the death rate up to 1 year were noted for 3 years and mean values were calculated. In order to determine the effect of the breeding season on the number of offspring born, bitches mated in spring and summer months were assigned into the first group (n:10) and those that mated in autumn and winter months were assigned into the second group (n:11) and the number of offspring, the number of female puppies, stillbirth rates and the number of offspring that died after less than a year were noted down. The bitches that were confirmed to be in oestrus by vaginal smear application were divided into 2 groups in order to determine whether the method of mating affect the offspring. Intravaginal artificial insemination using fresh sperm (2nd fraction) taken by the hand massage method which had spermatological values of motility ≥ 80 and normal morphology ≥%80 (Baran et al. 2003), was applied to the first group (n:6) at least 2 times with an interval of 2 days. After insemination the clitoris was massaged for at least 5 minutes. In the second group (n:14), natural inseminations were performed at least 2 times with an interval of 2 days. The pregnancy rates, the numbers of female puppies, the stillbirth rates and the total number of offspring were recorded.

Statistical analysis: Comparisons between the two groups were made using the Mann Whitney U Test. SPSS 21 package program was used in the analysis.

Results

The reproductive characteristics investigated within the scope of the study are given in Table 1. Accordingly, the age of first use in mating was found as 404.4 days, the average period between two estruses was found as 205.2 days, the average number of offspring was found as 3.4, the average number of male offspring was found as 2.15, the average number of female puppies was found as 1.4, the stillbirth rate was found as 1.4% and death rate up to 1 year 16.7%.

The effects of the mating season on reproduction are given in Table 2. It was determined that the mating season did not affect the number of female puppies, the number of stillborn offspring, and the

Table 1: Some reproductive characteristics of Pomeranian females

Investigated characteristics	Mean values
Age at sexual maturity (days)	404.4 ± 130.50
Interval between two oestrus (days)	205.2 ± 35.43
Duration of pregnancy (days)	62.4 ± 2.67
Number of offspring	3.4 ± 0.93
Number of male offspring	2.15 ± 0.86
Number of female offspring	1.4 ± 1.43
Number of stillborn (rate)	0.05 ± 0.22 (1.4%)
Number of death by 1 year old (rate)	0.6 ± 1.21 (16.7%)

Values are expressed as mean ± SD.

total number of offspring (P>0.05). However, the mortality rate up to the age of 1 was higher in the offspring of dogs mated in autumn-winter months than in the offspring of those mated in spring-summer months (P<0.05). The results of the effects of the insemination method on reproduction are given in Table 3. It was determined that there was no difference in the pregnancy rate, the number of female puppies, the number of stillborn offspring and the total number of offspring in naturally mated or artificially inseminated Pomeranian dogs (P>0.05).

Discussion

The reproductive characteristics of female dogs show a significant difference from those of other domesticated mammal species. Dogs are traditionally non-seasonal monoestric animals that show estrus once or twice a year. Considering that normal females show estrus every 5-7 months, it is calculated that they can be fertile for about 14 days a year (Grundy et al., 2002). Additionally, the uterus in dogs needs 2-6 months (130-150 days) for endometrial regeneration or involution whether they are pregnant or not (Al-Bassam et al., 1981; Freshman, 1991). The interval between two estruses varies between 5 and 11 months, with an average of 7 months in domestic dogs and 12 months in non-domestic dogs. Female dogs enter an obligatory anestrus period of approximately 4-5 months following the dioestrus, which lasts approximately 58-75 days (Soderberg, 1986; Beijerinka et al., 2003; Nak et al., 2012). In our study, the average time between 2 estruses was found to be 205.2 days, which is approximately 7 months. The prolongation of the estrus cycle and the variability of the time between estruses may be due to the varying durations of anestruses in different dog breeds as indicated by Cirit et al. (2017).

Female dogs reach puberty between 6-24 months of age and show their first estrus at approximately 10-12 months of age (Soderberg, 1986; Nak et al., 2012). In our study, it was determined that the average age of bitches used in the first mating was 404.4 days.

Table 2. Some reproductive characteristics of Pomeranian females according to estrus season

Investigated characteristics	Estrus season		P
	Spring-Summer	Autumn-Winter	
Number of female offspring	1.40 ± 0.54	1.36 ± 0.36	> 0.05
Number of stillborn (rate)	0.0 ± 0.00 (%0)	0.09 ± 0.091 (2.9%)	> 0.05
Total offspring number	3.7 ± 0.33	3.2 ± 0.23	> 0.05
Number of death by 1 year old (rate)	0.10 ± 0.10 (2.7%)	1.0 ± 0.47 (31.4%)	< 0.01

Values are expressed as mean ± SE.

Table 3. Effects of natural breeding and artificial insemination on reproductive performance

Investigated characteristics	Natural mating	Artificial insemination	P
Pregnancy rate	100%	100%	> 0.05
Number of female offspring (rate)	1.3 ± 0.36 (43%)	1.7 ± 0.67 (38%)	> 0.05
Number of stillborn (rate)	0.1 ± 0.07 (2%)	0.0 ± 0.00 (0%)	> 0.05
Total number of offspring	3.3 ± 0.25	3.8 ± 0.31	> 0.05

Values are expressed as mean ± SE.

The gestation period in dogs is considered to be the time between the first mating and parturition. This period has a very wide range and varies between 57 days and 72 days, but takes an average of 62-64 days (Concannon et al., 1983). Although the length of these periods can differ depending mainly on breed, the age of the animal, the number of parturition and the number of offspring born as well as the season, inconsistent results were reported in some previous studies (Jöchle and Andersen, 1977). Although in a previous study (Okkens et al., 1993) it has been reported that the gestation period may vary in dogs of different breeds, no effect of breed on gestation period has also been reported in another study conducted by Linde-Forsberg et al. (1999). Okkens et al. (2000) reported that the gestation period was 62.8 ±1 days for West Highland White Terriers, 60.4 ±1.7 days for German Shepherds, 60.9 ±1.5 days for Labrador Retrievers, and 61.4 ± 1.0 days for Dobermanns in their study of dogs of different breeds. Baran et al. (2003) reported that the average gestation period after insemination with fresh semen

was found as 64.62 days for German shepherds, Labradors and Pointer dogs in their study, similar with the above mentioned studies.

Linde-Forsberg (2000) reported that the yield of offspring obtained from artificial insemination and natural breeding are similar. In our study, the pregnancy rates, the average number of female puppies, the number of stillbirths and the total number of offsprings were found to be similar in females who were mated and artificially inseminated (P>0.05). However, although there was no statistical difference, the total number of offspring (3.8 and 3.3) and the rate of female puppies (43% and 38%) of females who underwent artificial insemination were found to be numerically higher than the naturally mated females (P>0.05). The numerically higher pregnancy rate in artificially inseminated females may be due to the fact that the artificial insemination was repeated at least 2 times with an interval of 2 days according to the vaginal smear results, and clitoral massage was performed by lifting the back of the dog for 5 minutes after AI. It has been reported that the massage applied to the clitoris after insemination gives a feeling of mating (Baran et al. 2003), causes oxytocin release and contractions in the smooth muscles of the genital system (Çoyan and Tekeli, 1996). Smith et al. (2019) reported that the breeding characteristics of domestic dog breeds show differences, and the average number of offspring born at a single birth in 60 dog breeds was 5.55 wherease this number was 2.4 for Pomeranian dogs. Evans and Adams (2010) reported the average number of offspring for Pomeranian dogs as 1.93 (168/87). In our study, the average number of offspring of artificially inseminated dogs was found as 3.4. The reason for higher average number of offspring in our study may be due to the fact that, because of the enterprise is for commercial purposes, careful monitoring of estrus and the fact that the bitches are mated or inseminated at least two times with an interval of two days. There is no data that can explain why female offspring was slightly higher in numbers in artificially inseminated bitches than matings in our study.

In our study, 14% of estrus in female dogs occurred in February, 29% in March, 10% in April, 5% in July, 5% in August, 14% in September, 14% in October, 5% in November and 5% in December. While no estrus was observed in January, May and June, approximately one-third of all estruses throughout the year were observed in March. Seasonally, 47.6% of the dogs showed estrus in the spring-summer months and 52.4% showed estrus in the autumn-winter months (P>0.05). Seasons of estruses did not affect the number of female offspring, the stillbirth rate or the total number of offspring (P>0.05). Compared to the spring-summer seasons, the offspring of the females showing estrus in the autumn-winter seasons were found to have a significantly higher mortality rate under the age of 1 (P<0.01). This may be due to the adverse effects of the winter season, such as cold and rainy weather conditions.

Conclusion

At the end of the present study, the mean values for investigated characteristics of Pomeranian bitches were determined. The duration of pregnancy was 62.4 days, the age of sexual maturity was 404.4 days, the length of interval between two estruses was 205.2 days, the number of offspring was 3.4, the number of male puppies was 2.15, the number of female puppies was 1.4, the stillbirth rate was 1.4%, and death rate up to 1 year 16.7%. In addition, the mating season did not affect the number of female puppies, the number of stillborn and the total number of offspring (P>0.05) but that the mortality rate up to the age of 1 was higher in the offspring of dogs mated in autumn-winter seasons than in the offspring of those that mated in spring-summer seasons (P<0.05). There was no difference between the naturally mated and the artificially inseminated Pomeranian bitches in the pregnancy rates, the number of female puppies, the number of stillborn and the total number of offspring (P>0.05), but it was determined that numerically more offspring could be obtained by application of artificial insemination with fresh semen to Pomeranian dogs with confirmed heat.

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Factors affecting fertility traits and milk yield of Holstein cattle with different origins raised in Trakya Region

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ABSTRACT

This study was conducted with Holstein heifers imported from the United States (US), Germany, and the Czech Republic to a private dairy farm in Kırklareli/Lüleburgaz region in Turkey to evaluate their adaptation in terms of milk production and fertility performance. The first insemination age of the herd was 490.62 days, and the first calving age was 804.24 days. The number of inseminations required per pregnancy was calculated as 3.99, gestation length as 279.72 days, and service period as 213.99 days. The rate of abortion and twin births were higher in those of US origin. Increased first calving age was observed in heifers of German origin due to delayed insemination. The mean actual lactation milk yield in the first lactation periods was 11834.75 Lt, 305 days milk yield was 8573.31 Lt, lactation length was 419.61 days, the dry period was 63.02 days. Milk yield performance of Holsteins of US origin was higher in the first lactation period. In conclusion, milk production was profitable; however, the fertility performance of the herd was poor in general. Poor fertility performance was due to poor herd management and adaptation problems. Therefore, after the calving period, more attention should be paid to oestrus monitoring and insemination activities. In Turkey, the success of live animal imports should be well investigated; in particular, problems occurring during animal selection for importation should be evaluated, and short-/long-term national strategies should be developed.

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Introduction

The cattle population in Turkey has reached 17 million heads in 2018 with an increase of 73% compared to the beginning of the 2000s. While the presence of culture cattle breeds increased by 350% and crossbred cattle by 61%, the presence of local breeds cattle decreased by 56% (MMB, 2018).

Animal imports to Turkey have started from the beginning of the 2000s to increase the ratio of culture and crossbred cattle in the cattle population and to improve the productivity in farming. Cattle imports constitute the largest part of livestock imports. While a total of 48000 cattle were imported in 2014, this figure increased to 132000 in 2018 (MMB, 2018). Live animal and food product imports, including live cattle imports

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to Turkey, were worth 6.9 billion; 6.5 billion; 6.4 billion, 8.3 billion, 8.8 billion and 8.6 billion between the years of 2014-2019, respectively. Germany, Austria, the United States (US), and the Czech Republic are among the most preferred countries for importation of live cattle. These imports were mostly made to the Aegean, East Marmara, Mediterranean, and West Marmara regions, respectively (TURKSTAT , 2020).

The effect of livestock imports on the national economy is a controversial issue. Although it is thought that live animal imports would increase productivity, opposite views are also available, suggesting that live animal imports would not be beneficial in the long term. Imports of live cattle can only be a short-term solution to Turkey's livestock problems. Live animal imports can bring meat and milk prices under control in the short term, but this effect will not be permanent, and in the long run, imports may cause an economic damage as these imports will cause a decrease in the financial reserves of Turkey. It is reported that animal breeders in Turkey do not have enough economic power to raise high-quality breeder cattle, their technical knowledge is generally not sufficient, therefore live animal imports will have a negative impact on small family businesses. Furthermore, following imports, fluctuations in raw milk prices in Turkey will cause breeder cattle to be sent to slaughterhouses, and the import-induced economic loss may be massive (Aydın et al., 2011).

The levels of reproductive and productive traits of the herd are closely related to the adaptation abilities of the animals to the climatic and environmental conditions of the region where they are raised. If animals are brought to a newly established farm from another region, or if the animals are imported from abroad, this change in the environmental conditions (e.g., climate, geography change) may be a stress factor for the animals, suppress the immune system, increase the incidence of diseases, and finally reduce the level of productivity (Yalçın, 1981).

The improvements made in the shelter conditions, especially the improvements in the micro-environmental parameters, as well as the improvements in the herd management increase the adaptability of the animals. As a result, these improvements indirectly cause an increase in milk yield (Kant et al., 2017). Poor welfare conditions in regionally displaced dairy cattle increase stress and aggression. It has been reported that old and non-pregnant cows have higher adaptation abilities than young and pregnant cows, and they adapt more easily to new environmental conditions (Broucek et al., 2013).

The aim of this study was to determine the fertility and milk yield levels of Holstein cattle imported from the US, Germany, and the Czech Republic to a private cattle farm in the Lüleburgaz/Kırklareli region and to compare their adaptation capabilities in terms of fertility and milk yield in the first production period.

Materials and Methods

This study was carried out in a newly established private dairy cattle farm in Lüleburgaz district of Kırklareli province in Turkey. The material of this research was 581 Holstein cattle imported to the enterprise as pregnant heifers from disease-free breeding farms in three different countries. Of these heifers, 182 were imported from the Czech Republic, 119 from Germany, and 280 from the US. Cows imported from the US were transported both by sea and land, whereas cows imported from the Czech Republic and Germany were transported by land.

All animals were milked twice a day with an automatic milking machine. Cows were grouped according to their reproductive status and milk production levels and were fed with different rations. The contents of these rations are presented in Table 1.

Table 1. Content of rations according to yield and pregnancy status of cows

	High Yield (kg/day)	**Moderate Yield** (kg/day)	**Low Yield** (kg/day)	**Pregnant Heifers** (kg/day)	**Close-up Dry Pregnancy** (kg/day)	**Far-off Dry Pregnancy** (kg/day)
Milch Factory Feed	15	11.5	10.5	-	-	-
Silage	24.8	19	19	10	12	12
Clover	3.5	3.75	2.5	1	1	1
Meadow Grass	1.8	2	2.5	2	-	-
Sodium bicarbonate	0.2	0.2	0.2	-	-	-
Straw	0.2	0	1	3	1	3.4
Canola Pellets	1	-	-	-	-	-
Dry Period Feed	-	-	-	3	-	4.5
Trans Feed	-	-	-	-	5.75	-
By-Pass Fat	0.4	0.2	-	-	0.1	-

Table 2. Some reproduction parameters and significance control in Holstein cows of different origins in the first production year (%).

Characteristics	CZ		DE		US		Overall		Significance
	n	%	n	%	n	%	n	%	
Cows pregnant at 1st insemination	43	23.6	24	20.2	79	28.2	146	25.1	NS
Cows pregnant at 2nd insemination	27	14.8	21	17.6	61	21.8	109	18.8	NS
Cows pregnant at 3rd insemination	29	15.9	18	15.1	44	15.7	91	15.7	NS
Cows pregnant at 4th + inseminations	83	45.6[a]	56	47.1[a]	96	34.3[b]	235	40.4	*
Total	182	100.0	119	100.0	280	100.0	581	100.0	-
Cows with stillbirth	4	2.2	3	2.5	8	2.9	15	2.6	NS
Cows with abort	3	1.6	1	0.8	15	5.4	19	3.3	NS
Cows with twin birth	0	0.0	0	0.0	4	1.4	4	0.7	-
Cows with single birth	175	96.2	115	96.6	253	90.4	543	93.5	NS
Total	182	100.0	119	100.0	280	100.0	581	100.0	-

CZ: Czech Republic; DE: Germany; US: United States

a, b: Differences between means marked with different letters in the same column are significant

NS: Not Significant (P>0.05); *: P<0.05

Investigated reproductive traits included age at first insemination, age at first calving, insemination number, first insemination interval, service period, gestation length, calving interval, and yearly fertility parameters expressed in proportion. Milk yield was evaluated with the following parameters: actual milk yield, 305-day milk yield, lactation period, dry period, and persistence of lactation. The effect of origin (the Czech Republic, Germany, or the US), birth/calving month at first production year, and their interactions on these parameters were determined.

Age at first insemination and age at first calving were categorized into subgroups according to the origin and the cow's month of birth, while other reproductive parameters and milk yield traits were categorized into subgroups according to the origin and calving month.

The difference between the groups was analyzed by ANOVA using the general linear model (GLM) procedure. The statistical model used was as follows:

$Yijk = \mu + ai + bj + abij + eijk$

where Yijk was the individual observation, μ was the overall mean, ai was the effect of origin (i = Czech Republic, Germany or the US), bj was the effect of month of calving/birth (j = January, February, …), abij was the effect of origin and month of calving/birth interaction, and eijk was random error. The differences between subgroup means were determined with Duncan's test. In order to determine the persistence, individual daily milk levels were grouped for every fifteen days and differences between origins were analyzed. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY.

Results

Fertility: The insemination, pregnancy, and calving status of Holstein cattle of different origins in the first production period including fertility parameters expressed in proportion are presented in Table 2. In the first production period, 93.5% of the cows gave single birth, 2.6% stillbirth, and 3.3% aborted. Twin births were only observed in Holstein heifers of US origin. No statistical difference was detected between different-origin Holsteins in terms of a single birth, stillbirth, or abort rates. While the rate of conception was similar for all groups in cows that got pregnant after 1st, 2nd, or 3rd inseminations, in the group of cows that became pregnant after four or more inseminations, Holsteins of Czech and German origin had a higher rate of pregnancy than those of US origin (P<0.05). When all the cows were evaluated together, the pregnancy rate in the first insemination was determined as 25.1%.

The findings regarding the first insemination and first calving ages of Holstein heifers of different origins are presented in Table 3. The mean age at first insemination of Holstein heifers was 490.62 days. Holstein heifers of Czech Republic origin reached the first insemination age at an earlier age, heifers of German origin at a later age, and a statistically significant difference was found between the first

Table 3. Some reproduction parameters and significance control according to the origin and calving month in Holstein cows of different origins

Factors		Insemination number		Gestation length		First insemination Interval		Service period		Calving interval		Age at first insemination [f]		Age at first calving [f]	
	n	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$
Origin															
CZ	182	4.18	0.283	278.6^{b}	0.622	96.6	5.450	229.21^{a}	12.169	507.9^{a}	12.300	471.2^{c}	6.989	776.8^{b}	8.512
DE	119	4.34	0.348	279.5^{a,b}	0.761	103.7	11.877	222.3^{a,b}	14.892	501.7^{a,b}	15.053	517.4^{a}	7.367	842.3^{a}	8.972
US	280	3.44	0.178	280.9^{a}	0.388	89.3	3.091	190.4^{b}	7.596	471.4^{b}	7.677	491.7^{b}	3.179	796.6^{a}	3.872
Calving month															
January	70	3.77	0.367	280.9	0.805	84.8	5.992	208.1	15.754	489.1	15.923	469.4^{c,d,e}	6.330	762.2^{c,d}	7.709
February	60	3.68	0.390	281.4	0.856	96.3	7.383	218.1	16.766	499.6	16.946	449.9^{e}	17.205	821.6^{d}	20.953
March	66	4.32	0.348	280.1	0.764	97.1	5.864	229.9	14.953	510.1	15.114	452.6^{d,e}	12.819	803.5^{d}	15.612
April	20	5.23	0.612	278.9	1.342	99.1	13.053	243.1	26.279	521.9	26.562	470.8^{c,d,e}	12.508	797.3^{b,c,d}	15.233
May	15	5.04	0.721	280.5	1.582	72.1	11.807	283.2	30.973	563.7	31.307	512.8^{a,b}	13.885	785.5^{a,b,c}	16.910
June	24	3.75	0.599	276.4	1.314	76.1	9.848	192.4	25.731	468.9	26.009	513.2^{a,b}	13.682	846.9^{a,b,c}	16.663
July	34	4.92	0.528	279.1	1.142	83.1	8.221	232.5	22.360	511.7	22.601	500.8^{a,b,c}	17.484	828.1^{a,b,c}	21.293
August	28	3.01	0.591	280.6	1.297	90.6	17.440	156.5	25.392	437.1	25.666	508.8^{a,b}	11.086	822.9^{a,b}	13.501
September	11	3.28	0.854	279.8	1.874	114.4	24.767	187.1	36.687	466.8	37.082	525.7^{a}	8.280	828.1^{a}	10.084
October	50	3.46	0.435	279.6	0.932	115.3	7.213	219.6	18.242	499.2	18.438	515.7^{a,b}	6.294	810.8^{a,b}	7.665
November	77	3.73	0.342	279.1	0.750	90.2	7.013	203.3	14.679	482.3	14.837	487.8^{b,c,d}	5.710	771.4^{b,c,d}	6.955
December	126	3.63	0.248	279.8	0.544	91.1	4.295	193.8	10.658	473.6	10.773	488.7^{b,c}	9.432	758.1^{b,c,d}	11.487
Overall	581	3.99	0.174	279.7	0.382	92.9	3.172	213.9	7.482	493.7	7.562	490.6	3.506	804.2	4.270
Significance															
Origin		NS		*		NS		*		*		***		*	
Calving Month		NS		NS		NS		NS		NS		***		*	
Origin x Calving Month		NS		NS		*		NS		NS		***		*	

$\bar{x}$: Mean; $\sigma_{\bar{x}}$: Standard error. CZ: Czech Republic; DE: Germany; US: United States. a, b, c, d, e: Differences between means marked with different letters in the same column are significant (P<0.05). f: The first insemination and calving ages were compared according to the month of birth. NS: Not Significant (P>0.05); *: P<0.05; ***: P<0.001

significant difference was found between the first insemination ages for all origins (P<0.001). The lowest mean age at first insemination was observed in heifers born in February. The mean age at the first calving of heifers of all three origins was 804.24 days. The mean age of the first calving of Holstein cows from Germany and the Czech Republic was older than those of US origin (P<0.001). The mean age of the first calving of heifers born in January was 762.2 days, and this duration was significantly shorter than those born in August, September, and October (P<0.001). The interaction between origin and month of birth was statistically significant in terms of both first insemination age and first calving age (P<0.001).

Insemination number, gestation length, first insemination interval, service period, and calving interval of Holstein heifers of different origins are presented in Table 3. The overall inseminations number of Holstein heifers in the first production period was 3.99, gestation length was 279.72 days, first insemination interval was 92.92 days, service period was 213.99 days, and calving interval was 493.71 days. The effect of origin and calving month on the number of inseminations was not statistically significant (P>0.05). A statistically significant difference was found only between Holstein cows originating from the Czech Republic and the US in terms of gestation length, and it was determined that the mean gestation length of cows originating from the US was approximately 2 days longer (P<0.05). The effect of calving month and origin × calving month interaction on the length of gestation was not significant (P>0.05). The effect of origin and calving month on the first insemination interval was not significant as well. The mean service periods of Holstein cows originating from the Czech Republic, Germany, and the US were calculated as 229.2 days, 222.3 days, and 190.5 days, respectively. A significant difference was found between Holstein cows originating from the

Czech Republic, Germany, and the US were calculated as 229.2 days, 222.3 days, and 190.5 days, respectively. A significant difference was found between Holstein cows originating from the Czech Republic and the US, and it was observed that the mean service period of cows originating from the US was shorter than the others (P<0.05). Calving month did not have an effect on the service period (P>0.05). The mean first calving intervals of Holstein cows originating from the Czech Republic, Germany, and the US were calculated as 507.9 days, 501.8 days, and 471.4 days, respectively. The mean first calving interval was shorter in Holstein cows from the US than in cows from the Czech Republic (P<0.05).

Milk Yield: The actual milk yield and 305-day milk yield parameters of Holstein Friesian cows from the Czech Republic, Germany and US are presented in Table 4. In the first lactation period, the highest mean actual milk yield was calculated as 12206 lt in Holstein cows of US origin, followed by Czech origin (12033 lt) and German origin (10664 lt). However, a significant difference was not found in terms of origin in actual milk yield values (P>0.05). When the 305-day milk yield in the first lactation was evaluated, it was found that Holstein cows of US origin (8919 lt) produced significantly more milk than cows of German (8073 lt) and Czech (8357 lt) origin (P<0.05). In cows of different origins, it was observed that calving month did not have a significant effect on actual milk yield or 305-day milk yield (P>0.05).

The mean overall lactation period of Holstein cows was 419.61 days, and the mean dry period was 63.02 days (Table 4). It was found that the effect of origin and calving month on the lactation length of cows of

Table 4. Some lactation parameters and significance control according to the origin and calving month in Holstein heifers of different origins

Factors		**Actual milk yield** (litres)		**305 Days milk yield** (litres)		**Lactation period** (days)		**Dry period** (days)	
Origin	**n**	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$	$\bar{x}$	$\sigma_{\bar{x}}$
CZ	182	12033.1	856.14	8357.2^{b}	288.47	443.4	23.71	63.3^{b}	2.71
DE	119	10664.5	1008.86	8073.4^{b}	339.93	396.3	27.94	97.7^{a}	5.92
US	280	12206.6	263.78	8919.6^{a}	88.88	415.4	7.31	48.38	1.38
Calving month									
January	70	12572.9	492.57	8822.2	165.97	433.1	13.64	56.9	2.81
February	60	11933.3	694.91	8630.2	234.14	422.9	19.25	56.9	2.91
March	66	13300.1	523.81	9064.3	176.49	445.4	14.51	56.3	2.87
April	20	11582.2	2058.14	9438.8	693.47	376.1	57.01	55.5	6.35
May	15	13858.5	1035.91	9266.6	349.04	456.8	28.69	47.4	5.89
June	24	11494.2	818.96	8585.8	275.94	413.9	22.68	49.7	4.45
July	34	13328.4	688.06	8703.2	231.84	461.7	19.06	48.2	3.87
August	28	9367.1	1472.02	7599.8	495.98	365.8	40.77	86.9	8.63
September	11	10638.7	2103.93	8295.4	708.90	389.0	58.28	53.2	12.27
October	50	12081.7	1393.65	7989.0	469.58	471.8	38.60	74.2	3.20
November	77	11813.8	591.17	8619.4	199.19	413.2	16.37	67.5	3.39
December	126	11625.6	368.25	8606.6	124.08	405.8	10.20	73.6	2.09
OVERALL	581	11834.7	352.03	8579.3	118.61	419.6	9.75	63.1	1.63
Significance									
Origin		NS		*		NS		*	
Calving Month		NS		NS		NS		NS	
Origin x Calving Month		NS		*		NS		NS	

x: Mean; $\sigma\bar{x}$: Standard error. CZ: Czech Republic; DE: Germany; US: United States. a, b, c: Differences between means marked with different letters in the same column are significant (P<0.05). NS: Not Significant (P>0.05); *: P<0.05

Table 5. Milk yield in 15-day periods and comparisons according to origin in the first lactation in Holstein cows of different origins

		DAYS																			
	N	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**	**195**	**210**	**225**	**240**	**255**	**270**	**285**	**305**
CZ	182	20.6	25.9	28.4	30.2	31.2	31.2	31.4	31.4	31.1	30.4	29.6	29.3	28.8^{b}	27.9^{b}	27.7^{b}	27.5	27.2	26.8$^{a, b}$	27.1	26.8
DE	119	20.8	26.9	29.2	30.7	30.9	31.5	31.4	30.5	30.7	30.6	30.6	30.4	30.6^{a}	29.7^{a}	29.4^{a}	28.7	27.2	26.1^{b}	25.5	25.3
US	280	21.1	26.2	29.4	31.1	31.9	32.2	31.7	31.4	31.1	30.8	30.5	30.2	29.8$^{a, b}$	29.3^{a}	29.1^{a}	28.7	28.2	27.8^{a}	26.9	26.3
Significance		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS	*	NS	NS

CZ: Czech Republic; DE: Germany; US: United States; NS: Not significant; *:P<0.05.

different origins was not statistically significant (P>0.05). The effect of the origin on the dry period was found to be significant. In the first lactation period, the shortest mean dry period was detected in Holstein cows of US origin, followed by cows of the Czech Republic and Germany origins, respectively (P<0.001). The effect of calving month on the dry period was found to be nonsignificant (P>0.05).

The daily mean milk yield of Holstein cows of the Czech Republic, Germany, and US origin during the first lactation period are presented in Table 5 and Figure 1 as 15-day periods. In the first lactation period, Holstein cows of German and US origin reached the peak level in the period between 76-90th days with a mean milk yield of 31.5 and 32.2 lt, respectively, while the Holstein cows of Czech origin reached the peak level in the period between 101-105th days with a mean milk yield of 31.4 lt. In the first lactation, the mean milk yield was higher in Holstein cows of German origin than in cows of other origins on 195, 210, and 225th days, while the yield decreased below the mean milk yield level of other origins on the 270th day (P<0.05). Cows of US origin showed a more stable lactation persistency during the onset of lactation, peak period, and dry period.

Discussion

Fertility: For all origins, the conception rates were found to be similar in the first, second, and third insemination, while the rate of conception of Holstein cows of Czech and German origin was found to be higher than those of US origin in the cows that became pregnant after four or more inseminations. In industrial dairy cattle farms, the aim of the conception rate for the first insemination is below 50%. In this present study, pregnancy rates of cows with different origins varied between 20.2% and 28.2% in the first insemination and these results are far behind compared to the industrial target. The stillbirth rate (2.6%) determined in the present study was found to be lower than that reported by Bayram et al. (2015). The stillbirth rate determined in the present study was found to be higher than the first production period and lower than the second production period in the reports of Deliömeroğlu (1993) and Özcan (1994). The abort rate (3.3%) determined in this study was higher than that reported by Deliömeroğlu (1993). The abort rate calculated in this study was higher than the abort rate calculated in the first production period and lower than the second production period in the report of Özcan (1994). The twin birth rate obtained in this present study (0.7%) was found to be higher than the first production period and lower than the second production period in the report of Özcan (1994). Although the fertility parameters expressed in proportion remain below the recommended ideal level to make an efficient breeding, it can be accepted that they are similar to the literature reported for Holstein cows in Turkey in general.

The mean age at first insemination of young heifers grown in industrial dairy cattle farms and to be included in production for the first time is ideally expected to be between 15–16 months (450–480 days) (Oğan et al. 2011). In this study, the mean age of first insemination of Holstein cows of Czech Republic, Germany, and US origin was calculated as 471.3 days, 517.5 days, and 491.8 days, respectively. Considering the ideal age at first insemination of the Holstein heifers and the mean age at first insemination calculated in this study, it can be said that the age at first insemination of the heifers of US and Czech Republic origin were within the normal limits, but the heifers of German origin were inseminated in a period later than the ideal insemination age. When the mean age at first insemination calculated for the Holstein cows of German origin (517.5 days, approximately 17.3 months) was compared with the information reported in the literature, it was seen that the data obtained in this study was higher than the data reported by Orman (2003), Akbaş and Şahin (2008), and less than the data reported by Özcan (1994), Duru and Tuncel (2002), Aslan and Altınel (1992), and Özçelik and Arpacık (1996). In this study, the lowest mean age of first

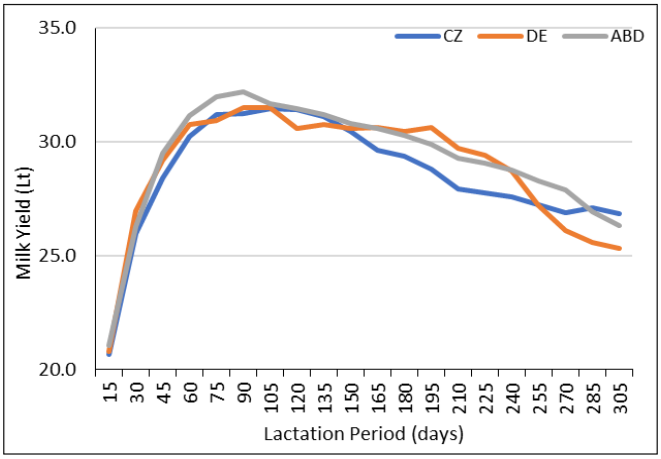

Figure 1. Milk yield in 15-day periods in the first lactation in Holstein cows of different origins
CZ: Czech Republic; DE: Germany; US: United States.

insemination was observed in Holstein heifers born in February. It was observed that the Holstein heifers born in February reached the age at first insemination significantly earlier than the heifers born in May, June, July, August, September, October, November, and December with an average of 449.96 days. Contrary to the result obtained in this study, Özcan (1994) found that the effect of the year and season on the age at first insemination in Holstein heifers was nonsignificant. Koçak et al. (2007) similarly found the effect of the season on the age at first insemination to be nonsignificant in Holstein cows. The reason for these differences may be due to the differences in the geographical conditions, climate or breeding techniques in which the studies were conducted.

The age at first calving in dairy cattle enterprises is ideally expected to be between 24-25 months (720-750 days) (Oğan et al. 2011). However, some researchers report that prolongation of this period up to 30 months should be considered normal (Kumlu and Akman, 1999). In this present study, the mean age at first calving for the whole herd was 804 days (26.8 months). The lowest mean age at first calving was determined in Holstein heifers of Czech origin as 777 days (25.9 months), followed by Holstein heifers of US origin as 797 days (26.6 months) and those of German origin as 842 days (28.1 months). It was determined that the mean age at first calving of Holstein heifers of Czech origin was shorter than those of German and US origin. Considering that the mean age at first insemination of heifers imported from Germany is also higher, this difference may be related to the insemination strategies applied in various regions/ territories/enterprises. For the heifers of German origin, calving at later ages may be due to the late insemination of pregnant heifers in the farm where they were imported from. When the studies conducted with Holstein cattle bred in Turkey were reviewed, it was observed that the mean age at first calving varied between 26.1 months (Orman, 2003) and 36.9 months (Akbulut et al., 1992). In this study, the mean age at first calving for Holstein cows of Czech Republic origin was younger than the mean age at first calving in all examined reports. The same parameter for Holstein heifers of German origin was only longer than those reported by Orman (2003) but shorter than those reported by Duru and Tuncel (2002), Karakaş (1996), Koçak et al. (2008), Özcan (1994), Alpan et al. (1976), and Akbulut et al. (1992). The mean age at first calving of the Holstein heifers of US origin was longer than those reported by Orman (2003), Duru and Tuncel (2002) and Karakaş (1996), and shorter than those reported by Koçak et al. (2008), Özcan (1994), Alpan et al. (1976), and Akbulut et al. (1992). Studies conducted with Holstein cattle outside of Turkey indicate that the youngest mean age at first calving was reported in Finland with 25.6 months (Mantysaari et al., 2002), and the oldest was reported in Ghana with 36 months (Osei et al., 1991).

Both the age at first insemination and first calving determined in this study were evaluated as far from the ideally accepted targets. This suggests an inadequate selection of heifers imported to the enterprise in the farms that they are imported from. Failure to carefully examine the pedigree information during the selection of pregnant heifers, and failure to apply scientific body condition scoring and breeding value criteria may have led to these results.

Insemination number below 1.5 is considered to be good, between 1.5-2.0 is considered fair, and above 2.0 is considered to be problematic reproductive performance (Alpan and Aksoy, 2012). Oğan et al. (2011) reported that the insemination number that can be considered ideal for dairy cattle should be less than 2. In the present study, the mean insemination number of Holstein cows of Czech Republic, Germany, and US origin were 4.2, 4.3, and 3.4, respectively. When the mean insemination number was compared with the relevant literature reports, the findings in this study were higher than all reported mean insemination number for Holstein cows. In the reviewed literature, the lowest mean insemination number (1.3) was reported by Duru and Tuncel (2002), and the highest mean insemination number (2.4) was reported by Özcan (1994). Similar to the results found in this study, many studies are reporting that the effect of the year and season on the mean insemination number in Holstein cows is insignificant (Özçelik and Arpacık, 1996; Orman, 2003).

Pregnancy is expected to last for an average of 279±5 days in Holstein cows (Yalçın, 1981; Alpan, 2012). In the present study, it was observed that the

gestation length was 279.7 days in the first production period. It was found that the mean gestation length in Holstein cows of Czech origin was about 2 days shorter than those of US origin. This difference may be regarded as reasonable. For Holstein cows of different origins, it can be said that the pregnancy durations observed in the present study are consistent with those reported determined in similar scientific studies. The shortest gestation length was determined in heifers calving in June in the first production period, The longest gestation length was observed in heifers calving in February. However, the effect of calving months on gestation length was insignificant. Even if the effect of the calving month was statistically insignificant, the shortest gestation length observed in Holstein cows calving in warm months indicates that pregnancies in warm months can end earlier. Similarly, longer pregnancies have been reported in Holstein cows calving in winter compared to those calving in summer (Özçelik and Arpacık, 1996). Similar to the results obtained in this study, many researchers reported that the effect of environmental factors on pregnancy duration was insignificant (Özcan, 1994; Balcı, 1999; Orman, 2003).

In order to perform a profitable breeding in dairy cattle production, it should be aimed to inseminate the cow in the first oestrus period and 60 days after calving (Alpan and Aksoy, 2012). These values are recommended to be between 45-80 days for ideal breeding (Oğan et al. 2011). Although no statistically significant difference was found between the origins, the shortest first insemination interval was observed in cows of US origin in the first lactation period. For all three origins, the first insemination intervals of Holstein cows in the first production period were found to be above the recommended values. In the present study conducted with Holstein cows of different origins, the value of the mean first insemination interval obtained in the first production period was found to be higher than the reports of Pelister et al. (2000), Özcan (1994), and Aydın and Deveci (2001), and lower than the report of Orman (2003).

In this study, the mean service period of Holstein cows for the whole herd was 214 days. The length of the service period detected in the first production period remained above normal. When the results of the studies conducted with Holstein cows in Turkey are examined, it is seen that the mean service period varies between 93 days (Duru and Tuncel, 2002) and 218 days (Halıcıoğlu, 1989). The mean service period of the herd in this study was similar to the longest service period reported in Turkey. This result suggests that the first production period in this farm was far from an economic breeding in terms of the service period. In this study, it was determined that the effect of calving month on the service period in Holstein cows was insignificant. However, contrary to the result obtained in this study, Özcan (1994), Pelister et al. (2000), Orman (2003), and Özçelik and Arpacık (1996) found that the year or season had a significant effect on the service period.

In heifers calving for the first time and in high-yielding cows, prolongation of calving interval up to 400 days is considered normal (Noakes, 1997). Oğan et al. reported the target calving interval for dairy cows as 365-405 days (Oğan et al., 2011). Among the reviewed literature reports, the shortest mean calving interval was 369 days (Duru and Tuncel, 2002) and the longest calving interval was 438 days (Koçak et al., 2008) in the studies conducted with Holstein cows in Turkey. The shortest mean calving interval calculated in the present study was found in Holstein cows of US origin (471 days), but even this period is longer than the longest reported calving interval in Turkey. This shows that an economic breeding could not be performed in the first period in terms of calving interval in the enterprise where cows of different origins were bred. The technical failure in oestrus monitoring and insemination in the enterprise caused the service period and calving interval periods to be prolonged. Since this situation was observed in all cows of different origins in the first period, it would be more reasonable to explain it not with genetics and adaptation problems of the cows to the region, but with the management of the herd in the enterprise, the qualification of the personnel dealing with the cows, and the technical inadequacies in the insemination. In this study, it was concluded that the calving month did not have an effect on the calving intervals of Holstein cows. However, there are also studies reporting that factors such as year and season have a significant effect on the average calving interval in Holstein cows. While Pelister et al. (2000) reported that the effect of the year was significant in Holstein cows, Özçelik and Arpacık (1996) reported that the year and season, and Özcan (1994) reported that the season had an effect on the calving interval.

Milk Yield: The actual milk yield of cows of different origins, which were maintained and fed under similar conditions, was 11,835 lt in the first lactation period for the overall herd. A significant difference was observed between the origins in the first lactation period. In terms of mean 305-day milk yields, Holstein cows of US origin produced significantly more milk than other origins. The actual milk yield was reported as 3934 lt by Karakçı (1990), 4556.4 lt by Pelister (2000), 5350.7 lt by

Özcan (1994), 7473.5 lt by Şahin and Ulutaş (2010), and 8379.9 lt by Kaya and Bardakçıoğlu (2016) conducted with Holstein cows of different origins raised in different regions in Turkey. Alkoyak (2018) reported that the mean actual milk yields were 9034 lt in Holstein cows of Estonian origin and 8383 lt in those of US origin. It is seen that the mean actual milk yields for the Holstein cows bred in Turkey vary between 3934 and 9034 lt. In the present study, the actual milk yields calculated for the US, Czech, and German-origin Holstein cows are higher than the average values reported in Turkey for all three origins. It can be said that this situation is associated with the delay of the second pregnancy of the cows in the enterprise. The delay in the next pregnancy of cows prolongs the length of lactation, and naturally, the actual milk yields also show a relative increase. Although this situation prolongs the lactation period in enterprises and increases the actual milk yields, which is an uncorrected lactation parameter, it should be kept in mind that it may negatively affect the duration of the cows' stay in the herd and their lifetime productivity.

For the overall herd, the 305-day milk yield for cows of different origins was calculated as 8579 lt in the first lactation period. It was observed that the cows of US origin produced more milk than those of the Czech Republic and German origin, and this difference was statistically significant. The 305-day milk yields of Holstein breed cows raised in Turkey were reported by Halıcıoğlu (1989) as 3171 lt, 2771 lt, and 3500 lt for the Holstein cows of US, Netherlands, and Karacabey origin, respectively; by Karakçı (1990) as 5119 lt for those of Israeli origin, 4394 lt for German origin, and 4382 lt for US origin; by Pelister (2000) as 4455 lt for German origin; by Özcan (1994) as 4711 lt; by Şahin and Ulutaş (2010) as 6976 lt; by Alkoyak and Çetin (2018) as 7450 lt and 6738 lt for Holstein cows of the US and Estonian origin, respectively, and by Kaya and Bardakçıoğlu (2016) as 7909 lt. Our literature search indicates that the 305-day milk yields of Holstein cows grown in Turkey vary between 2771 and 7909 lt. In the present study, the 305-day mean milk yield values obtained with Holstein cows were higher for all three origins than those reported in the studies conducted in Turkey. This gives the impression that all imported Holstein cows have high genetic capacities in terms of milk yield capability. Similar to the results obtained in this study, Van Dorp et al. (1998) reported a mean milk yield of 8519 lt for 305 days in their study in Canada. In a study conducted with cows of US origin in Poland, 305-day milk yields were reported to be lower than the results obtained in this study. In the study conducted in Poland, the mean milk yield of the cows of US origin in the first three lactation periods was 3955, 4011, and 4014 lt, respectively (Zarnecki, 1991). However, there are also studies in the US conducted with Holstein cows reporting a higher 305-day mean milk yield (109,78 lt) than our study (Gröhn, 1999).

It has been reported that the ideal lactation period for a profitable management in dairy cattle breeding is between 300-320 days (Oğan et al., 2011). In this study, the mean lactation length for the first lactation period was 420 days for the overall herd, and 443 days, 396 days, and 415 days for cows of Czech Republic, Germany, and US origin, respectively. The effect of origin and month of calving was insignificant on the lactation period. However, the mean lactation period of this study was above the ideal lactation period limits. This shows that cows had problems during the conception process. The main fertility parameters and lactation periods reveal that pregnancies are delayed in the enterprise and this situation prolongs the lactation periods. This can be explained by herd management problems rather than the genetic predisposition and adaptability of cows. The mean lactation periods in the studies conducted with Holstein cows raised in various regions in Turkey were reported by Duru and Tuncel (2002) as 304.4 days, Kaya and Bardakçıoğlu (2016) as 319.5 days, Şahin and Ulutaş (2010) as 326.5 days, Özcan (1994) as 355.7 days, and Alkoyak (2018) as 352 days for the overall herd, 296 days for cows of US origin, and 370 days for those of Estonian origin. In the present study conducted with Holstein cows of different origins, the mean lactation periods were longer than the lactation periods reported in the literature.

It is reported that milk production should be stopped at least two months before calving and the optimal dry period should be between 42-75 days in order to achieve the expected milk yield in the next lactation period and to prepare the cow for the calving (Oğan et al., 2011; Dinç, 2016). In the present study, the mean dry periods were within the ideal limits reported in the literature. The mean dry period of the Holstein cows of German origin was longer than recommended ideal dry period however, the optimal dry period was reached in the cows of Czech origin. Cows of US origin were found to have a short dry period. Since the milk yield capabilities of cows of US origin were at a higher level than cows of German and Czech origin, the short duration of the dry period can be interpreted as not having a suppressive effect. Cows of US origin that reach the ideal level of dry period, may achieve a higher milk yield performance than determined in the present study and may reflect their complete genetic potential. In the studies conducted with Holstein cows raised in different regions in Turkey, the mean dry period was reported by Çetinkaya (2006)

as 62 days, by Kaya and Bardakçıoğlu (2016) as 62.7 days, by Özcan (1994) as 67.8 days, and by Şahin and Ulutaş (2010) as 82.2 days. From these reports, it is understood that the mean dry period of the cows in Turkey varies between 62 days and 82 days. The mean dry period of the overall herd observed in the present study was longer than those reported by Çetinkaya, (2006) and Kaya and Bardakçıoğlu, (2016), and shorter than those reported by Özcan (1994), Şahin and Ulutaş (2010).

In terms of persistency, no difference was observed between origins in general. The highest milk yields were observed in the 90-105 day periods. The lactation persistency values calculated for all three origins examined in the present study were generally consistent with the reports of Kaya and Kaya (2003), Küçük Baykan and Özcan (2017), Cummins et al. (2012), and Hagiya et al. (2014). In the present study, although the data obtained with Holstein cows of different origins were similar to the study of Küçük Baykan and Özcan (2017) on Brown and Simmental breed cows in terms of the time to reach the highest milk yield in the first lactation period, it was observed that the highest milk yield amounts observed in the present study were higher than those reported by Küçük Baykan and Özcan (2017).

Conclusions

In conclusion, generally, the milk yield performances of cows of Czech, German, and US origin were evaluated as good, while their reproductive performances were evaluated as problematic in the private enterprise, which started production by importing the first animal material from three different countries for intensive dairy cattle breeding in Kırklareli province. Providing appropriate breeding and feeding may help the cows to reflect their genetic potential so they can maintain their milk yield performance and even reach higher production levels. Utmost care should be taken in the oestrus monitoring and insemination activity of all cows of the three origins with regard to reproduction, since the failures detected in this study, are due to the adaptation difficulties of imported cows as well as the inadequacies in herd management. Therefore, fertility parameters should be meticulously monitored, appropriate insemination techniques should be applied, monitoring for diseases that may affect fertility and milk yield should be performed, and the animals should be fed with adequate rations and thus possible negative effects on adaptation should be minimized. In addition, the success of animal imports in Turkey should be investigated with large scale and well-designed studies on a country, region, province, and enterprise basis; in particular, the problems experienced in the first animal selection and its consequences should be examined, and new short and long-term strategies should be developed on a national basis in a way that will ultimately constitute an alternative to livestock import policies.

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Feline fibroepithelial hyperplasia and current treatment protocols

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ABSTRACT

Due to prolonged exposure to daylight, the seasonal oestrus cycle in cats has reached the point where it can spread almost throughout the year. This situation has brought many problems with it. Mammary tumors and mammary gland hyperplasia are the most common conditions in mammary tissue as a result of exogenous progesterone applications for reproductive control. In this review, information about the etiology, pathogenesis, clinical detection and especially current and successful treatment options for mammary gland hyperplasia in cats is given.

Keywords: aglepristone,mammary, ovariectomy, ovariohysterectectomy, progesterone

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Introduction

In cats, there are 4 to 5 pairs of mammary complexes, arranged in a symmetrical fashion, starting from the thorax and extending to the inguinal region. These structures are called axillary, thoracic, abdominal and inguinal mammary complexes according to their location (Gultiken, 2016). Approximately 80% of the masses in feline mammary glands are neoplastic (Görlinger et al., 2002). This makes the regular examination of the mammary tissue very important.

Mammary gland formations in cats are classified as hyperplasias and dysplasias, benign epithelial neoplasms, malignant epithelial neoplasms, special types of malignant epithelial tumors, malignant mesenchymal tumors, carcinosarcoma, hyperplasia/ dysplasia of the teat, neoplasms of the teat, non-neplastic lesions of the mammary gland (Zappulli et al., 2019). Approximately 20% of non-tumoral masses in cats are often present as mammary gland hyperplasia (Görlinger et al., 2002). In addition, atypical FEH of the teats in a Syphnx cat was reported by the reseachers (Ozenc & Bozkurt, 2014). Therefore, it is very important to make the differential diagnosis by using suitable diagnostic techniques.

Mammary hyperplasia in cats is one of the side effects of progestins (Enginler and Senünver, 2011). It was first identified in 1973 and was called feline mammary hypertrophy (Allen, 1973) but they are commonly known as fibroepithelial hyperplasia (FEH) or feline mammary hyperplasia (Görlinger et al., 2002). It is formed as a result of benign, progesterone-induced fibroglanduler proliferation of the mammary gland (Gultiken, 2016). In other words, it is also considered a form of mammary dysplasia characterized by rapid, abnormal growth of one or more mammary glands without milk production (Solano-Gallego & Masserdotti, 2016).

Fibroepithelial hyperplasia occurs mostly in young female cats (Uçmak et al., 2011) whereas FEH was reported also in male cats (Küçükbekir et al., 2020) although it can be observed in adult and geriatric cats.

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Fibroepithelial hyperplasia most often occurs between 13 weeks and 2 years of age, during the first oestrus, pregnancy or pseudo-pregnancy (Uçmak et al., 2011). However, the frequently reported age range for FEH is 6 months to 10 years (Concannon & Myers-Wallen, 1991). In chronic cases, ulcerations may occur due to the enlargement of the mammary lobe and exposure to trauma. Although it is considered a benign formation, ulcerative lesions resemble mammary tumors, especially in cases of advanced FEH (Payan-Carreira, 2013). Since mammary tumors in cats are approximately 85% malignant, differential diagnosis is very important at this point (Loretti et al., 2005). Fibroepithelial hyperplasia is a luteal stage disease and it is reversible (Payan-Carreira, 2013). The mammary lobes tend to shrink when the progestative effect disappears (Giménez et al., 2010; Leidinger et al., 2011). Spontaneous regression of the mammary lobes is usually observed as a result of luteolysis, ovariectomy/ovariohysterectomy, parturition, or natural abortion (Johnston et al., 2001; Keskin et al., 2008).

Epidemiyology and Pathogenesis: No racial predisposition has been detected in cats in mammary gland hyperplasia, and it is observed that the incidence is higher in domestic and crossbred cats in uncontrolled breeding areas when environmental and climatic conditions are taken into account (Allen, 1973)

It can occur in all female and male cats treated with progesterone, regardless of age (Hayden et al., 1981; Görlinger et al., 2002). The risk of mammary gland hyperplasia in complex with mammary tumors become higher with increasing age. In such cases, biopsy can be applied as a differential diagnosis (Murphy, 2009).

Progesterone and its synthetic analogues play an active role in many cases (Hinton & Gaskell, 1977; Bethlehem & Van der Luer, 1993; Souza et al., 2002; MacDougall, 2003; Loretti et al., 2005). Various progestins are used to prevent or suppress sexual activity in female cats (Romagnoli & Concannon, 2003). Cases of FEH have also been reported in male cats treated with medroxyprogesterone acetate (MPA) and megestrol acetate (MA) applications (MacDougall, 2003; Burstyn, 2010).

The growth and development of the mammary gland is under the control of progesterone. Progesterone exerts this effect mostly on progesterone receptors on epithelial and stromal cells. Local activation of progesterone receptors triggers structures that are specific for each mammary gland and stimulate mammary gland cellular proliferation (Payan-Carreira, 2013). Cyclic changes between estrogen and progesterone in physiological conditions stimulate or suppress the cyclic activation of progesterone receptor-mediated mechanisms (Conneely et al., 2003). The decrease in progesterone receptors is directly related to the decrease in progesterone activity. In a recent study, two isoforms of progesterone receptors (A and B) were detected at high rates in the epithelium of the mammary ducts in tissue samples taken from FEH lesions. However, when the progesterone receptor amounts in samples from mammary carcinomas and FEH lesions were compared, the rate in FEH samples was quite high (Mol et al., 2012). Progesterone dominance causes down-regulation of estrogen receptors (De Las Mulas et al., 2000). Therefore, it is possible that the duration and amount of circulating progesterone may affect the presence of estrogen receptors in the mammary tissue. Enginler and Senünver (2011) proved that significantly higher estrogen receptor labelling in mammary tissues of healthy cats were observed than MPA induced group.

The mammary gland is known as a non-pituitary source of growth hormone (GH) (Mol et al., 1996; de Melo et al.,2021). Progesterone releases insulin-like growth factors, which are active mitogens, usually released from mammary gland fibroblasts, and induces the release of GH. Therefore, FEH can also occur in the mammary glands with a synergism between progesterone, GH, and insulin-like growth factor (Ordás et al., 2004; de Melo et al.,2021).

Clinical presentation: Initial lesions are usually soft, fluctuant, jelly-like and sharply circumscribed structures (Uçmak et al., 2011). Indicated swollen mammary gland volumes are between 1.5 and 18 cm (Seixas Travassos, 2006). Generally, non-pregnant cats have asymmetrical swellings, while pregnant cats have more homogeneous swollen lesions (Payan-Carreira, 2013). In advanced cases, the lesions are edematous, inflamed, and gradually darkening in color due to the gradual swelling of the breast lobes and their anatomical location in an area open to trauma. It can also be seen that alopecia is formed in these areas. Severe inflammation, bleeding areas and necrosis can be observed with increasing trauma. Necrotic areas are most often observed in the axillary and inguinal lobes. While initial lesions do not cause pain, advanced swelling may cause severe pain and related conditions such as loss of appetite and reluctance to walk. Regardless of size, when FEH develops in pregnant cats, there is no milk production in the mammary glands (Görlinger et al., 2002). After birth, kittens are lost due to not being fed. Again, ulceration in advanced lesions make the mammary gland prone to mastitis and abscess, resulting in systemic diseases (Burstyn, 2010).

No FEH-specific conditions are observed in most laboratory tests (Leidinger et al., 2011). If mastitis and ulceration are present, increased leukocyte count and anemia may be observed. While urea, creatinine and liver enzymes in blood biochemistry are within normal limits at the initial level, they may increase due to systemic infection in advanced cases (Payan-Carreira, 2013).

Diagnosis: Diagnosis should always be based on clinical findings and anamnesis (Gaviria et al., 2010). Mammary tumors (benign or malign) should definitely be taken into consideration in the differential diagnosis. While evaluating in terms of the tumoral structures, injuries in the mammary tissue, ulceration and general condition evaluation should be taken into consideration seriously (Mayayo et al., 2018). Generally, it is not difficult to diagnose in cases where more than one mammary gland is swollen. Contrary to tumoral formations, the affected glands in FEH cases show swelling of almost similar sizes (Lana et al., 2007). The age of the animal can often be considered as a clue, but it should not be forgotten that FEH can be observed in advanced ages. Progestin applications to prevent urine spray in tomcats that have reached sexual maturity and are kept indoors are valuable in the diagnosis of FEH (Küçükbekir et al., 2020). In pregnancy, there is no enlargement of the mammary glands until the last period under normal conditions. The presence of swollen mammary glands in the early or mid-term pregnancy may be deceptive, and this situation may be considered physiological, and it may be quite late in diagnosing FEH. It should be noted that there will be rapid loss of offsprings due to inability of feding. If there is no advanced pregnancy, the swellings in the mammary glands should be carefully examined. In case of swelling developed in a single mammary gland, the distinction between FEH and mammary tumor should be made very carefully in middle and/or advanced-aged cats. Although not a judgement, FEH lesions are more voluminous and softer than mammary gland tumors (Sorenmo, 2011). In addition, measurement of blood progesterone level can also help in diagnosis, as FEH formation shows that the ovulation has taken place or that it has been treated with progestin (Payan-Carreira, 2013). However, although FEH formation is associated with progesterone, serum progesterone measurement is not a diagnostic method with sufficient sensitivity (de Melo et al., 2021).

Cytology or biopsy is preferred in histopathological examinations. In cytological diagnosis, FEH lesions should exhibit the following two criteria: One of the single type epithelial cells and one of the spindle-shaped mesenchymal cells should be found, as well as moderate anisocytosis and anisokaryosis should be observed. Abundant eosinophilic extracellular matrix can be expected to be observed in close proximity to cells. It is difficult to distinguish between benign and malignant structures. Therefore, cytological analyzes should be evaluated together with the patient's anamnesis and clinical findings (Leidinger et al., 2011). In addition, FEH lesions are highly proliferative (Pereira, et al., 2004) and it should not be forgotten that they may display a malignant appearance in a misleading way (Allen, 1973). For all these reasons, fine needle aspiration biopsy should be preferred, although it is more costly, when a definitive diagnosis is requested (Wehrend et al., 2001; Vitasek &Dendisova, 2006).

Besides mammary ultrasonography is also helpful in the diagnosis of FEH, as well as a fast and reliable method in the evaluation of the structure of the mammary gland. In cases of FEH the mammary gland has a higher echogenity than the normal and lactating ones (Payan-Carreira, 2013).

Radiological examination is usually performed in the laterolateral position. Radiologic examination in FEH lesions is not a diagnostic method of much interest, as this imaging only shows enlargement of the mammary glands, intact abdominal wall, and homogeneity within the diseased mammary glands (Burstyn, 2010).

The response to aglepristone application is also evaluated for the diagnosis of FEH. From the third day of the application, reduction of the swellings in the mammary glands and clinical improvement are also considered in the diagnosis (Payan-Carreira, 2013).

Treatment:Primarily, in most animals diagnosed with FEH, swelling of the mammary glands, the possibility of infection and necrosis may require treatment, but is generally considered a benign disease state (Görlinger et al., 2002). However, spontaneous recovery is rarely observed and this process can take weeks or even months (Loretti et al., 2004).

Since it is a progesterone-related disorder, the first step in the treatment approach of FEH should be to eliminate the progesterone effect on the tissue. Therefore, if there is any ongoing hormone therapy, it should be discontinued (Payan-Carreira, 2013).

The determined treatment approach, treatment costs, recovery time and the possibility of recurrence should be discussed with the cat owners, and in cases of mastitis, ulcer, abscess or systemic infection in addition to FEH, treatment should be started after the owner is adequately informed. In addition to the chosen treatment protocol, broad-spectrum antimicrobial therapy (such as amoxicillinclavulanic acid / cephalosporin) and fluid replacement may be required. In painful cases, short-term treatment with

non-steroidal anti-inflammatory drugs (such as meloxicam, ketoprofen or carprofen) can be used to relieve symptoms (Payan-Carreira, 2013).

In the treatment, only antiprogestin will be used, or ovariectomy or ovariohysterectomy can be applied (Johnston et al., 2001; Keskin et al., 2008). Until the late 90's, ovariectomy or ovariohysterectomy was used mostly as a treatment option (Wehrend et al., 2001). Especially in the operation, with the lateral (left flank) approach, further damage to the already traumatic mammary tissue is prevented. In most of the patients treated in this way, shrinkage and healing were observed in the mammary tissues after 3 to 4 weeks post-operatively, but some cases that did not show any regression from time to time reported (Görlinger et al., 2002).

Although mastectomy has been tried as the first approach in cases in ancient times, it is not recommended today due to the delay in healing, the size of the operation wound and surgical complications. It is only applied as a last option in cats who do not respond to ovariohysterectomy and medical treatment (Payan-Carreira,2013; Küçükbekir et al., 2020).

Today, medical treatment approaches are available in most countries. Economic constraints may affect the drug chosen and this may change the recovery time. It also takes longer for progestin to regress if exogenous antiprogesterone drugs are not chosen. Many studies have proven that a progesterone receptor blocker, Aglepristone (Alizine®, Virbac, France), can successfully reverse FEH with a variety of treatment protocols (Görlinger et al., 2002; Nak et al., 2004; Sontas et al., 2008; Jurka & Max, 2009; Enginler and Senünver, 2011; Uçmak et al., 2011). Aglepristone binds to progesterone receptors in target tissues with 9 times more affinity than natural progesterone without activating the hormone response, and its residence time in the organism is 6 days, according to the manufacturer's information (Payan-Carreira, 2013) . Although not licensed for use in cats, aglepristone is widely used in abort induction and pyometra treatment.

In FEH cases, Aglepristone is administered subcutaneously at a dose of 15 mg/kg, divided into two equal parts and administered subcutaneously from the inside of the hind legs. There are basically two reasons for this application. Firstly, aglepristone is stored in the fat tissue, the inner part of the hind legs is poor in fat, and secondly, the hair loss can occur in the area where aglepristone is applied, the inner part of the hind legs is a sparse and anatomically inconspicuous region (Görlinger et al., 2002).

Aglepristone is often used alone (Little, 2011). In FEH cases with also lactation, aglepristone can be combined with a dopamine agonists such as cabergoline (Uçmak et al., 2011). Cabergoline is a prolactin inhibitory dopamine agonist that is used to stop milk production in lactating animals. It is given orally once a day at a dose of 5µg/kg in cats and dogs, and it is usually sufficient for 5 to 7 days (Uçmak et al., 2011). Cabergoline is also used in the treatment of mastitis. It should not be forgotten that it can lead to severe nausea, vomiting and hypotension during its use.

Bromocriptine is a similarly effective dopamine agonist as cabergoline, but its behavioral side effects are more severe than cabergoline. It is used in cats at a dose of 0.25 mg / cat / day (Payan-Carreira, 2013).

Aglepristone has been used in many studies on FEH, and as a result, the most frequently used and recommended forms of use are as Table 1.

Some authors have reported that ovariohysterectomy is the primary treatment for FEH (Medeiros et al., 2007; Motta & Silveira, 2009). Although mammary gland regression may occur up to 5 to 6 months after ovariectomy, the lesion tends to subside 3 to 4 weeks after ovaries are removed (Amorim, 2007; Gimenéz et al., 2010). However, it was also noted that 53 days after surgical sterilization, the lesions in the mammary tissue still persisted and the mastectomy had been performed due to the recurrence of FEH on 7 months after the surgical sterilization (Motta & Silveira, 2009).

Considering all these reasons, it seems that the first treatment option for FEH is the use of antiprogestin, but in the absence of an adequate response, the next most prudent alternative treatment is ovariectomy/ovariohysterectomy. This is a less invasive, well tolerated and effective method with lower mortality compared to surgical techniques such as mastectomy. If the cat is pregnant, the abortion can form as a result of treatment with antiprogestin. To avoid the pregnancy loss, conservative treatment should be managed until the pregnancy is over. Although mastectomy, reported as the surgical treatment of FEH, is still used indiscriminately, it currently does not provide effective protection for FEH (Silva, 2008). However, this technique is only indicated when there is no response to clinical therapy, extensive ulceration and/or necrosis, or associated with carcinoma of the mammary gland (Vasconcellos, 2003; Medeiros et al., 2007). Such a procedure is generally not recommended for young cats, as mastectomy is a complicated surgical procedure and possible post-operative complications are more likely to occur (Gimenéz et al., 2010).

Table 1. Drugs and treatment protocols for FEH and brief information about the studies.

Authors	Drugs and protocols	Brief information about the study
Uçmak et al., 2011	15 mg/kg aglepristone twice a week for 3 weeks and cabergoline 5µg/kg once a day for 1 week every day	In the examination at the end of three weeks, significant shrinkage of the mammary glands and cessation of milk secretion were observed. After 6 weeks from the beginning of the treatment, it was observed that the mammary glands completely shrunk, and at the end of the study, it was determined that aglepristone and cabergoline could be used successfully in cats with FEH.
Jurka & Max, 2009	10 mg/kg/day aglepristone was administered as 2 doses 24 hours apart and the treatment was continued until the mammary tissue was completely healed	In the study, aglepristone treatment was started with 14 cats at the specified dose, and complete regression of the mammary tissue was achieved in an average of 3.9 weeks in the treatment. As a result of the study, a 5 week treatment with aglepristone was recommended for cats with FEH.
Sontas et al., 2008	10 mg/kg/day aglepristone was applied for 4 or 5 conecutive days, and then the last dose was administered on the 7th day	There was a decrease in the size of the mammary tissue 2 days after the begining of the treatment, and significant regression was observed from the 6th day. It took 4 weeks for the mammary tissue to return to normal. No side effects were observed during the treatment and it was concluded successfully.
Görlinger et al., 2002	20 mg/kg/day aglepristone was administered once a week as a single dose and the treatment continued for 4 weeks	Aglepristone was administered subcutaneously at a dose of 20 m g/kg/day once a week to 7 cats with common symptoms of tachycardia, weakness, skin ulcers and anorexia, and all cats except 1 cat showed complete and permanent improvement in symptoms after 1-4 weeks of treatment. Skin irritation at the injection site was observed in 2 cats in the study, and abortion and subsequent endometritis was observed in 2 pregnant cats. In conclusion, this study shows that cats were successfully treated with aglepristone.
Wehrend et al., 2001	10 mg/kg/day aglepristone administered for 4 or 5 consecutive days	About 5 days after the first injection, significant reductions were observed in the size and tissue stiffness of the mammary gland, and it took 3-4 weeks for the tissue to fully return to normal. Similar results were obtained when compared to ovariectomized animals, and no side effects were observed in the treatment.
Nak et al., 2004	10 mg/kg/day aglepristone administered on days 1, 2 and 7	In the study, aglepristone was applied at the indicated days and doses, and it was observed that the mammary tissues were completely healed on the 21st day without any side effects. Ovariohysterectomy was performed due to the risk of recurrence.
Vitasek & Dendisova, 2006	10 mg/kg/day aglepristone administered on days 1, 2, 7, 14 and 21	A seven month old female cat was treated with 10 mg/ kg of aglepristone subcutaneously on days 1, 2, 7, 14, and 21, with complete regression of the mammary glands observed within 6 weeks. No adverse events were observed.

Conclusions

In this review, etiology, pathogenesis, clinical detection of mammary gland hyperplasia in cats and current treatment options are reported. As a result, FEH is common in intact, exogenous progesterone administered, pregnant or non-pregnant cats, usually young cats. It has also been reported to occur in female and male cats. These cases can be successfully treated with various aglepristone protocols. Cabergoline can be added to the treatment protocol if there is milk secretion. Another treatment options are ovariectomy, ovariohysterectomy and mastectomy which is now abandoned except for mandatory conditions. Preventing the use of progestin, which is one of the effective factors in the formation of FEH, is also important for both female and male cats.

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The investigation of the presence of *Listeria* species in poultry farms and antimicrobial resistance profiles of *Listeria monocytogenes* strains

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ABSTRACT

Listeria species are widespread in nature and found in various environments. In this study, the presence of *Listeria* species in poultry farms environments was investigated. For this purpose, a total of 332 samples including feces, feed, drinking water and nipple swab from 5 broiler flocks reared on the floor and 352 samples including feces, feed, drinking water and eggshell swab from 5 layer flocks reared in cages were obtained. A modified version of the USDA-FSIS MLG 8.13 method was used for the isolation of *Listeria* species from samples. As a result of the study, *Listeria* spp. was isolated from all broiler and layer flocks. Isolation of *Listeria* spp. was carried out from 18 of 190 feces samples (9.4%), 5 of 15 drinking water samples (33.3%), 3 of 102 nipple swab samples (2.9%) in broiler flocks, and 22 of 167 feces samples (13.1%), 2 of 25 feed samples (8%), 3 of 15 drinking water samples (20%), 3 of 145 eggshell swab samples (2%) in layer flocks. Isolates were identified by cultural and biochemical characters, and a total of 56 *Listeria* isolates were identified as 15 *L. monocytogenes,* 3 *L. ivanovii,* 19 *L. innocua,* 13 *L. seeligeri,* 2 *L. welshimeri,* and 4 *L. grayi*. The antibiotic resistance profiles of *L. monocytogenes* isolates to eleven antibiotics were detected by the disc diffusion method. *L. monocytogenes* isolates were found to exhibit the highest resistance to ciprofloxacin (33.3%) among eleven antibiotics, and three isolates (20%) were also multidrug resistant. Consequently, it was determined that *Listeria* species in poultry farms environments are common.

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Introduction

The bacteria of the genus *Listeria* are found in various environments including soil, water, vegetation, sewage, animal feeds and farm environments. The genus *Listeria* currently includes 17 recognized species; *Listeria monocytogenes* (*L. monocytogenes), L. ivanovii*, *L. grayi, L. innocua, L. seeligeri, L. welshimeri*, *L. marthii*, *L. rocourtiae, L. fleischmannii*, *L. weihenstephanensis*, *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, *L. grandensis*, *L. newyorkensis and L. booriae*. Only two of these species, *L. monocytogenes* and *L. ivanovii*, are pathogenic for animals and humans (Orsi and Wiedmann, 2016). However, sporadic human infections due to *L. seeligeri* and *L. innocua* have also been reported (Rocourt et al., 1986; Perrin et al., 2003).

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L. monocytogenes is one of the important foodborne pathogens and threats public health in many countries (Datta and Burall, 2018). Human listeriosis can occur sporadically or epidemically; in both cases, contaminated foods are the primary means of transmission. Direct contact with animals/birds is of less significance in the transmission/spread of *Listeri*a, except for highly susceptible individuals (Dhama et al., 2015). Many different food items, such as dairy products, red meat, poultry meat, seafood, vegetables and ready-to-eat prepared foods have been implicated as vehicles for *L. monocy*togenes transmission (Lunde´n et al., 2004; Şanlıbaba et al., 2018). The severity of listeriosis can range from mild gastroenteritis to severe disease conditions linked to septicemia, encephalitis, meningitis, miscarriage, and may even lead to death. The mortality rate can reach values of around 30%. Some people have a higher risk of listeriosis than others, such as newborns, the elderly, immunocompromised people and pregnant women (Zhu et al., 2017). In the treatment of listeriosis with antibiotics, β-lactam antibiotics (e.g., penicillin or ampicillin) alone or in combination with an aminoglycoside (e.g., gentamicin) is frequently used as the first treatment of choice. The association of trimethoprim and a sulfonamide (e.g. sulfamethoxazole) is considered to be a second-choice therapy, especially for patients allergic to β-lactams. However, vancomycin, erythromycin, rifampicin, chloramphenicol, tetracycline and fluoroquinolones are also used to treat cases of listeriosis (Olaimat et al., 2018). In recent years, *L. monocytogenes* isolated from foods, the environment and human sources have been found to be resistant to commonly used antibiotics (Alonso-Hernando et al., 2012; Soni et al., 2013; Şanlıbaba et al., 2018; Arslan and Baytur, 2019). But, the levels and type of resistance are affected by regional differences and antimicrobial usage in humans and animals. Thus, investigation and monitoring of the antibiotic susceptibility of *Listeria* species isolated from different sources are very important for public health (Dhama et al., 2015).

Listeriosis has been reported in a wide range of species of domestic and wild animals, including birds. In birds, even though outbreaks of listeriosis are infrequent, commonly affected species include chickens, turkey, geese, ducks, pigeons, parrots, wood grouse, snowy owls, eagles and canaries (Seifi, 2012; Dhama et al., 2013). Chickens are thought to be the carriers of *Listeria* and thus contaminate the litter and environment of the poultry production systems. Intestinal colonization and the presence of *L. monocytogenes* in the feces of poultry play a significant role in the spread of listeriosis in domestic animals (Dhama et al., 2015). However, few studies have investigated and characterized *Listeria* species in poultry farm environments (Dahshan et al., 2016; Locatelli et al., 2017). Most studies have focused on investigating the presence of *L. monocytogenes* in final products and in food-processing and retail environments thought to be the main sources of contamination for the final products (Ojeniyi et al., 1996; Oliveira et al., 2018). In a farm-to-fork approach, it is necessary to assess the presence of *L. monocytogenes* throughout the entire poultry production chain, including the farm environment and the primary production stage (Locatelli et al., 2017). In Türkiye, numerous studies have investigated the prevalence of *L. monocytogenes* in chicken carcases or fresh chicken meats (Siriken et al., 2014; Gücükoğlu et al., 2020), but to the best of our knowledge, there is no data on the presence of *Listeria* spp. from poultry farms and environments. This study was aimed to investigate the presence of *Listeria* spp. in broiler and laying hen flocks environment and also determine the antimicrobial resistance profiles of *L. monocytogenes* isolated.

Materials and Methods

Sample collection: A total of 684 samples were taken from 5 broiler flocks reared on the floor and 5 layer flocks reared in cages located in Bandirma, Türkiye. Each of the flocks was determined randomly, and the samples were taken from each flock in one go. Chickens were clinically healthy appearance and there was no record of listeriosis cases in all flocks. From the broiler flocks, a total of 332 samples including feces, feed, drinking water and nipple swabs were collected. Also, a total of 352 samples including feces, feed, drinking water and eggshell swabs were collected from the layer flocks. The details of the samples and the flock's information are given in Table 1. Feces (fresh and not trampled) samples were randomly collected by using sterile swabs from the surface of the litter in broiler houses and from the cages (only one sample from each cage) in layer houses, and fecal samples were placed in sterile screw-top vials. The feed samples (approximately 200 g each) were taken from five different points on the feeding stations in each house. The drinking water samples (1 liter each) from each poultry flock were collected from the water tank in the house (two samples) and from the water tank on the outside of the house (one sample). Swab samples were randomly taken from nipple drinkers in broiler houses. In layer houses, eggs were randomly chosen directly from the collecting conveyor and then

swab samples were taken from eggshell. All samples were taken aseptically and quickly transported to the laboratory under chilled conditions, and stored at 4 °C until analyzed. Samples were analyzed within 3 h. of being taken.

Isolation and identification of *Listeria* spp.: The *Listeria* strains were isolated according to the US Department of Agriculture (USDA/FSIS) method (USDA, 2021) with some modification. Briefly, each of the swab samples was directly inoculated into 100 mL of University of Vermont *Listeria* Enrichment broth (UVM I, Oxoid, CM863, SR142). Twenty-five grams of each feed sample were inoculated into 225 mL of UVM I. The water samples were filtered through 0.45 µm membrane filters (Millipore, GSWG047S1) using a membrane filtration system (Sartorius AG) and the filters were placed in 100 mL of UVM I broth. All enrichments were homogenized and then incubated at 30 °C for 24 h. One mL of primary enrichments was transfered to 9 mL UVM II broth (Oxoid, CM863, SR143) and Fraser broth (Oxoid, CM895, SR156), and incubated at 35 °C for 24 h. Secondary enrichments were streaked onto modified Oxford agar (Oxoid, CM856, SR206) and BrillanceTM *Listeria* agar (Oxoid, CM1080, SR227), and incubated at 37 °C for 48 h. All plates were examined for typical *Listeria* colonies, and colonies that were grayish surrounded with a dark zone on modified Oxford agar, and blue with or without opaque white halos on BrillanceTM *Listeria* agar were accepted as suspects for *Listeria* spp. Five suspected *Listeria* colonies were picked up from each sample and transferred to Tryptone Soya agar (Oxoid, CM131) plates supplemented with 0.6% Yeast extract (Oxoid, L21) for pure culture, and incubated at 37 °C for 24. All the isolates were confirmed to the standard identification and biochemical tests including Gram staining, catalase and oxidase reaction, H2S production, indole test, urease activity, motility at 25°C and 37°C, β-haemolysis, nitrate reduction, methyl-red-Voges Proskauer test, CAMP test with control strains of *Staphylococcus aureus* and *Rhodococcus equi*, and acide production from rhamnose, xylose, mannitol and α-methyl-D-mannopyranoside (Gasanov et al., 2005). All media used were prepared according to the manufacturer's directions (Oxoid). The reference strain *L. monocytogenes* ATCC7644 was used in all biochemical tests.

Antibiotic susceptibility test: All *L. monocytogenes* isolates were tested for antimicrobial susceptibility using the disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014). Briefly, a single colony of each isolate was inoculated into 10 mL of tryptic soy broth containing 0.6% yeast extract. After incubation at 30 °C for 24 h., the culture was diluted to a turbidity equivalent to the McFarland 0.5 standard in sterile physiological saline solution. Then, using the sterile swab, inoculation was conducted on Mueller-Hinton agar (Oxoid, CM337) containing 5% sheep blood. Later on, antibiotic discs were placed and the plates were incubated at 35°C for 24 h. After the incubation, the diameters of the inhibition zones were measured with calipers and compared to the breakpoints for *Staphylococcus* spp. as recommended by the CLSI (CLSI, 2014). Currently, there are no resistance criteria for *Listeria* susceptibility testing in the CLSI guidelines, with the exception of susceptibility breakpoints for ampicillin and penicillin. The strains were classified as resistant, intermediate resistant, and susceptible. The eleven antimicrobial agents used and their corresponding concentrations were as follows: penicillin G (10 U), ampicillin (10 µg), gentamycin (10 µg), tetracycline (30 µg), erythromycin (15 µg), streptomycin (10 µg), vancomycin (30 µg), chloramphenicol (30 µg), rifampicin (5 µg), ciprofloxacin (5 µg) and trimethoprim/sulphamethoxazole (1.25/23.75 µg) (Oxoid, UK). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 25922 were used for quality control strains and tested in each replicate.

Table 1. The information regarding the samples taken and flocks

	Broiler flocks					Layer flocks				
		No of samples					No of samples			
Flock No	Flock age (day)/ No of chickens	Feces	Feed	Drinking water	Nipple swab	Flock age(week)/ No of chickens	Feces	Feed	Drinking water	Eggshell swab
1	32/9.300	34	5	3	20	23/10.650	30	5	3	25
2	28/11.700	40	5	3	22	45/11.230	32	5	3	27
3	25/13.100	40	5	3	20	53/21.400	40	5	3	35
4	35/11.150	38	5	3	20	42/17.600	35	5	3	33
5	41/12.480	38	5	3	20	36/9.800	30	5	3	25
Total		190	25	15	102		167	25	15	145

Table 2: Prevalence of *Listeria* spp. isolated from broiler and layer flocks

Type of samples	Number of samples	*Listeria* spp. n (%)	*L. monocytogenes* n (%)	*L. İvanovii* n (%)	*L. innocua* n (%)	*L. seeligeri* n (%)	*L. welshimeri* n (%)	*L. grayi* n (%)
Broiler flocks								
Feces	190	18 (9.4)	4 (2.1)	-	7 (3.6)	6 (3.1)	1 (0.5)	-
Feed	25	-	-	-	-	-	-	-
Drinking water	15	5 (33.3)	2 (13.3)	1 (6.6)	-	-	-	2 (13.3)
Nipple swab	102	3 (2.9)	-	-	2 (19.6)	1 (0.9)	-	-
Total (%)	332	26 (7.8)	6 (1.8)	1 (0.3)	9 (2.7)	7 (2.1)	1 (0.3)	2 (0.6)
Layer flocks								
Feces	167	22 (13.1)	8 (4.7)	-	8 (4.7)	3 (1.7)	1 (0.5)	2 (1.1)
Feed	25	2 (8)	-	-	1 (4)	1 (4)	-	-
Drinking water	15	3 (20)	1 (6.6)	2 (13.3)	-	-	-	-
Eggshell swab	145	3 (2)	-	-	1 (0.6)	2 (1.3)	-	-
Total (%)	352	30 (8.5)	9 (2.5)	2 (0.5)	10 (2.8)	6 (1.7)	1 (0.2)	2 (0.5)

Results

Isolation and identification of *Listeria* spp.: In this study, a total of 56 *Listeria* spp. were isolated from 684 poultry farm environment samples. The prevalence of positive samples was 8.1%. Also, the prevalence of *Listeria* spp. in layer flocks as 8.5% (30/352) was higher than that in broiler flocks with the ratio of 7.8% (26/332) (Table 2). In broiler flocks, twenty-six *Listeria* spp. were isolated from feces and drinking water samples of five flocks and nipple swab samples of two flocks, but not isolated from the feed samples. These isolates were identified as: 6 *L. monocytogenes* (4 from feces of three flocks and 2 from drinking water of two flocks), 1 *L. ivanovii* (from drinking water of one flock), 9 *L. innocua* (7 from feces of four flocks and 2 from a nipple swab of two flocks), 7 *L. seeligeri* (6 from feces of five flocks and 1 from a nipple swab of one flock), 1 *L. welshimeri* (from feces of one flock) and 2 *L. grayi* (from drinking water of two flocks). In layer flocks, thirty *Listeria* spp. were isolated from feces samples of five flocks, drinking water and eggshell samples of three flocks, and feed samples of two flocks. These isolates were identified as: 9 *L. monocytogenes* (8 from feces of five flocks and 1 from drinking water of one flock), 2 *L. ivanovii* (from drinking water of two flocks), 10 *L. innocua* (8 from feces of five flocks, 2 from eggshell swab and feed of one flock), 6 *L. seeligeri* (3 from feces of three flocks, 2 from eggshell swab and 1 from feed of one flock), 1 *L. welshimeri* (from feces of one flock) and 2 *L. grayi* (from feces of two flocks). The isolation and identification results are given in Table 2.

Antibiotic susceptibility: Isolates of *L. monocytogenes* recovered from the feces and drinking water samples of the broiler and layer flocks were tested for their antibiotic susceptibility. Among the 15 *L. monocytogenes* isolates, 9 isolates (60%) were susceptible to eleven tested antimicrobial agents. The remaining 6 isolates (40%) were resistant to ciprofloxacin (five isolates, 33.3%, resistance or intermediate), to penicillin G (two isolates, 13.4%), to trimethoprim/sulphamethoxazole (two isolates, 13.4%), to ampicillin (one isolate, 6.7%), to vancomycin (one isolate, 6.7%), to rifampicin (one isolate, 6.7%) and to gentamycin (one isolate, 6.7%, intermediate). These 6 isolates resistant to antimicrobial agents were isolated from feces samples of broiler and layer flocks. The number of antimicrobials to which an isolate was resistant ranged from one to three. However, multidrug-resistance, i.e., resistance to three or more antimicrobial agents, was observed in 3 isolates (20%). Of these multidrug-resistant isolates, one isolate (from broiler feces) was resistant to ampicillin, ciprofloxacin, and trimethoprim/sulphamethoxazole, and the other two isolates (from layer feces) were resistant to ciprofloxacin, rifampicin, trimethoprim/ sulphamethoxazole and ciprofloxacin, penicillin G, vancomycin, respectively. The antimicrobial resistance of *L. monocytogenes* isolates was shown in Table 3.

Discussion

The Bandirma district, where the study was conducted, is located in the Southern Marmara Region of Türkiye, and domestic animal breeding is done quite intensively in this region. Also, the poultry sector is clustered in this region, where poultry farms are very intense, and about 12% of the broiler and 3% of the layer hens in our country are raised in this region. Therefore, this region is located in the front rows of chicken meat and table egg production in Türkiye (Anonymous, 2020). This study was planned due to the absence of a previous study on the subject of the presence of *Listeria* spp. in poultry farms in this region and Türkiye,

Table 3. Antimicrobial resistance profiles of *L. monocytogenes* isolates

Antimicrobial agent	Number of isolates (%) n=15		
	Resistant	Intermediate	Susceptible
Penicillin G	2 (13.4)	-	13 (86.6)
Ampicillin	1 (6.7)	-	14 (93.3)
Gentamycin	0 (0)	1 (6.7)	14 (93.3)
Tetracycline	0 (0)	-	15 (100)
Erythromycin	0 (0)	-	15 (100)
Streptomycin	0 (0)	-	15 (100)
Vancomycin	1 (6.7)	-	14 (93.3)
Chloramphenicol	0 (0)	-	15 (100)
Rifampicin	1 (6.7)	-	14 (93.3)
Ciprofloxacin	4 (26.7)	1 (6.7)	10 (66.6)
Trimethoprim/ Sulphamethoxazole	2 (13.4)	-	13 (86.6)

and has been carried out in broiler and layer flocks. In the study, the presence of *Listeria* spp. was determined at least one of the samples taken from each broiler and layer flock examined, and the overall prevalence of *Listeria* spp. was found as 8.1%, while 7.8% in broiler and 8.5% in layer flocks. In a study conducted by Dahshan et al. (2016) in Egypt, 48.3% of broiler farm samples including litter, feed and water were found to be contaminated with *Listeria* spp. Petersen and Madsen (2000) reported the prevalence of *Listeria* spp. in Danish broiler farm litter and fecal samples as 14%. In a study conducted by Milillo et al. (2012) in pasture-reared poultry demonstrated that environmental samples including cecal, soil and grass collected from the pasture before broiler introduction and samples collected after broiler exposure were found to harbour 5% and 53% *Listeria* spp., respectively. Those reported by the authors were found to be higher than our results. In a study performed by Iida et al. (1991) in Japan, a lower prevalence of *Listeria* spp. (4.7%) than our result was found in four chicken farm samples, including fresh feces. Likewise, Schwaiger et al. (2010) reported that the low *Listeria* spp. prevalence in cloacal swab samples from organic and conventional laying hen flocks was 1.3% and 1.8%, respectively, and only *L. innocua* was isolated. In previous studies, *L. innocua* has been shown to be the predominant species isolated from the poultry farms and environment (Dhama et al., 2013; Dahshan et al., 2016; Locatelli et al., 2017). However, other *Listeria* spp. such as *L. ivanovii*, *L. welshimeri* and *L. seeligeri* were also isolated less frequently (Dahshan et al., 2016), but most studies only focused on *L. monocytogenes* (Chemaly et al., 2008; Esteban et al., 2008, Kanarat et al., 2011). In this study, *L. innocua* was the predominant *Listeria* species and isolated at the highest rates in both production type flocks (2.7% in broilers and 2.8% in layers), and was also more prevalent than *L. monocytogenes* (1.8% in broilers and 2.5% in layers). Petersen and Madsen (2000) reported that non-pathogenic *L. innocua* (13%) was more prevalent than *L. monocytogenes* (3%) in broilers. *L. innocua* was declared to be important because it was closely related to *L. monocytogenes* and commonly used by the food industry as an indicator to identify environmental conditions allowing the presence, growth, and persistence of the relevant human pathogen *L. monocytogenes* (Gwida et al., 2020), and also because it has been reported that it may be an antimicrobial resistance reservoir for *L. monocytogenes* due to its availability in similar habitats (Bertrand et al., 2005). Also, other studies indicated that *L. innocua* was the most common species in chicken meat (Yücel et al., 2005; Fallah et al., 2012; Arslan and Baytur, 2019).

L. monocytogenes has been isolated on poultry farms and environments from litter, dust, feed, water, feces and cecal or cloacal swab samples, with an overall prevalence rate ranging from 0% to 91.5% in those samples (Skovgaard and Morgen, 1988; Petersen and Madsen, 2000; Chemaly et al., 2008; Schwaiger et al., 2010; Kanarat et al., 2011; Dahshan et al., 2016; Ishola et al., 2016; Locatelli et al., 2017; Gwida et al., 2020). In this study, *L. monocytogenes* was the second predominant species, with an average 2.2% prevalence rate, and it was isolated from feces and drinking water samples in both production type flocks. In a study conducted by Ishola et al. (2016) in Nigeria, the overall prevalance of *L. monocytogenes* contamination was found to be 91.5% in cloacal swabs from broilers and layers, and cloacal samples from broilers (98.8%) had significantly higher than those from layers (89.8%). Skovgard and Morgan (1988) reported that 33% harboured *L. monocytogenes* in the fecal samples taken from poultry in a dairy farm and from cages used for transporting poultry to slaughter. In another study, *L. monocytogenes* was detected in 10.5% of the feces samples from laying hen flocks, while in 34.6% of the feces samples when only *L. monocytogenes*-positive flocks were considered (Chemaly et al., 2008). In a study performed by Ojeniyi et al. (1996), *L. monocytogenes* was detected at a rate of 4.7% in caecal samples taken from broiler breeder flocks, but was not detected in 2078 caecal samples taken from 90 randomly selected broiler flocks. However, in some studies, *L. monocytogenes* was not isolated in cloacal or faecal swabs taken at the slaughterhouse but was

detected in chicken meat products (Iida et al., 1991; Cox et al., 1997; Kanarat et al., 2011). Whereas, in a study conducted by Kalender (2003), it was reported that *L. monocytogenes* was isolated from faecal samples of chickens on the slaughter line of abattoir. In our study, we isolated *L. monocytogenes* from 2.1% and 4.7% of feces samples in broiler and layer flocks, respectively. Since no cloacal swabs were taken in this study, cross-contamination of feces with *Listeria* spp. from the environment could not be ruled out. All these results showed that this variation in prevalence of *L. monocytogenes* might be attributed to various factors such as sample type (litter, dust, feces, cecal or cloacal swab), type of production systems and contact with other livestock farms, but also did not clarify whether the feces was a source for *L. monocytogenes* contamination in poultry products.

Drinking water has often been considered a risk factor for *L. monocytogenes* contamination in poultry flocks (Cooper et al., 1992; Aury et al., 2011; Seifi, 2012). In previous studies carried out on broiler chickens, it has been reported the presence of *C. jejuni* in biofilms in the water systems (Zimmer et al., 2003) and the isolation of *C. jejuni* from nipple swab samples, which may cause flock contamination (Cokal et al., 2011). With these, also taking into account the biofilm-forming ability of *L. monocytogenes* isolated from broilers and layers (Osman et al., 2020) and *L. monocytogenes*' ability to form biofilms in tap water (Gião et al., 2014), we collected nipple swab samples in broiler flocks. However, *L. monocytogenes* was not isolated, while *L. innocua* and *L. seeligeri* were isolated in these samples. Aury et al. (2011) reported that the nipples have a protective effect against *L. monocytogenes* contamination in broiler flocks. However, we isolated *L. ivanovii* in drinking water samples in both production type flocks. Similarly, Dahshan et al. (2016) also reported that *L. ivanovii* isolation was performed from water samples as well as litter, feed and raw chicken meat samples from broiler farms. Obviously, isolation from drinking water samples did not surprise us as *Listeria* spp. are highly adapted to soil, water and vegetation. In a study in different environmental ecosystems, it was reported that the most dominant species in soil and water samples were *L. seeligeri*, *L. innocua*, and *L. ivanovii* (Linke et al., 2014). The poultry farms in this study provided their drinking water from groundwater sources. Contamination of groundwater sources with *Listeria* spp. may be possible by prolonged rainfall and flooding (Linke et al., 2014). Further comprehensive studies are needed to provide a better understanding of the presence of *Listeria* spp. in the water systems of poultry farms. Also, in this study, *L. innocua* and *L. seeligeri* were isolated from feed and eggshell swab samples in layer flocks. These findings were comparable to the results reported by other investigators (Farber et al., 1992; Schwaiger et al., 2010; Gwida et al., 2020). It has been reported that feed meal was found to increase the risk of *L. monocytogenes* contamination in laying hen flocks (Aury et al., 2011).

L. monocytogenes is generally susceptible to a wide range of antibiotics (Hof et al., 1997). However, *L. monocytogenes* strains resistant to one or more antibiotics have been isolated from foodstuffs, environmental, animal and human samples. (Bertrand et al., 2005; Olaimat et al., 2018, Şanlıbaba et al., 2018). A few studies examined antibiotic susceptibility patterns of *L. monocytogenes* strains isolated from poultry farms (Dahshan et al., 2016; Ishola et al., 2016). In the present study, we detected that 40% of the *L. monocytogenes* isolates exhibited resistance or intermediate resistance to at least one antibiotic tested. This resistance rate was close to the 47.4% found in the study by Oliveira et al. (2018), higher than the 0.6% found in the study by Walsh et al. (2001) and lower than the 100% found in the study by Gücükoğlu et al. (2020). The highest resistance has been found against ciprofloxacin (33.3%) in this study. Şanlıbaba et al. (2018) reported a resistance rate (35.3%) to this antibiotic close to our finding. Arslan and Baytur (2019) reported a lower resistance rate (3%) to ciprofloxacin than our finding, while Dashan et al. (2016) and Ishola et al. (2016) reported higher resistance rates (50% and 56.2%). On the other hand, Yücel et al. (2005) and Oliveira et al. (2018) indicated that no resistance was detected to ciprofloxacin in their studies. The high resistance rate to ciprofloxacin in this study could be explained by the frequent use of this antibiotic to treat in infection in poultry farms in Türkiye. In the treatment of listeriosis, ampicillin or penicillin G alone or in combination with an aminoglycoside is considered the most effective therapy (Soni et al., 2013). The results of this study indicated that *L. monocytogenes* isolates are resistant to ampicillin (6.7%), penicillin G (13.4%) and gentamycin (6.7%, intermediate). Our levels of resistance to ampicillin, penicillin G and gentamycin were lower than those found by Fallah et al. (2012) at rates of 44.9%, 41.8% and 10.2%, respectively. In their study, Şanlıbaba et al. (2018) reported that all *L. monocytogenes* strains were resistant to ampicillin and penicillin, and susceptible to gentamycin, whereas Wang et al. (2015) reported that all *L. monocytogenes* strains were susceptible to these antibiotics. In this study, 13.4% of *L. monocytogenes*

strains were found to be resistant to trimethoprim/ sulphamethoxazole, which is a second-choice therapy for listeriosis. Similar findings have been reported by other researchers for this antibiotic (Şanlıbaba et al., 2018; Arslan and Baytur, 2019; Gücükoğlu et al., 2020). The percentage of isolates resistant to trimethoprim/ sulphamethoxazole detected by Wang et al. (2015) was 100%, while Fallah et al. (2012) reported that all *L. monocytogenes* strains were susceptible to that antibiotic in their study. In the present study, it has also been detected that *L. monocytogenes* isolates were resistant to vancomycin (6.7%), rifampicin (6.7%). Vancomycin and rifampicin are also used treat human listeriosis (Olaimat et al., 2018; Şanlıbaba et al., 2018). Other studies also reported that *L. monocytogenes* isolates were resistant to vancomycin (Siriken et al., 2014; Gücükoğlu et al., 2020), and rifampicin (Fallah et al., 2012; Şanlıbaba et al., 2018), similar to our results. However, vancomycin resistance of *L. monocytogenes* isolates was not reported in some studies (Fallah et al., 2012; Şanlıbaba et al., 2018; Arslan and Baytur, 2019). In addition, the present study showed that all *L. monocytogenes* isolates were susceptible to erythromycin, streptomycin, tetracycline and chloramphenicol. These results agree with those previously reported by some authors (Yücel et al., 2005; Soni et al., 2013; Siriken et al., 2014; Arslan and Baytur, 2019). Antibiotic susceptibility patterns of *L. monocytogenes* isolates to different antibiotics might be influenced by many factors such as strain variation, sampling sites, geographic origin and factors that trigger gene transfer (Walsh et al., 2001; Olaimat et al., 2018; Osman et al., 2020). Therefore, the present study comprising results that were different from those of some other researchers.

The evidence of the emergence of *L. monocytogenes* strains' multidrug resistance has been documented in recent years (Alonso-Hernando et al., 2012; Soni et al., 2013). In a study conducted by Alonso-Hernando et al. (2012) in Spain, multidrug resistance of *L. monocytogenes* strains from poultry products was reported to be more common in 2006 (84%) as compared to 1993 (18.6%). In India, Dhanashree et al. (2003) reported that all the isolates of *L. monocytogenes* were sensitive to antibiotics tested, while Soni et al. (2013) reported that all *L. monocytogenes* isolates were resistant to multiple antibiotics. In Türkiye, in a study performed by Siriken et al. (2014), it was reported that 1.9% and 5.6% of *L. monocytogenes* isolates from raw chicken meat were resistant to five and six antimicrobial agents, respectively. Şanlıbaba et al. (2018) investigated resistance of 17 *L. monocytogenes* isolates from raw ready-to-eat food to 23 antibiotics and reported that all isolates had multidrug resistant. However, multidrug resistance of *L. monocytogenes* isolates has also been reported in other countries (Fallah et al., 2012; Wang et al., 2015; Dahshan et al., 2016). In this study, we found 3 (20%) of 15 *L. monocytogenes* isolates from poultry farms had multidrug resistance. These results showed that there was multidrug resistance in *L. monocytogenes* strains isolated from various sources around the world, and this could be a potential public health hazard.

Conclusion

Listeria species including *L. monocytogenes* are quite common in poultry farms environments. *L. monocytogenes* is one of the important foodborne pathogens, and sporadic and isolated cases of listeriosis have been attributed to poultry. *L. monocytogenes* is isolated from other stages of the poultry production chain. Therefore, *Listeria* spp. found in the poultry farm environments can be a potential source of contamination for the poultry processing environments. Studies are needed to reveal the relationship between the presence of *Listeria* in poultry farms and *Listeria* contamination of poultry processing environments. Furthermore, the fact that *L. monocytogenes* strains isolated from poultry farms were resistant to antibiotics used in the treatment of listeriosis and that some of these strains were multidrug resistant poses a potential risk to public health. Therefore, taking preventive measures against *Listeria* contamination in poultry farms and continuous surveillance of antibiotic sensitivity are absolutely necessary for future risk assessment and consumer protection, i.e., public health protection.

Ethical approval

The authors declare no ethical approval is required.

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Determination of estrogen receptor, progesterone receptor and epidermal growth factor receptor in canine vaginal tumors and vaginal fold prolapse: A preliminary study

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ABSTRACT

The objective of this study was to determine steroid receptors and epidermal growth factor receptor in canine vaginal tumors and vaginal fold prolapse. Eight dogs with a vaginal mass were incorporated into the study. According to the vaginal examination (vaginoscopy, exfoliative vaginal cytology) and histopathology, the groups were designed as vaginal fold prolapse (Group VFP; n=3) and vaginal tumor (Group VT; n=5). Vaginal tissue samples were homogenized. Tissue homogenates were analyzed with an enzyme-linked immunosorbent assay (ELISA) to determine the levels of estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor (EGFR). The mean ER level was significantly higher ($P<0.05$) in group VT (8.06±0.45) compared to the group VFP (5.93±0.36). However, PR and EGFR levels did not show significance related to the groups ($P>0.05$). In conclusion, significant differences were obtained between the bitches with VT and VFP related to ER levels although both groups had pathological conditions. Further studies are needed to discuss these pathologies with healthy bitches in terms of steroid receptor and EGFR levels in both vaginal tissue and blood sera.

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Introduction

The vaginal masses in bitches are seen frequently as vaginal fold prolapse (VFP), vaginal tumor, or urethral neoplasia protruding into the vagina (Manothaiudom and Johnston, 1991). In bitches, VFP is defined as a protrusion of edematous vaginal tissue through the opening of the vulva which appears due to the excessive response of the vaginal mucosa to estrogens (Sontas et al., 2010). Clinically, VFP is classified into three different types based on the degree of vaginal tissue protrusion. A slight to moderate eversion of the vaginal floor to the urethral opening is defined as type I VFP. In type II, the vaginal fold is prolapsed through the

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vulvar lips thus becoming visible from outside. When the entire circumference of the vagina protrudes through the vulva, it is described as type III (Shuttle, 1967; Anya et al., 2020). Tumors affecting canine vaginal vestibule or vulva are rare (Brodey and Roszel, 1967; McEntee, 2002). hyperplasias). Vaginal tumors usually appear in sexually intact bitches (Thacher and Bradley, 1983). The occurrence ages of vaginal tumors in bitches are reported to be ranging from 5 to 16 years of age (Cotchin, 1954). Tumors affecting the canine vaginal tissue have a mostly benign character such as leiomyomas and fibromas (Brodey and Roszel, 1967; McEntee, 2002). In dogs with benign tumors, surgical excision of the tumor combined with ovariohysterectomy provides the treatment and prevention of the recurrence of the disease (Thacher and Bradley, 1983). However, the use of steroidal hormones receptor antagonists was reported as an alternative treatment option because the growth of these masses may be stimulated by ovarian steroids (Rollón et al., 2008; Sathya and Linn, 2014). Ferré-Dolcet et al. (2020) reported a case of progesterone-sensitive vaginal leiomyoma in which they emphasized the importance of identifying progesterone-related conditions in order to decide on effective treatment as a combined medical and surgical approach.

The aim of the present study was to evaluate the differences in the amount of estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor (EGFR) in tissue homogenates of the bitches with VFP and VT.

Materials and Methods

Animals and study design: All animal procedures were carried out in accordance with the approval of the Istanbul University-Cerrahpaşa Animal Experiments Local Ethical Committee (HADYEK) (Approval number: 2021/256807). Eight dogs with a vaginal mass were incorporated into the study. All bitches were clinically and gynaecologically examined. The groups were designed according to vaginal examination (vaginoscopy, exfoliative vaginal cytology) and histopathology. Vaginal smear was obtained for cytological examination of the vagina for both groups. The samples were collected with a cotton swab from all the bitches. The smears were stained with Diff Quick method according to manufacturers' instructions (ADR Group®, Mediko Kimya, Turkey). The slides were examined by using a light microscope (BAB -LAM®, BAB, Turkey) at x400 magnification. The bitches were divided into 2 groups which consist of vaginal fold prolapse (Group VFP; n=3) and vaginal tumor (Group VT; n=5). Clinical staging of VFP (Type I, Type II, Type III) was performed as Schutte (1967). The ages of the bitches in group VFP and group VT ranged from 1 to 6 years of age and from 6 to 12 years, respectively. The breeds of the eight bitches were Cocker spaniel, Golden retriever, English Setter, Pekingese, Akita Inu, King Charles, and mixed breed. The bitches in group VT had three-view thoracic radiography and abdominal ultrasonography to determine the any metastasis to the distant organs.

Histopathological examination: Vaginal tissue samples in group VFP and group VT were fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (HE) for histopathological examination by light microscopy. The vaginal tumors were classified according to Baba and Catoi, 2007.

Sample preparation and enzyme-linked immunosorbent assay (ELISA): Vaginal tissue samples were handled with PBS (PH 7.4) (1 ml PBS for 100mg tissue). They were homogenized by grinders and then centrifuged 20 min at the speed of 2000-3000 r.p.m. At the end of this procedure, supernatant was collected into a sterile eppendorf tube. Homogenized tissue samples were stored at - 20°C until ELISA were applied. Levels of ER, PR and EGFR in tissue homogenates were determined with commercially available canine-specific ELISA kits according to the manufacturer's instructions (ER Catalogue no: E0414Ca, PR Catalogue no: E0058Ca, EGFR Catalogue no: E0301Ca Bioassay Technology Laboratory, Shangai, China).

Statistical analysis: Statistical analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, Illinois, USA). The comparison of the groups in terms of the ER, PR and EGFR was performed by Mann Whitney-U test. The significance level was accepted as $P<0.05$.

Results

The mean ages and standard errors of the mean (SEM) in group VFP and group VT were 2.66 ± 1.66 years and 9.20 ± 1.06 years; respectively. In vaginal cytology

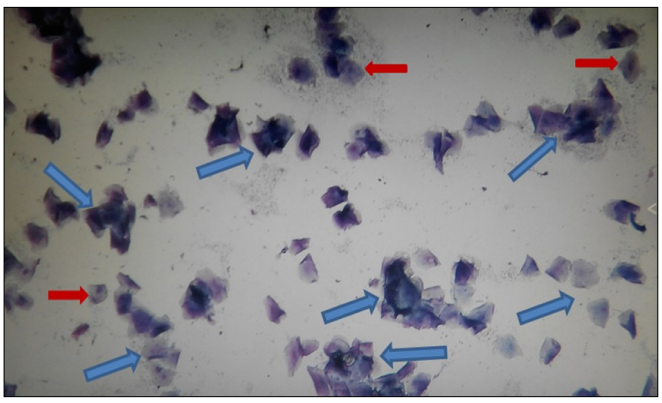

Figure 1. Superficial cells in the smear (red arrows). Keratinized (anuclear) superficial cells (blue arrows) in the smear.

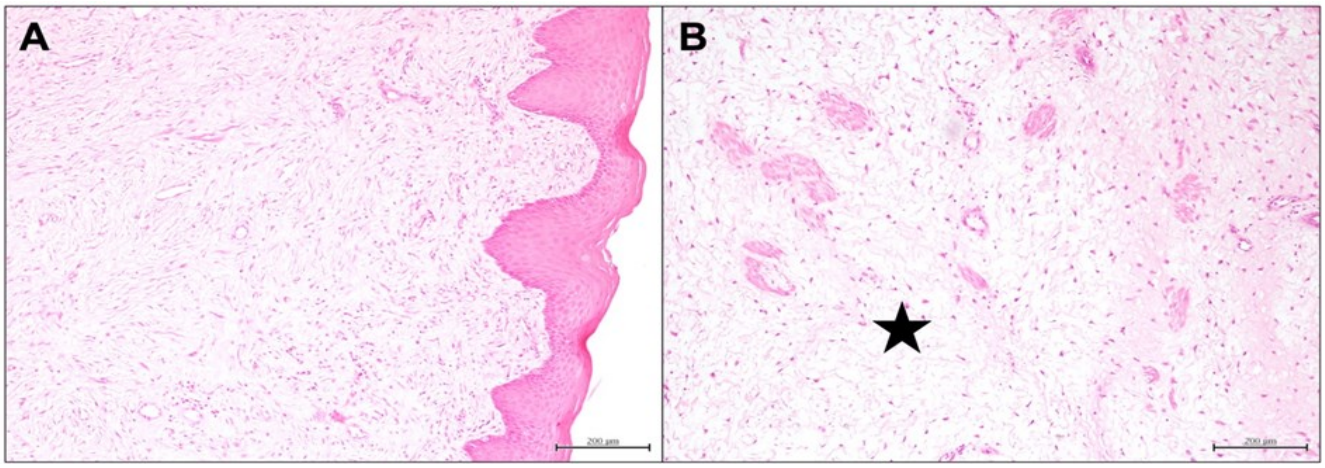

Figure 2. Vagina. Hyperplasia. A. Mild submucosal mononuclear inflammatory cellular infiltration, edema, and increased vascularization, H&E, Bar = 200 µm. B. Marked submucosal edema (star), H&E, Bar = 200 µm.

smears of all bitches incorporated into the study, keratinized superficial cells constituted 75% of all the cells in the slide (Figure 1) and they had their proestrus bleeding 7-14 days before they presented to the clinic. According to these data, it was determined that all bitches were in estrous in this study. In group VFP, two bitches had type 2 VFP and one bitch had type 3 VFP. Histopathological examination revealed vaginal hyperplasia characterized by inflammatory changes within the submucosa indicating marked edema, hyperemia, neovascularization, and mild infiltration of mononuclear cells (Figure 2). In group VT, three bitches had leiomyoma, which was composed of a circumscribed proliferation of monomorphic smooth muscle cells having abundant eosinophilic cytoplasm without mitotic figures (Figure 3) and two bitches had leiomyosarcoma, which showed interwoven histological pattern composed of the pleomorphic spindle to ovoid cells with numerous atypical mitoses (Figure 4). As a result of examination with diagnostic imaging techniques (radiography and ultrasonography), any metastases were not observed to the distant organs in group VT. According to gynaecological examination, accumulation of the pus into the uterine lumen, purulent vaginal discharge, and increase in uterine diameter were determined in bitches with vaginal leiomyoma. Mean, minimum and maximum levels of ER, PR, and EGFR related to groups were given in Table 1. The mean level of ER was significantly higher in group VT compared to the group VFP ($P < 0.05$). However, PR and EGFR levels were not shown a significance related to the groups ($P > 0.05$).

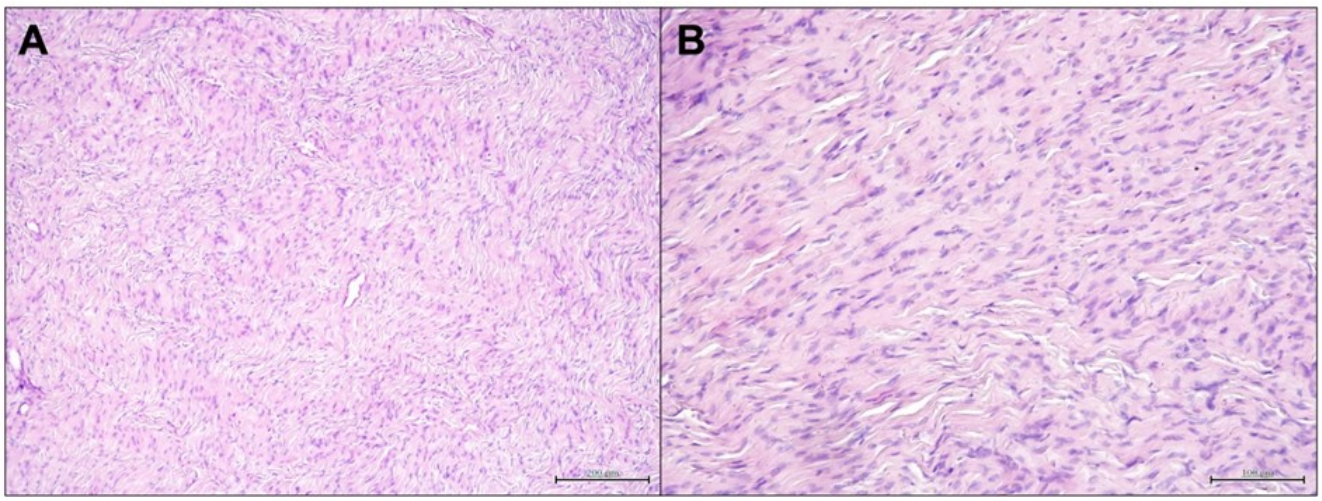

Figure 3. Vagina. Leiomyoma of the vaginal wall. A. Interwoven fascicles of the spindle cells, H&E, Bar = 200 µm. B. Higher magnification of well differentiated spindle cells, H&E, Bar = 100 µm.

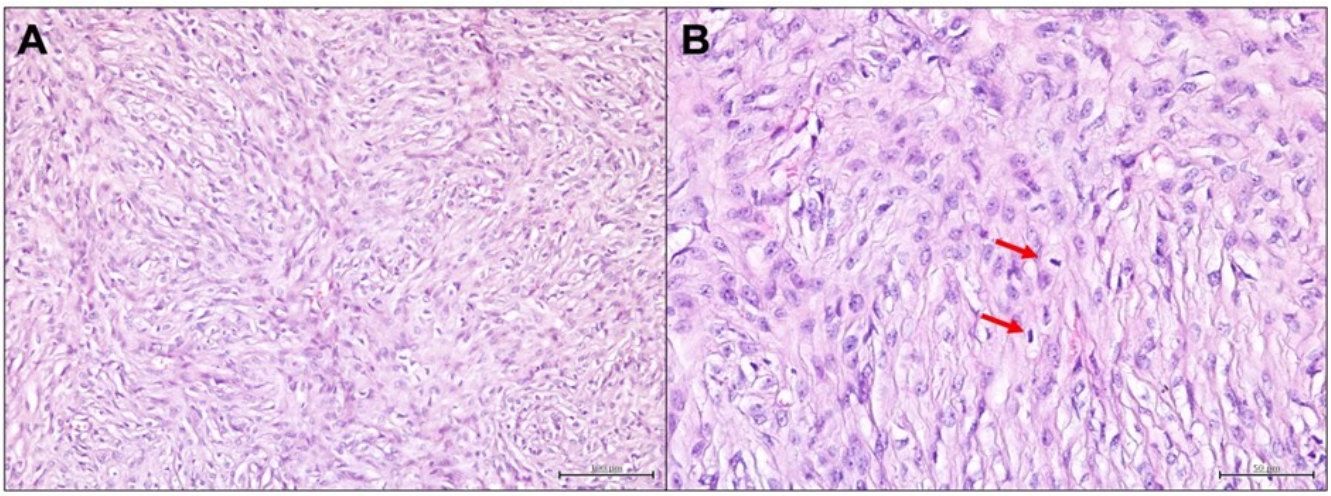

Figure 4. Vagina. Leiomyosarcoma of the vaginal wall. A. Proliferation of the neoplastic spindle cells arranged as interwoven fascicles, H&E, Bar = 100 µm. B. Higher magnification of neoplastic cells showing marked pleomorphism, numerous atypical mitosis (arrows), H&E, Bar = 50 µm.

Discussion

Vaginal fold prolapse occurs due to the elevated levels of estrogens during the proestrus and estrous stages of the sexual cycle (Sontas et al., 2010; Anila et al., 2020). The development and growth of the vaginal masses are considered to be stimulated by ovarian steroids and growth factors (Zhao et al., 2003; Ferré-Dolcet et al., 2020). The stage of the estrous cycle can be interpreted by vaginal cytology. Estrous is cytologically defined by 100% cornification with greater than 50% anuclear squames in epithelial cells (Root Kustritz, 2006). Similar to Root Kustritz (2006), in the current study, keratinized superficial cells constituted 75% of all epithelial cells in all cytology smears.

Anila et al. (2020) reported that nearly 75% of all cases of Type II and Type III vaginal prolapse were recorded in medium-sized breeds. Likewise the previous report, group VFP which had Type II and Type III vaginal prolapse, constituted of medium-sized breeds in this study. Although Boxers are the most represented breed for vaginal leiomyoma incidence, there is no apparent breed predisposition reported for vaginal leiomyosarcoma (Cooper and Valentine, 2016). In the current study, no breed predisposition was observed in group VT as reported by Copper and Valentine (2016).

The highest occurrence age of VFP was reported to be between 1.5 to 2.7 years of age (Schutte, 1967), while Greenberg et al. (2002) indicated the occurrence of VFP in older bitches. Although the mean age of the bitches in group VFP was 2.66 years old in this study as reported by Schutte (1967), in one of the VFP cases, the affected bitch was 5 years old as indicated by Greenberg et al (2002). The smooth muscle tumors, leiomyomas and leiomyosarcomas, are observed in the vagina of the bitches, with leiomyomas being the most encountered ones

Table 1. Mean, minimum and maximum levels of ER, PR and EGFR related to groups.

Groups	Parameters	Minimum	Maximum	Mean ± SEM
Group VFP	ER (ng/ml)	5.236	6.467	5.93 ± 0.36[a]
	PR (ng/ml)	8.741	15.217	11.01 ± 2.10
	EGFR (ng/ml)	7.839	19.381	12.49 ± 3.51
Group VT	ER (ng/ml)	6.764	8.832	8.06 ± 0.45[b]
	PR (ng/ml)	6.486	14.209	10.21 ± 1.44
	EGFR (ng/ml)	8.102	17.414	11.14 ± 1.10

VFP: Vaginal fold prolapse. VT: Vaginal tumor. ER: Estrogen receptor. PR: Progesteron receptor. EGFR: Epidermal growth factor receptor. SEM: Standard error of mean. [a,b] Different letters in ER lines indicate the significance (P<0.05).

(Manothaiudom, & Johnston, 1991). The incidence age of the bitches with vaginal leiomyomas and leiomyosarcomas are averaging 10.8 years old (Klein, 2001). Consistent with the previous report, vaginal tumors were observed in older intact bitches in this study.

Leiomyomas are non-invasive and non-metastatic vaginal tumors (Cooper and Valentine, 2016) whereas canine vaginal leiomyosarcomas can metastasize to the spleen (Brodey and Roszel, 1967). Macroscopic features of the vaginal leiomyomas are polypoid and/ or pedunculated (Brodey and Roszel, 1967). In accordance with the researchers (Brodey and Roszel, 1967; Cooper and Valentine, 2016), leiomyomas and leiomyosarcomas had the similar clinical and histological characteristics but as opposed to Brodey and Roszel (1967), metastasis was not observed in any of the leiomyosarcomas in this study. Enginler et al. (2014) reported a vaginal leiomyosarcoma following pyometra in a 10-year old Labrador Retriever bitch. In this study, pyometra was diagnosed in group VT but the bitches with pyometra had vaginal leiomyoma as opposed to Enginler et al. (2014).

It was reported that VFP occurs due to a strong response of vaginal mucosa to the estrogens during pro-estrous and early estrus (Sontas et al., 2010; Alan et al., 2007). In healthy bitches, reduction of ER expression in vaginal tissue was detected from pro-estrus and late estrus (Ithurralde et al., 2013). de Brito et al. (2006) compared ER-a in the vaginal epithelium of bitches with transmissible venereal tumor (TVT) or control bitches, and they indicated that there was a close relationship between neoplastic cells and cells of the vaginal stroma, which were highly positive for ER-a. Even though tumor types were different from the previous report (de Brito et al., 2006), the mean ER level of the vaginal tissue homogenates was higher in group VT than group VFP. A decrease in ER in bitches with VFP could be explained by the research of Uchima et al. (1987) reported that continuous exposure to estradiol resulted in a reduction in cytosolic estrogen receptors associated with nuclear accumulation of estrogen receptors.

In canine vaginal wall, both ER and PR are localized in the nuclei of the epithelial cells, stromal cells of the mucosa, and smooth muscle cells of the muscular layer. When the epithelium has a multilayered squamous stratified structure during pro -estrus and estrus, ER and PR receptors are predominantly observed in the basal and parabasal cell layers. Immunohistochemical intensity scores for PR in the canine vaginal epithelium, stroma and muscular layer were higher in pro-estrus and estrus than in met-estrus (Vermeirsch et al., 2002). A positive correlation between estrogen concentration and PR expression in vaginal stroma and epithelium was observed in bitches and rats (Vermeirsch et al., 2002; Ohta et al., 1993). Benign tumors of the vagina that have progesterone receptors such as fibroleiomyoma can be reduced in size by using the progesterone receptor antagonist aglepristone (Rollón et al., 2008). In canine vaginal leiomyoma and leiomyosarcoma, PR expressions were determined as 82.1% and 100%; respectively (Millian et al., 2007). In this study, tissue PR levels were not different between the groups ($P > 0.05$). It was hypothesized that insignificant results were obtained due to the increase of tissue PR levels in both VFP and VT.

Sağsöz et al. (2019) reported that EGFR expression in the bovine vagina, which alters according to the phase of the oestrous cycle, and a significant increase in EGFR expression had been detected in the follicular phase compared to the luteal phase. Iguchi et al. (1993) indicated that reduction of EGFR level in the vagina correlates with persistent proliferation and keratinization of the vagina in mice. Neonatally diethylstilbestrol-exposed mice had lower EGFR levels in the vagina than untreated mice (Iguchi et al., 1993). Mukku et al. (1985) emphasized that estrogen induction of physiological processes, including proliferation and differentiation, is mediated by EGF-EGF receptor interaction. In humans, EGFR is frequently expressed in invasive squamous cell carcinoma of the vagina. However, there was no statistically significant relationship between EGFR expression and clinical stage, grading, and tumor size in vaginal cancer (Brunner et al., 2011). In this study, EGFR levels did not show an alteration related to the groups (P>0.05). Because all bitches in the present study were in estrus and they were influenced by estrogen. As in previous reports (Mukku et al. 1985; Iguchi et al. 1993; Brunner et al. 2011; Sağsöz et al. 2019), in which the increase in EGFR in the follicular phase and in carcinogenic cases were noted, insignificant results in terms of EGFR levels were obtained between the group VFP and group VT in the present study.

Conclusions

In conclusion, significant differences were obtained between the bitches with VT and VFP related to ER levels although both groups had pathological conditions. Further studies are needed to discuss these pathologies with healthy bitches in terms of steroid receptors and EGFR levels in both vaginal tissue and blood sera.

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

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Trends towards the use of natural anesthetics in fish

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ABSTRACT

Anesthesia is generally defined as the loss of sensation caused by a pharmacological agent resulting from the suppression of the nervous system. Anesthetic agents are used to reduce stress, facilitate surgical operations requiring prolonged immobilization, in transportation, classification, handling, sorting, tagging, grading, weighing, measuring or vaccination in fish. In order to talk about an adequate level of anesthesia, signs such as loss of balance, relaxation in muscle tone, decreased respiration and inability to respond to stimuli must be observed in the fish. A good anesthetic agent must not have toxic side effects, be able to be eliminated from the body in a short time, not have permanent physiological, immunological or behavioral effects. In order to determine the optimal anesthesia dose, exposure time and maximize the drug's efficacy in fish, the size of the fish and the characteristics of the water it is in need to be determined beforehand. There are two types of commercial anesthetics, natural and synthetic. Although chemical anesthetics are commonly used for fish, there has been a recent trend towards the use of natural anesthetics due to safety, residue problems, accumulation in the fish body and side effects. These new herbal anesthetics have more favorable properties for the health of both fish species and the people who consume them.

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Introduction

Anesthesia is the suppression of the functions of a part or whole of the body due to loss of sensitivity by a natural or synthetic pharmacological agent (Aktop et al., 2019). Anesthetic drugs can be used for sedative, reducing stress and struggle, minimizing physiological changes, rendering them amenable to handling during invasive procedures such as blood sampling or injection and for euthanasia in fish (Aktop et al., 2019; Neiffer and Stamper, 2009; Rairat et al., 2021). Most fish encounter anesthetic agents at some point in their lives for the reasons stated above (Readman et al., 2013).

Due to the potential harms of chemical anesthetics, the interest in herbal anesthetics in aquaculture is increasing day by day (Can and Sümer, 2019). Herbal anesthetics are considered safe agents

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due to their natural ingredients, not causing any side effects, not having carcinogenic properties and not accumulating in tissues (Aktop et al., 2019).

The aim of this study is to compare and evaluate chemical anesthetics and natural anesthetics used in fish.

There are about 30,000 different fish species. Teleosts account for almost 96% of this. For this reason, most anesthesia studies are performed with teleosts. To a lesser extent, sharks, elasmobranchs such as stingrays, and other fish species are used in these studies. There are anatomical, physiological and behavioral differences between fish breeds. Given these differences, extrapolation of limited data on the anesthetics used could potentially carry risks. It provides a better understanding of anesthetic agents developed on unknown fish breeds and drug combinations, and their natural history and taxonomic relationship. (Neiffer and Stamper, 2009).

Properties of a good anesthetics in fish: In the selection of an ideal anesthetics for fish, some of the most important criteria to be considered are to be easy to use, not to be toxic or harmful living things and the environment, to be economical, having a rapid induction with quick excretion and recovery and not producing residues (Hamackova et al., 2006; Schroeder et al., 2021). The optimal dose of a fish anesthetic depends on species, fish size, environmental factors and process to perform (Javahery et al., 2012). The time for the fish to enter anesthesia should not exceed 3 minutes, and the time for exit from anesthesia should not exceed 5 minutes. It should show sufficient effect at low doses. It should be low cost and easily available (Aktop et al., 2019).

In general, the anesthesia phase should be short, and loss of balance and muscle tone in the fish should occur rapidly for handling (measuring, vaccination, grading) and the recovery stage should be calm and rapid (Bodur et al., 2018). Recovery from anesthesia can be observed when fish are returned to non-medicated water. Main signs are operculum movement and motions of swimming and balance (Schroeder et al., 2021).

Comparison of chemical and natural anesthetic agents: Commonly used synthetic anesthetics in fish are MS-222 (Tricaine methane sulfonate), benzocaine, isoeugenol, AQUI-S, 2-phenoxy ethanol, metomidate, quinaldine and carbon dioxide. The recommended induction and recovery time of less than 5 minutes for good anesthesia is among the values close to synthetic anesthetics in natural anesthetics (Husen et al., 2014; Purbosaria et al., 2019; Sneddon, 2012)

Essential oils of different plants such as Lamiaceae, Verbenaceae, Lau-raceae and Myrtaceae are mainly studied in order to benefit from their anesthetic effects in fish. In addition to these, herbal compounds such as menthol, linalool, myrcene, cineole, globulol, spathulenol, guaiol, caryophyllene oxide, terpinen-4-ol and dehydrofuquinone have been investigated for their anesthetic effects in recent years (Hoseini et al., 2019; Can and Sümer, 2019).

Clove oil is an effective, local and natural anesthetic that is widely used in fish today (Javahery et al., 2012). It is obtained by distillation of the flowers, stems and leaves of the clove tree Syzygium aromaticum (Husen and Sharma, 2014). Clove oil is less expensive and more cost-effective than MS-222, a chemical anesthetic. Oregano and eucalyptus oils are also inexpensive products like clove oil (Bodur et al., 2018). In fact, it can be said that Clove oil and eugenol are relatively more effective natural anesthetic agents compared to synthetics in terms of induction and recovery times (Purbosaria et al., 2019). The disadvantage of clove oil as an anesthetic is that its dosage is sensitive during application. A small variation in dosage can result in fish death or slow recovery time (Kamble et al., 2014).

Another example of essential oil that has been studied is coriander oil (Coriandrum sativum), which is derived from coriander mot. Coriander contains the active ingredient linalool, which is anesthetized in 50-70% of the main components. Depending on whether it is dry or wet, the essential oil content of coriander (C. sativum L.) fruits varies between 0.03-2.60% and is used for anesthetic purposes in fish (Aktop et al., 2019; Can et al., 2019).

Ocimum gratissimum was also found to be an effective and safe anesthetic in fish (Silva et al., 2012). It is commonly known as alfavaca or tree basil. It is often used as a spice and is also used as a sedative and cure for stress and headaches in human medicine (Albuquerque et al., 2007).

Linalool and myrcene are other substances used in fish anesthesia (Mirghaed et al., 2016). Citronellal anesthetic activity is higher than linalool and lower than eugenol. However, it can be preferred in terms of fish health as it causes less tissue problems (Yousefia et al., 2018).

Lavender essential oil has been found to be

effective in anesthetizing fish similar to clove extract without any side effects (Raisi et al., 2020).

Tricaine methanesulfonate (MS-222) is a synthetic agent frequently used in fish anesthesia (Pubosari et al., 2019; Harmon, 2009). However, tricaine methanesulfonate (MS-222) has been reported to have pharmacokinetic and pharmacodynamic differences in its substance (Topic et al., 2012). It has also been reported that this compound has a repulsive negative effect for some fish species (Readman et al., 2017). Other studies have shown that MS-222 may cause state changes in the immune system and biochemical parameters such as increased cortisol, and altered oxidative and immune responses in different fish species during transport (Cao et al., 2019, Kenter et al., 2019).

A number of studies have been conducted on the effects of anesthetics such as propofol (2,6-diisopropyl phenol), a GABA A agonist that is widely used for human and veterinary anesthesia, in different fish species (Fleming et al., 2003, Gressler et al., 2015). Propofol was reported to be a short-acting, rapid healing, and rapidly metabolized anesthetic agent without cumulative effects in a study conducted with the Nile tilapia (Oreochromis niloticus) species (Valença-Silva et al., 2014). Due to between- and within-species drug responses differences, anesthetics must be investigated on a species-by- species basis to assure its safe use (Toutain et al., 2010). At the end of transport in a study, propofol increased glucose levels while decreasing hematocrit (HCT) and lactate levels. Compared to naïve animals, animals carried with MS-222 had decreased levels of HCT and lactate, while cortisol levels increased. Cortisol levels were also increased in the control animals and were still elevated 24 hours later. In addition, there was a decrease in gill ROS levels and GST activity after transport with propofol compared to the control group. However, fillet quality and histopathology of the gills did not change in all tested groups (Félix et al., 2021).

Use of high-dose anesthetic for euthanasia : Overdose of immobilization drugs can be used for fish euthanasia (AVMA 2007). The most commonly preferred option for fish euthanasia is the use of immersion drugs (especially MS-222) between five and ten times the anesthetic concentrate for a given species. However, injectable agents are also effective (Ross and Ross, 1984). In large fish breeds, immersion and bathing method is not appropriate and practical. Dipping medicine for fish is poured directly over the gills. (Harms and Bakal 1995). With the exception of some breeds, generally keeping the fish in the anesthetic solution for 5 to 10 minutes after the cessation of opercular movement is sufficient for euthanasia. Fish myocardial cells do not need blood sugar for energy and use local glycogen stores. Therefore, cardiac asystole typically lags behind brain death (Stetter 2001). Doppler probes, ultrasonography, or electrocardiography may be used to confirm cardiac asystole. In addition, intravenous (in the heart or caudal vein) anesthetic drug or pentobarbitone administration can be done to ensure cardiac asystole (Ross 2001). Spinal transection or cranial concussion, blood loss applications are also alternative methods in deeply anesthetized fish (Harms 1999). Although the effectiveness of using carbon dioxide for immobilization has been reported, it is difficult to control concentrations of this substance in water. It is also known to have negative effects on oxygen levels, blood gases, and acid-base balance. (Gelwicks and Zafft 1998; Harms 1999; Prince et al. 1995).

Conclusion

Since compounds that are aversive in fish anesthesia cannot be routinely used in practice, herbal anesthetics play a key role as alternative. It is known that there are about thirty thousand fish breeds. It is impossible to perform anesthesia studies on all fish breeds. However, using as many different types and anesthetic agents as possible will increase the knowledge and experience in this matter. In line with the studies and results obtained, it has been reported that MS-222, a synthetic agent frequently used in fish anesthesia, may endanger the health and welfare of fish. It is reported that anesthetics of natural origin have a more positive effect on welfare during fish transportation. However, more research is needed to clarify this situation in the light of developing technology and information.

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