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Investigation of the Presence and Levels of Aflatoxin M1 in Labneh Cheese from Different Producers in Afyonkarahisar Using ELISA

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ABSTRACT

Aflatoxins are toxic compounds produced by *Aspergillus* species and are among the most dangerous mycotoxins. They can cause serious health effects such as cancer, birth defects, and genetic damage. Humans are mainly exposed through contaminated food and animal products like milk, dairy, and eggs. AFM1 is the metabolite of AFB1 found in milk and dairy products resulting from the consumption of contaminated feed containing AFB1 by dairy animals. In this study conducted for this purpose, the presence and level of AFM1 in labneh cheeses of different brands were investigated. In this context, 40 labneh cheese samples sold in markets were collected, and the presence of AFM1 was investigated using the ELISA method. AFM1 level was detected between 0.026-0.033 µg/kg in 10% (4/40) of the labneh cheese samples analysed. All of the samples were found below the Turkish Food Codex Contaminants Regulations limit (0.050 µg/kg). In conclusion, it is recommended to raise awareness about the potential health risks associated with the consumption of milk and dairy products contaminated with AFM1, which is an important group among Aflatoxins that harbour many health risks that may affect the general public, and to implement measures to prevent contamination in the food supply chain.

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INTRODUCTION

The term "mycotoxin," derived from the Greek word "mykes" (fungus) and the Latin "toxicum" (poison), refers to toxic secondary metabolites produced by certain fungal species (Öksüztepe and Erkan, 2016; Türel and Calapoglu, 2017). Mycotoxins can develop in a variety of foods and feeds due to the increased moisture content and temperature of the environment and can lead to toxin production by fungi. Among the significant types of mycotoxins, aflatoxins are primarily produced by Aspergillus flavus and Aspergillus parasiticus molds (Dinçel et al., 2012). Depending on exposure and dose, aflatoxins can exhibit acute or chronic effects, causing teratogenic, mutagenic, toxigenic, and carcinogenic impacts on both humans and animals (Erdoğan, 2004; Fazekas and Tarakovacs, 2005; Sengun et al., 2008). Within the food chain, humans and animals can ingest aflatoxins through contaminated food and feed. Particularly, humans are exposed to aflatoxins via milk, dairy products, and eggs derived from animals fed aflatoxin-contaminated feed (Dinçel, 2012; Akgül, 2021).

Aflatoxins are named based on their fluorescence under ultraviolet (UV) light. AFB1 and AFB2, produced by A. flavus, emit blue fluorescence, while AFB1, AFB2 (blue), AFG1, and AFG2 (green) are produced by A. parasiticus. Aflatoxin M1 (AFM1), also known as the "milk toxin," exhibits a blue-violet fluorescence and is found in milk and dairy products derived from animals fed AFB1-contaminated feed (Kocasarı Şahindokuyucu, 2014; Akgül, 2021). After ingestion of AFB1-contaminated feed, AFB1 is hydroxylated in the liver and converted into AFM1, which is classified as a probable human carcinogen (Group 2B) by the International Agency for Research on Cancer (Karaoğlan et al., 2022; Mortaş et al., 2022).

Due to its harmful effects, particularly on children, the presence of AFM1 in food is regulated by food safety standards. The Turkish Food Codex Contaminants Regulation limits AFM1 levels to 0.05 µg/kg in raw milk, heat-treated milk, and milk used for dairy production, and 0.025 µg/kg in infant formulas (Turkish Food Codex, December 29, 2011, Issue: 28157). Cheese, a concentrated food product, can contain AFM1 levels 3-4 times higher than the milk used in its production (Mortaş et al., 2022).

Labneh cheese, originating from the Middle East and Egypt, is traditionally made by straining yogurt made from buffalo milk or a buffalo-cow milk mixture. This process involves salting the yogurt, straining it in cheesecloth for 12-24 hours, and storing the resulting smooth, creamy, acidic, and milk-white cheese under refrigeration (Abou-Donia, 2008). Studies on this subject have shown that labneh cheese can be produced by adding yoghurt cultures, lowering the pH, adding salt and rapidly stirring the mixture before heat treating it at 85°C for five minutes and hot filling it into containers (Sönmez, 2019).

This study aimed to investigate the AFM1 levels in labneh cheese samples available in Afyonkarahisar using the Enzyme-Linked Immunosorbent Assay (ELISA) method, identifying potential public health risks.

MATERIAL and METHODS

Study Material

A total of 40 randomise labneh cheese samples, representing 20 different brands and two batch numbers per brand, were collected from retail outlets in Afyonkarahisar between July and September 2023. Samples were transported to the laboratory under cold chain conditions.

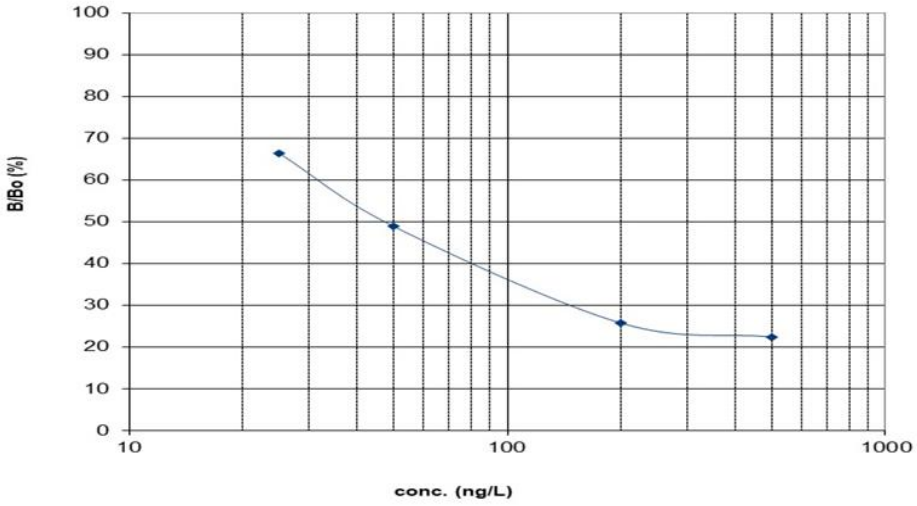
Sample Preparation

One gram of cheese was homogenized with 4 mL of 70% methanol and centrifuged at 10°C, 3000 rpm, for 10 minutes. Following centrifugation, 0.4 mL of the fat-free aqueous phase was transferred into sterile 15 mL containers, mixed with 0.4 mL of hexane, and vortexed for 10 seconds. The mixture was centrifuged again under the same conditions. The aqueous phase was diluted at a 1:5 ratio, and 100 µL of the final solution was used for analysis (Akgül and Kara, 2021; Aksoy and Sezer, 2019).

ELISA for Aflatoxin M1 Detection

AFM1 levels in the collected labneh cheese samples were measured using an ELISA reader (Thermo MultiScan) and a commercially available ELISA kit (AflaM1, Celer AFLA M1 500; detection limit: 25 ng/L). Absorbance values obtained from standards with known concentrations (0, 25, 50, 200, and 500 ng/L) were used to calculate results. The standard curve for aflatoxin concentrations is shown in Figure. Before use, ELISA kits and reagents were equilibrated to room temperature. Standards and extracted sample solutions (100 µL each) were added to the respective wells as per the manufacturer's instructions. The ELISA plate was covered with a transparent film, manually shaken for 5 seconds, and incubated at room temperature for 10 minutes. After discarding the liquid from the wells, they were washed three times with washing buffer. Subsequently, 100 µL of enzyme conjugate solution was added to each well, covered with a transparent film, and incubated for 5 minutes at room temperature. The washing step was repeated, and all wells were filled with the substrate solution using a multichannel micropipette. The plate was covered with a transparent film, gently shaken for 5 seconds, and incubated for 5 minutes to allow colour development. Finally, 50 µL of stop solution was added to each well, and absorbance values were measured at 450 nm.

Figure. Standard curve for Aflatoxin M1 concentration.



RESULTS

Analysis of the 40 labneh cheese samples revealed that AFM1 was detected in four samples (10%), with concentrations ranging from 0.026 to 0.033 µg/kg. All samples were within the AFM1 limit set by the Turkish Food Codex Contaminants Regulation (0.050 µg/kg) (Table 1). Other samples were below the detection limit.

Table 1. AFM1 levels in positive samples

Samples	**AFM1 Levels**	
	ng/kg	**µg /kg**
1	26,347	0,026
2	27,225	0,027
3	32,475	0,032
4	32,709	0,033

N:40

DISCUSSION

The presence of AFM1 in milk and dairy products is a significant concern, particularly in developing countries (Iqbal et al., 2015). Manetta et al. (2009) reported a direct relationship between the level of AFM1 in milk and its concentration in final dairy products. Anfossi et al. (2012) found that Italian cheeses made from goat's and sheep's milk were less contaminated with AFM1 than those made from cows' milk. Cheese is a potential source of AFM1 due to its association with the casein fraction of milk. Industrial producers typically use a greater variety of milk sources, thereby reducing the likelihood of high AFM1 contamination. In contrast, small dairies often rely on a limited number of sources, which may contribute to higher contamination (Anfossi et al., 2012). Furthermore, labneh cheese, which is generally produced industrially from various milk sources, is expected to pose a lower risk of AFM1 contamination; however, its AFM1 level can be up to three times higher than that of the milk from which it is made (Ardıç et al., 2009). AFM1 levels are minimally affected by heat treatments (e.g., pasteurization, thermization), refrigeration, freezing, sterilization, or fermentation processes used in dairy technology (Prandini et al., 2009). Seasonal temperature variations and improper feed storage conditions influence aflatoxin levels in feed. AFM1 can be detected in milk as early as 12 hours after ingestion of AFB1-contaminated feed. Due to its strong binding to casein, AFM1 is found at concentrations 3-7 times higher in dairy products than in milk, posing severe health risks (Toptaş and Erköse Genç, 2023; Erol, 2022). Consumption of AFM1-contaminated dairy products by lactating women has also been reported to pose risks to infants (Benkerroum and Amir, 2022).

Studies conducted in Türkiye on AFM1 levels in cheese and other dairy products have reported numerous cases exceeding and falling below regulatory limits. For example, Mortaş et al. (2022) found that 1.2% of 83 cheese samples from Ankara contained AFM1 levels above the Turkish Food Codex limits. Similarly, Aksoy and Sezer (2019) reported measurable AFM1 levels in 60 out of 150 cheese samples, although none exceeded the legal limit. However, in a study using ELISA to analyze 193 cheese samples from Erzurum, 26.4% exceeded the Turkish Food Codex limits (Ardıç et al., 2009). A study on 90 cheese samples from Diyarbakir revealed that 14.4% contained AFM1 levels above the limits (Erkan et al., 2009). Yaroğlu et al. (2005) investigated the levels of AFM1 in 600

cheese samples (200 Turkish white cheeses, 200 kashar cheeses and 200 cream cheeses) collected from various regions of Türkiye. AFM1 was detected in 5% of samples (30 in total), with an incidence rate of 5% for Turkish white cheese, 6% for kashar cheese and 4% for cream cheese. Variations in AFM1 levels between studies may result from differences in animal feeding practices, feed storage methods, and seasonal factors affecting aflatoxin levels (Karaoğlan et al., 2022). Studies and results on AFM1 levels in cheeses are shown in Table 2.

Table 2. Studies and results on AFM1 levels in cheeses

Source	Product	Samples (N)	Positivity	AFM1 Levels
Ardıç et al. (2009)	Turkish White Cheese	193	82.4% (159)	52–860 ng/kg (51 samples > 250 ng/kg)
Gücükoğlu et al. (2010)	Various local cheeses	64	-	11 samples above legal limit
Turgay et al. (2010)	Semi-Hard Cheeses	Total 46; (22 cows, 18 goats, 6 sheep)	Sheep 0%, Cow and Goat 80%	Cow: 0.06–1.20 ng/g, Goat: 0.06–0.22 ng/g
İşleyici et al. (2011)	Divle Tulum Cheese	55	18.18% (10)	5.15–26.44 ng/kg (Average: 10.83 ± 6.7)
Dinçel et al. (2012)	Civil, Mihalıç, Kars Kashar, Otlu, Urfa Cheeses	100 (20 each)	Urfa 50% (10)	Average 0.036 µg/kg
Rubio et al. (2011)	Curd, Manchego Cheese, Whey	-	-	Curd 2-2.7x, After Ripening 2.7-2.9x (139.0–221.3 ng/kg)
Manetta et al. (2009)	Grana Padano Cheese and Curd	-	-	Grana Padano 4.5x, Curd 3x higher

CONCLUSION

Studies on AFM1 levels in milk and dairy products have shown a wide range of concentrations. A review of previous studies on AFM1 levels in cheeses worldwide reveals a consistent trend of higher levels in developing and least developed countries with temperate or tropical climates, particularly during warm seasons. This phenomenon may be attributed to variations in feed storage conditions. It is hypothesised that industrial cheeses, which are manufactured using milk sourced from multiple producers, contain lower levels of AFM1 than local cheeses made from milk provided by a limited number of producers. Given that labneh cheeses in Türkiye are typically produced on an industrial scale, a positivity rate of 10% for AFM1 is considered to be high. In this study, AFM1 was detected in only four of the 40 labneh cheese samples, all within the Turkish Food Codex limits. Considering the potential public health risks associated with AFM1, preventive measures should be implemented throughout the food chain, from raw material production to final consumer delivery. Strict hygiene regulations and penalties for non-compliance, along with producer education, are crucial to reducing AFM1 levels.

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Ethics Approval

Ethics committee decision is not required.

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Conflict of Interest

There is no conflict of interest in the study.

Author Contributions

Research Design (CRediT 1) Author 1 (%40) – Author 2 (%20) – Author 3 (%20) – Author 4 (%20)
Data Collection (CRediT 2) Author 1 (%20) – Author 2 (%20) – Author 3 (%30) – Author 4 (%30)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%30) – Author 2 (%30) – Author 3 (%20) – Author 4 (%20)
Writing the Article (CRediT 12-13) Author 1 (%30) – Author 2 (%20) – Author 3 (%20) – Author 4 (%20)
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Sustainable Development Goals (SDG)

2 Zero Hunger
3 Good Health and Well-Being

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Determining Forage and Quality Traits of Some Sorghum Genotypes under the Ecological Conditions of Muş

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ABSTRACT

This study was conducted to determine the forage yield and quality of sorghum genotypes to be grown in Muş province. The research was carried out in 2022 in Muş province. The aim of the research was to determine the grass yield and quality of (Akdarı, 26, Mataco, Talisman, Karaca Melez, 305, Uzun, Hulk, Gözde 80, Early Sumac, Rox, 310, Aldarı, Öğretmen Oğlu, 314, Leoti, Erdurmuş, Beydarı) genotypes in some 14 Sorghum and Sorghum Sudan grass hybrids an 4 candidate cultivars under the Muş ecological conditions, The experiment was designed in a randomized block design with three replications. The plants' height ranged from 96.33 to 243.66 cm, single plant weight ranged from 868.33 to 2418.33 g, dry matter yield per decare varied between 345.95 and 1057.18 kg, and crude protein content ranged from 6.08% to 10.02%. According to the results of this study, in terms of quality, the genotypes Leoti, Uzun, and Beydarı stood out, while in terms of yield, the genotypes Gözde 80, Uzun, and Mataco were more prominent. For obtaining good yields in the ecological conditions of Muş and similar regions, it is recommended to grow one of the Gözde 80, Uzun, or Mataco genotypes. Considering both yield and quality, the Uzun genotype, which is in the high statistical groups, would be the most suitable choice for cultivation.

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INTRODUCTION

Sorghum (Sorghum bicolor L.) is an annual warm-season cereal crop that has gained global importance due to its versatility and adaptability (Kumuk and Avcıoğlu, 1986). It ranks fifth worldwide in terms of production and utilization, following barley, maize, wheat, and rice (Ebrahim, 2014). Sorghum is extensively cultivated in the United States, as well as in parts of Africa, Asia, and Central and South America, covering approximately 40 million hectares with an annual production of around 58 million tons. Major producers include the United States, Nigeria, Ethiopia, India, and Mexico. In terms of yield, countries such as Oman, Israel, Jordan, Uzbekistan, Austria, Italy, and Türkiye are among the top performers. According to the Turkish Statistical Institute (Anonymous, 2022a), sorghum production in Türkiye reached 117,093 tons across 29,263 decares.

Traditionally used as animal feed, sorghum has recently gained importance in human nutrition, particularly with the growing demand for gluten-free diets. It is a safe and nutrient-rich option for individuals with celiac disease and is increasingly used in products such as bread, cakes, cookies, breakfast cereals, and alcoholic beverages (Schober et al., 2005, Yousif et al., 2012, Aljobair, 2022). While 75% of global sorghum is used for human consumption, this figure varies significantly between regions. In developing countries, about 56% of sorghum is consumed by humans, compared to just 2% in developed countries, where its use as animal feed is more dominant (Anonymous, 1996; Fageria et al., 1997).

Sorghum stands out due to its drought tolerance, efficient water use, and high biomass production. It can be grown as fresh or dry forage and is suitable for hot, arid climates where irrigation is limited. Its rapid regrowth after cutting, resistance to pests and diseases, and high green fodder yield make it a strong alternative to maize (Çiğdem and Uzun, 2006). New silage-type sorghum cultivars have been developed that are taller, more productive, and comparable in quality to maize. Optimal harvesting time for silage sorghum is at the mid-dough grain stage when plant moisture content is between 65–70% (Undersander et al., 1990, Bulut et al., 2023).

Muş, located in eastern Türkiye, is among the provinces with the highest livestock potential. In 2022, it ranked 15th in registered cattle numbers and 12th in small ruminant population nationwide. However, livestock productivity in the region remains constrained by the high cost of feed, which accounts for 60–70% of total production expenses. Muş's land uses 42% agricultural land, 34% pasture, 11.5% natural meadow, 9% forest, and 3.5% non-arable land—along with its arid climate, presents a suitable environment for sorghum cultivation as an alternative to maize. Furthermore, sorghum can contribute to the rehabilitation of meadows and pastures by providing a cost-effective and locally adaptable roughage source (Anonymous, 1996).

This study was conducted in the ecologically significant Muş Plain to evaluate the forage yield and quality of 18 sorghum and sorghum–Sudan grass hybrid genotypes. The objective was to identify genotypes capable of producing high biomass with efficient water use under local climatic conditions, contributing to the sustainability of livestock farming through low-cost, drought-tolerant, and disease-resistant forage production.

MATERIAL and METHODS

Materials

Experimental site

The field trial was conducted during the 2022 growing season in Muş Province, Türkiye. The experiment was established in parcel number 3 of block 105, located in Muratgören village, approximately 15 km from the city center (Figure).

Figure. Experimental plant location

Climate characteristics of the experimental site

According to data from the Turkish State Meteorological Service, the average temperature during the trial period from June to September was recorded as 22.38 °C, compared to the long-term average of 22.46 °C. The total precipitation was 40.53 mm, while the long-term total precipitation was 55.80 mm (Anonymous, 2022b) (Table 1).

Table 1. Average precipitation and temperature data for Muş province from 1991 to 2022.

Month	Average Temperature (°C)		Precipitation Amount (mm)	
	1991-2022	2022	1991-2022	2022
June	20.16	19.88	27.10	21.43
July	24.75	24.16	7.70	1.15
August	24.98	25.54	5.40	0.77
September	19.96	19.94	15.60	17.18
Average/Total	22.46	22.38	55.80	40.53

Method

Experiment implementation

Soil companent

The soil of the experimental area is classified as clay loam, non-saline, and slightly acidic. It has moderate levels of plant-available phosphorus and organic matter, a medium lime content, and is rich in potassium (Aydeniz and Brohi, 1993).

Planting

In the study, seeds were manually sown in 4 rows with a 70 cm row spacing and a 4-5 cm sowing depth. Planting was done on June 1, 2022, by hand broadcasting, with a seeding rate of 1.5 kilograms per decare. The experiment was designed according to a Randomized Complete Block Design with three replications. The plot area was 5m × 2.8m = 14m² (Fernandez et al., 2012).

Maintenance

Since sorghum seeds are small, thinning and singling were performed after manual planting to achieve the desired plant density. A total of 14 kg of nitrogen (N) per decare (33% Ammonium Nitrate) was applied. Half of the nitrogen (7.0 kg/da) was applied at planting, and the remaining half (7.0 kg/da) was applied when plants reached a height of 40-50 cm. Additionally, 8 kg/da of P_2O_5 (Triple Superphosphate) was applied (Avcı et al., 2018). Weed control was performed by hoeing when the plants reached a height of 15-20 cm and further weed control and root collar filling were carried out when the plants reached 40-50 cm in height.

Irrigation

Irrigation is essential for improving plant productivity. For this purpose, the trial plots were irrigated using furrow irrigation after sprinkler irrigation when the plants reached a height of 100-120 cm.

Harvest

Sorghum varieties were harvested between September 10-11, 2022, during the dough stage. The central two rows and the two rows at the edges of the plot (30 cm from the edge) were excluded from the analysis as border effects. The harvested sorghum plants were weighed for fresh weight, and 10 randomly selected plants from the central two rows of each plot were set aside for observation and measurement.

Traits measured in the experiment

Observations on the plants were made on 10 randomly selected plants from the central two rows (Anonymous, 2010).

1. Plant height: The vertical distance from the surface to the tips of 10 randomly selected plants in the central two rows of each plot was measured (Anonymous, 2010).

2. Dry matter yield: The green biomass of 10 plants randomly selected from the central two rows

of each plot was weighed, then dried and reweighed to determine the Dry Matter Yield (DMY) (Anonymous, 2010).

3. Single plant weight: Ten plants, cut at a height of 5 cm from the soil surface, were weighed individually to determine their fresh weight (Anomymous, 2010).

4. Total plant crude protein content: For crude protein analysis, 10 plants from the central two rows of each plot were selected, four of which were taken as whole plants, ground, and processed. Protein content was determined using the Kjeldahl method under laboratory conditions. The plants were ground through a 1 mm sieve, and 0.5 grams of the sample were weighed. The total nitrogen content was determined by dry combustion. The total nitrogen value was then multiplied by a factor of 6.25 to calculate the crude protein content (Yavuz, 2011).

Data evaluation

The data obtained were analyzed using variance analysis according to the Randomized Complete Block Design with the JMP statistical software, and the Duncan test was used to compare differences between the groups (Düzgüneş et al. 1987).

RESULTS

Plant Height

The differences in plant height among the varieties were statistically significant at the 5% level. The plant heights of the varieties under investigation are presented in the table below (Table 2). As a result of the research, the average plant height was found to be 175.38 cm.

Table 2. Plant height of sorghum varieties in the study.

No	n	Genotypes	Mean±SE Plant Height (cm)
1	10	Akdarı	96.63±19.2^{ı}
2	10	26	128.3321.6^{ghı}
3	10	Mataco	216.00±26.7^{a-d}
4	10	Talisman	188.33±44^{b-e}
5	10	Karaca Melez	243.66±39.9^{a}
6	10	305	229.66±11.6^{abc}
7	10	Uzun	125.66±20.3^{hı}
8	10	Hulk	221.00±12.8^{a-d}
9	10	Gözde 80	231.33±19.2^{ab}
10	10	Early Sumac	175.66±14.9^{d-g}
11	10	Rox	153.00±25.2^{e-h}
12	10	310	226.66±9.39^{abc}
13	10	Aldarı	96.33±5.17^{ı}
14	10	Öğretmen Oğlu	123.00±9.81^{hı}
15	10	314	147.00±18.9^{e-h}
16	10	Leoti	182.00±13.5^{c-f}
17	10	Erdurmuş	237.33±54^{ab}
18	10	Beydarı	135.00±27.6$^{f-ı}$
Overally			175.38±29.68

+: Values marked with similar letters are not significantly different from each other at the P≤0.05 error level according to the Duncan test results.

Dry Matter Yield

As shown in the Table 3, there is a statistically significant difference in dry matter yield (DMY) among the varieties, with a significance level of 1%. The average DMY (dry matter yield) of the examined varieties is presented in the table below (Table 3). As a result of the study, the average dry matter yield (DMY) was found to be 755.37 kg per hectare.

Table 3. Dry matter yield in the study.

No	n	Genotypes	Mean±SE Dry Matter Yield (kg/da)
1	10	Akdarı	345.95±122^{h}
2	10	26	711.90±114^{c-f}
3	10	Mataco	973.69±101^{abc}
4	10	Talisman	811.17±120^{a-e}
5	10	Karaca Melez	766.15±188^{b-f}
6	10	305	774.90±106^{b-f}
7	10	Uzun	758.63±150^{b-f}
8	10	Hulk	976.75±70.2^{abc}
9	10	Gözde 80	1057.18±106^{a}
10	10	Early Sumac	603.84±163^{e-g}
11	10	Rox	635.50±258^{d-g}
12	10	310	875.77±70^{a-d}
13	10	Aldarı	421.26±63.5^{gh}
14	10	Öğretmen Oğlu	670.35±101^{d-g}
15	10	314	782.31±352^{b-f}
16	10	Leoti	884.64±99.8^{a-d}
17	10	Erdurmuş	1004.02±180^{ab}
18	10	Beydarı	542.82±198^{fgh}
Overally			755.37±161.50

+: Values marked with similar letters are not significantly different from each other at the P≤0.05 error level according to the Duncan test results.

Single Plant Weight (g)

When examining the single plant weight in Table 4, statistical analysis indicates a significant difference at the 1% level among the varieties. The single plant weight of the varieties in the study is shown in the table below. Based on the research results, the average single plant weight was found to be 1550.96 g.

Total Plant Crude Protein Content

As seen in the Table 5, there are statistically significant differences in the total crude protein content among the varieties at the 1% level. The crude protein content of the varieties examined is shown in the table below. Based on the research results, the average crude protein content was found to be 7.38%.

Table 4. Single plant weight analyzed in the study.

No	n	Genotypes	Mean±SE Single Plant Weight (gr)
1	10	Akdarı	1864.33±82^{bcd}
2	10	26	1683.33±79^{cde}
3	10	Mataco	1630.00±75.5^{cde}
4	10	Talisman	1531.66±253^{c-f}
5	10	Karaca Melez	1826.66±181^{bcd}
6	10	305	1370.00±153^{d-g}
7	10	Uzun	1505.00±286^{def}
8	10	Hulk	1515.00±204^{def}
9	10	Gözde 80	868.33±127^{g}
10	10	Early Sumac	2035.00±190^{abc}
11	10	Rox	1658.33±476^{cde}
12	10	310	1190.00±123^{efg}
13	10	Aldarı	911.66±108^{g}
14	10	Öğretmen Oğlu	1091.66±153^{fg}
15	10	314	2418.33±497^{a}
16	10	Leoti	2290.00±189^{ab}
17	10	Erdurmuş	1295.00±253^{efg}
18	10	Beydarı	1233.33±304^{efg}
Overally			1550.96±310.20

+: Values marked with similar letters are not significantly different from each other at the P≤0.05 error level according to the Duncan test results.

Table 5. Crude protein content analyzed in the study

No	n	Genotypes	Mean±SE Crude Protein Content %
1	10	Akdarı	8.83±0.13^{abc}
2	10	26	7.51±0.11^{cde}
3	10	Mataco	6.54±0.12^{def}
4	10	Talisman	6.76±0.21^{def}
5	10	Karaca Melez	6.08±0.16^{f}
6	10	305	6.77±0.27^{def}
7	10	Uzun	10.02±0.07^{a}
8	10	Hulk	6.23±0.113^{ef}
9	10	Gözde 80	6.52±0.07^{def}
10	10	Early Sumac	7.25±0.09^{def}
11	10	Rox	7.22±0.07^{def}
12	10	310	7.20±0.09^{def}
13	10	Aldarı	7.80±0.17^{bcd}
14	10	Öğretmen Oğlu	7.22±0.07^{def}
15	10	314	7.45±0.09^{c-f}
16	10	Leoti	7.46±0.09^{c-f}
17	10	Erdurmuş	6.93±0.08^{def}
18	10	Beydarı	9.10±0.06^{ab}
Overally			7.38±0.83

+: Values marked with similar letters are not significantly different from each other at the P≤0.05 error level according to the Duncan test results.

DISCUSSION

Statistically, the highest plant heights were obtained from varieties such as Karaca Melez 310,305, Erdurmuş, and Gözde 80, which formed the same statistical group. The lowest plant heights were recorded from varieties such as Akdarı, Aldarı, Öğretmen Oğlu, and Uzun (Table 2). Comparing the plant heights of different sorghum varieties: Malik et al. (2007) reported the highest plant height as 234 cm in Pakistan, Büyükburç et al. (1997) found plant heights between 157-213.9 cm in Tokat, Gül and Baytekin (1999) recorded the highest plant height as 114.60-135 cm, Geren and Kavut (2009) reported plant heights between 147.8-330 cm in Bornova, Karadaş (2008) observed heights of 210-218 cm in Konya, Bhale and Borikar (1982) found the highest plant heights between 93-132 cm, İptaş (1993) recorded a maximum of 198 cm in Tokat conditions, Blümmel et al. (2003) found plant heights ranging from 133-333 cm, Başaran (2011) reported the highest height as 189 cm, Uygur (2011) in Tokat found plant heights ranging from 215-281.7 cm, Güneş and Acar (2005) reported heights between 260-285 cm, Gül and Başbağ (2005) observed heights ranging from 139-248 cm, Özköse et al. (2014) found heights between 83-155 cm, Geren and Kavut (2009) reported heights between 148-330 cm, and Salman and Budak (2015) reported the highest plant height as 345 cm. The variation in these results may be attributed to differences in the varieties used in the trials, as well as factors such as temperature, total precipitation, ecological conditions, and irrigation.

Statistically, the highest yields were observed in the varieties Mataco, Gözde 80, Erdurmuş, and Hulk. The lowest dry matter yields were obtained from the varieties Akdarı, Aldarı, Beydarı, and Early Sumac. In studies by Acar and Yıldırım (2001), the dry matter yield was 2093 kg per hectare, while Çakmakçı et al. (1999) reported a yield of 2093 kg per hectare in Antalya. Kır and Şahan (2017) found the dry matter yield to range from 1352 to 2848 kg per hectare under the conditions of Kırşehir, and Çeçen et al. (2005) reported a range of 1248-1654 kg per hectare in Antalya. Kara et al. (2019) found a dry matter yield of 1334 kg per hectare, while Dündar et al. (2019) reported a range of 6006-3661 kg per hectare. The differences in these results may be attributed to the use of different varieties in the trials, as well as variations in temperature, total rainfall, ecological conditions, and irrigation practices.

Statistically, the highest single plant weights were observed in the varieties 314, Leoti, Early Sumac, and Akdarı. The lowest single plant weights were recorded in the varieties Gözde 80, Aldarı, 310, and Öğretmen Oğlu. In terms of single plant weights, Uygur (2012) reported the highest values of 385-457 g in Tokat, while Yılmaz et al. (2003) found a single plant weight of 599 g under the conditions of Hatay. Güneş and Acar (2005) determined the highest single plant weight to be 266 g in Karaman. The differences in these results may be attributed to the use of different varieties in the trials, as well as variations in temperature, total rainfall, ecological conditions, and irrigation practices.

Statistically, the highest crude protein content was observed in the varieties Uzun, Beydarı, Akdarı, and Aldarı. The lowest crude protein content was recorded in the varieties Karaca Melez, Hulk, Gözde 80, and Mataco (Table 5). In terms of total plant crude protein content, Parlak and Sevimay (2007) reported a value of 10% under the conditions of Ankara, while Çiğdem and Uzun (2006) found values ranging from 6% to 10.16% in Samsun. Büyükburç et al. (1997) reported a range of 8.5% to 10% in Tokat, and İptaş (1993) found a value of 6% in Tokat. Hosaflıoğlu (1998) reported values ranging from 7% to 8% under irrigated conditions in Van, while Cacares and Santana (1987) found a value of 10% in Cuba. Açıkgöz (1995) observed a range of 6% to 9%, and Yılmaz and Hoşafoğlu (2000) reported a range of 7% to 8% in their study in Van. Uygur (2012) found values between 8% and 12% in Tokat, while Torrecillas et al. (2011) observed values between 4% and 4.2%

in Argentina. The differences in these results may be attributed to the use of different varieties in the trials, as well as variations in temperature, total rainfall, ecological conditions, and irrigation practices

CONCLUSION

In conclusion, the average plant height of the sorghum varieties planted in this study was found to be 175.38 cm, with plant heights ranging from 96.33 cm to 243.66 cm. The average dry matter yield of the sorghum varieties was 755.37 kg per decare, with dry matter yield ranging from 345.95 kg/da to 1057.18 kg/da. The average single plant weight of the sorghum varieties was 1550.96 g, with single plant weights ranging from 868.33 g to 2418.33 g. The average total plant crude protein content of the sorghum varieties was found to be 7.38%, with crude protein content ranging from 6.08% to 10.02%. According to the results of this study, in terms of quality, the genotypes Leoti, Uzun, and Beydarı stood out, while in terms of yield, the genotypes Gözde 80, Uzun, and Mataco were more prominent. For obtaining good yields under the ecological conditions of Muş and similar regions, it is recommended to grow one of the Gözde 80, Uzun, or Mataco genotypes

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Ethics Approval

This project is not required ethical statement.

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Conflict of Interest

The authors have no relevant interests.

Author Contributions

Research Design (CRediT 1) Author 1 (%20) – Author 2 (%80)

Data Collection (CRediT 2) Author 1 (%20) – Author 2 (%80)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%20) – Author 2 (%80)

Writing the Article (CRediT 12-13) Author 1 (%20) – Author 2 (%80)

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Sustainable Development Goals (SDG)

12 Responsible Consumption and Production

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Examination of Lung Lesions in Parainfluenza-3 Infections in Ruminants with Pathological and Immunohistochemical Methods

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ABSTRACT

Parainfluenza 3 (PI3) infection is one of the most common viral respiratory diseases in domestic and wild ruminants. The most common finding is characteristic bronchointerstitial pneumonia. Concurrent viral and bacterial infections may exacerbate this condition and lead to fibrinopurulent-necrotic bronchopneumonia. The aim of this study was to investigate the histopathological findings of PI3 infection in ruminants, the release of mannose binding lectin (MBL) and surfactant protein B (SP-B) in lung tissue during inflammation, their role and importance in pathogenesis and their interrelationship. Paraffin-embedded lung tissue blocks were used from 30 ruminants with suspected viral pneumonia and 10 ruminants without lesions. Histopathologically, the acute destructive phase was observed in most cases (78%), whereas the chronic proliferative phase was observed in 4 cases (22%). Immunohistochemistry revealed anti-PI3 positivity in 15 animals (50%). In addition, MBL release was found to be high in acute cases but decreased in subacute or chronic cases. SP-B release was higher in subacute and chronic cases compared to acute cases. In conclusion, it was suggested that MBL release may be insufficient for recovery in cases of immunodeficiency or mixed infections, whereas early and accurate treatment could increase the chances of survival through the effects of SP-B.

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INTRODUCTION

Respiratory infections are a significant health issue for both human and animal health. These infections also result in severe economic losses due to reduced feed efficiency, weight gain, milk yield and working capacity, as well as increased costs for medication and treatment (Dawson et al., 1996; Friton et al., 2005; Gorkem et al., 2020; Jim et al., 1993; Sumen et al. 2023). Respiratory diseases are caused by viral, bacterial, fungal and parasitic agents. Among the viruses commonly encountered in the field, parainfluenza-3 (PI3) virus is prominent, affecting species such as cattle, sheep, goats and horses. PI3 virus belongs to the family Paramyxoviridae, subfamily Paramyxovirinae and genus Respirovirus and is an RNA virus. The virus enters the body via the airborne route and replicates in the upper respiratory tract, alveolar epithelial cells and macrophages. Transmission occurs via nasal mucus, ocular secretions and droplet infection (Yuzbasıgil, 2010). When the immune system is suppressed or defence mechanisms are inadequate, bronchointerstitial pneumonia develops with its characteristic inflammation, creating a predisposition to enzootic pneumonia. In mild cases, the disease manifests with clinical symptoms such as fever and nasal discharge leading to weight loss (Fenner et al., 1987; Gunn and Wilson, 1991; Yuzbasıgil, 2010). MBL (Mannose-binding lectin) binds to carbohydrate surfaces on pathogens, which facilitates opsonization and phagocytosis (Degn et al., 2007; Neth et al., 2000; Jack et al., 2001; Worthley et al., 2005). By promoting the recognition of pathogens by macrophages, MBL enhances phagocytosis (Jack et al., 2001; Stuart et al., 2005). Surfactant protein B (SP-B) is a hydrophilic surfactant protein secreted by type-II pneumocytes (Pérez, 2008). This phospholipid-structured protein complex coats the alveoli and airways. It is crucial for maintaining airway stability and providing the immunomodulation necessary for host defense (Mulugeta et al., 2006). In addition to preserving alveolar surface tension, it acts as a barrier against foreign pathogens (Whitsett et al., 2002). The aim of this study was to investigate the histopathological findings of PI3 infection in ruminants, the release of mannose binding lectin (MBL) and surfactant protein B (SP-B) in lung tissue during inflammation, their roles and importance in pathogenesis, and their interrelationships.

MATERIAL and METHODS

The material of this study consisted of paraffin-embedded lung tissue blocks from 40 ruminants (cattle, sheep, and goats) diagnosed with intersitital/bronchointerstitial pneumonia between 2017 and 2020, archived in the Department of Pathology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University.

The selected paraffin blocks from the study group, comprising 30 animals, included 18 small ruminants (6 males and 12 females) and 12 large ruminants (7 males and 5 females), of which all were adults except for three. The control group, consisting of 10 animals without any inflammatory reaction in the lungs, included 7 small ruminants (2 females and 5 males) and 3 large ruminants (all females).

Tissue sections with a thickness of 5 µm were taken from the blocks and mounted on standard and poly-L-lysine-coated slides. The sections on standard slides were stained with routine Hematoxylin-Eosin for microscopic evaluation of the lesions. The sections on poly-L-lysine-coated slides were subjected to immunoperoxidase techniques using a standard commercial Avidin-Biotin Complex Peroxidase (ABC-P) kit (Abcam, UK, ab236466 UltraVision Polyvalent Rabbit-Mouse HRP, TP-125-HL). The antibodies used were Polyclonal anti-Bovine Parainfluenza 3 (Moab a-BPI3-Biox Med), anti-MBL-2 [Anti-Mannan Binding Lectin antibody (ab203303)], and anti-Surfactant Protein B (SP-B) [Anti-Pro + Mature Surfactant Protein B antibody (ab40876)].

Histopathological evaluation method: Lesioned tissue sections scored the following criterias based on severity: Bronchitis, bronchiolitis, desquamation, bronchopneumonia, interstitial pneumonia, bronchial and bronchiolar hyperplasia, inclusion bodies, syncytial cell formations, and bronchiolitis obliterans. The scoring criteria is shown as Table 1. The scoring criteria and semiquantitative analysis method were derived from the study by Ozyıldız et al. (2018).

Table 1. Scoring criteria

Score	Bs/Bsl	Plts	D	ICAL	IASE	BH	İ	SCF	BO	IHC
3	5≥ foci	Diffuse	5≥ foci	5≥ foci	5≥ foci	5≥	5≥	5≥ foci	5≥ foci	Intense
2	3≥ foci	3≥ foci	3≥ foci	3≥ foci	3≥ foci	3≥	3≥	3≥ foci	3≥ foci	Moderate
1	1≥ foci	1≥ foci	1≥ foci	1≥ foci	1≥ foci	1≥	1≥	1≥ foci	1≥ foci	Mild
0	No lesion	No lesion	No lesion	No lesion	No lesion	No lesion	No lesion	No lesion	No lesion	Negative

Bs/Bsl: Bronchus/Bronchiole, Plts: Pleuritis, D: Desquamation, ICAL: Inflammatory cells in alveolar lumens, IASE: Interalveolar septal enlargement, BH: Bronchial hyperplasia, İ: Inclusion, SCF: Syncytial cell formation, BO: Bronchiolitis obliterans, IHC: Immunohistochemistry.

Immunohistochemical Evaluation Method

The lesions were examined for 5 different areas on x200 magnification under microscopy. The immunoreactivities of anti-bovine parainfluenza 3, anti-MBL, and anti-Surfactant Protein B markers in the lung tissue were determined. The scoring criteria is shown as Figure 1.

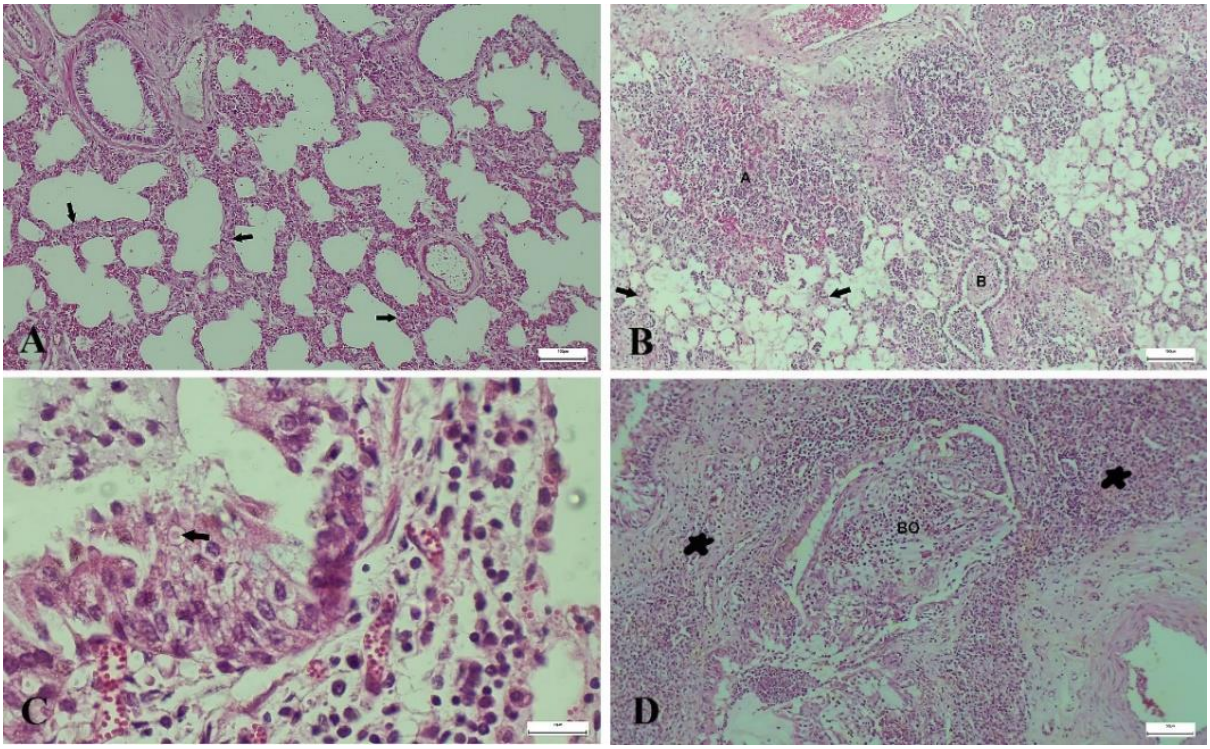
RESULTS

Histopathological Findings and Immunohistochemical Findings

Histochemical staining revealed lesions characterised as interstitial pneumonia and bronchopneumonia with secondary infections. Based on the composition of inflammatory cells, the lesions were classified as "acute destructive form" and "chronic proliferative form". In the acute form, tissue destruction and a fluid-cellular response were predominant. Interlobar, interlobular and interalveolar vessels showed severe hyperemia. In the acute destructive form, the interalveolar septal tissue is dilated due to oedema and infiltration consisting mainly of lymphocytes, histiocytes and macrophages (Fig. 1A). Alveolar lumens were mostly empty, with some containing pink homogeneous oedema fluid, desquamated type 1 pneumocytes, alveolar macrophages (Fig. 1B), a few neutrophils and syncytial cell formations. In some areas, pink eosinophilic inclusion bodies were seen in the bronchial-bronchiolar epithelium (Fig. 1C). In almost all acute cases there were areas of bronchopneumonia associated with bacterial contamination (enzootic pneumonia).

In the chronic proliferative form, the interalveolar, interlobular, and interlobar septa were markedly widened, and areas of atelectasis and emphysema were observed. The cellular composition of the interstitium included dense fibroblastic cells, lymphocyte infiltration, and histiocytes. Bronchial and bronchiolar epithelia in some areas were desquamated into the lumen. In some areas, there were wide fibrotic areas and some lumens of bronchioles were found to be completely obstructed by organized material (bronchiolitis obliterans) Fig. 1D). At this stage, it was noticed that the vital capacity of the lungs in the animals had significantly decreased due to increased fibroplasia and loss of parenchyma.

Figure 1. Histomorphological appearance of lung lesions.



A: Enlargements in the interalveolar septal tissue (arrows), H&E, X40, 100 µm. **B:** Broncho-interstitial pneumonia, thickening in the interalveolar septal tissue (arrows) with bronchiole (B) and alveolar lumens (A) filled with inflammatory cells, H&E, X40, 100 µm. **C:** Eosinophilic intracytoplasmic inclusion body (arrow) in bronchiolar epithelium, H&E, X400, 20 µm. **D:** Intense fibrous-histiocytic proliferation in the peribronchial region and atelectasis in the alveoli resulting in parenchymal loss (stars), with bronchiole lumen completely obstructed by inflammatory cells and fibrotic tissue (BO), H&E, X100, 50 µm.

To detect infection and its distribution, the localisation and intensity of anti-parainfluenza 3 immunoreactivity in the lesioned lung tissue was determined. The intensity of the immunopositive reactions increased proportionally with the severity of the inflammation (Figure 2A). In addition, areas with strong anti-Parainfluenza 3 positivity also showed intense positive reactions for anti-MBL protein (Fig. 2B). In cases of interstitial pneumonia, the most intense anti-PI3 and MBL immunopositive areas were observed in bronchial and bronchiolar epithelia and lumens, as well as in interalveolar and interlobular regions. Intense anti-PI3 and anti-MBL positivity was also seen in the cytoplasm of macrophages in these areas, in syncytial cell formations and in peribronchial lymph nodes.

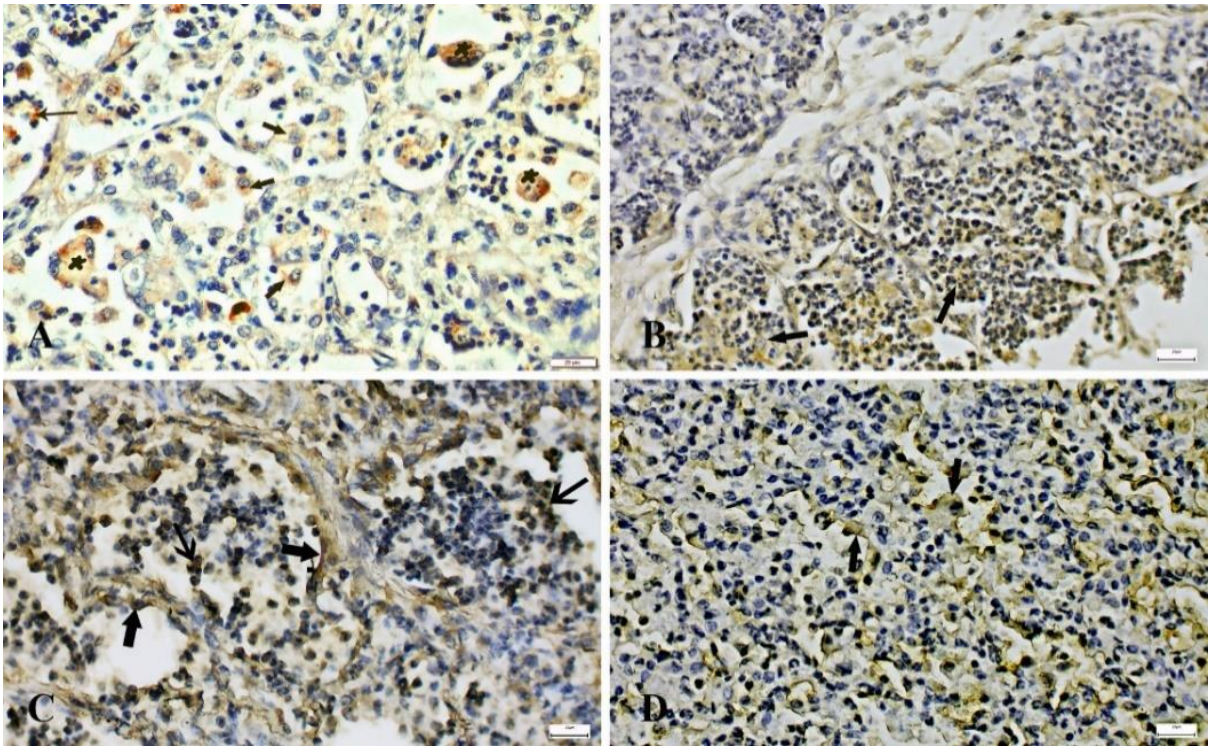
In contrast, anti-SP-B reactions were predominantly observed in the cytoplasm of phagocytic cells and alveolar and bronchiolar epithelia with mild to moderate (Fig. 2C).

In chronic progressive cases, anti-PI3 and anti-MBL immunopositivity was moderate in the cytoplasm of macrophages and interstitial regions, whereas it was mild on the luminal surfaces of bronchial and bronchiolar epithelia and in epithelial cells desquamated into the lumen. Intact alveoli showed strong anti-SP-B positivity, whereas no positivity was observed in atelectatic alveoli (Figure 2D).

In cases of mixed infection, strong anti-PI3 and anti-MBL immunopositivity was seen in the interstitium, bronchiolar and alveolar epithelia and lumens of phagocytic cells, whereas mild to moderate anti-SP-B positivity was observed.

No anti-PI3 or anti-MBL immunopositivity was found in the lung tissue of the control group. However, a very thin layer and mild anti-SP-B positivity were observed in the cytoplasm of type 2 pneumocytes in some alveolar walls.

Figure 2. Immunohistochemical characterization of lung lesions.

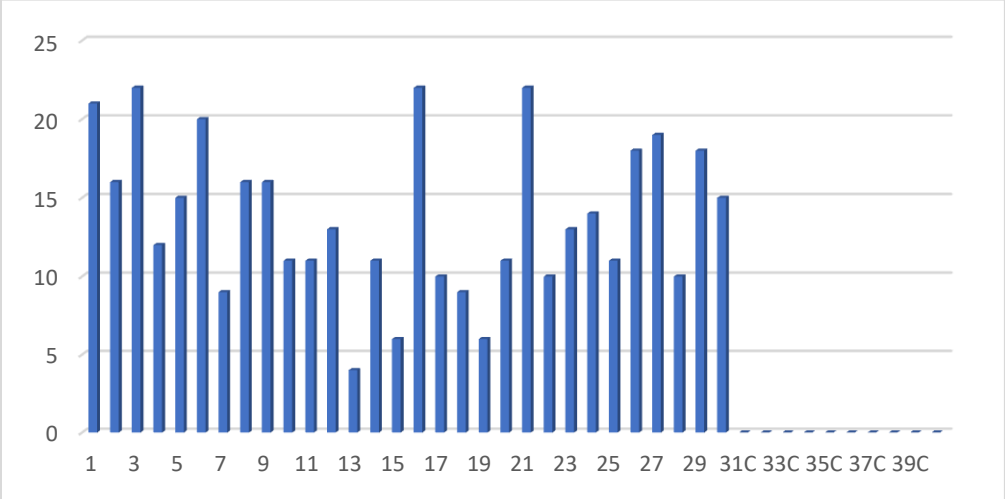


A: Anti-PI3 immunopositive reactions within neutrophils (thin arrow), macrophages (thick arrows), and syncytial cell formations (stars) in the alveolar lumens, ABC-P, X400, 20 µm. **B:** Anti-MBL positive reactions in the cytoplasm of phagocytic cells within the alveolar lumens (arrows), ABC-P, X400, 20 µm. **C:** Inflammatory cell cytoplasms in the alveolar lumens (thin arrows), with anti-SFB immunopositive areas in the alveolar walls and pneumocytes (thick arrows), ABC-P, X400, 20 µm. **D:** Chronic proliferative phase; anti-SFB positive immunoreactions in the remaining and partially atelectatic alveolar walls and type 2 pneumocytes (arrows), ABC-P, X400, 20 µm

Semi-Quantitative Analysis

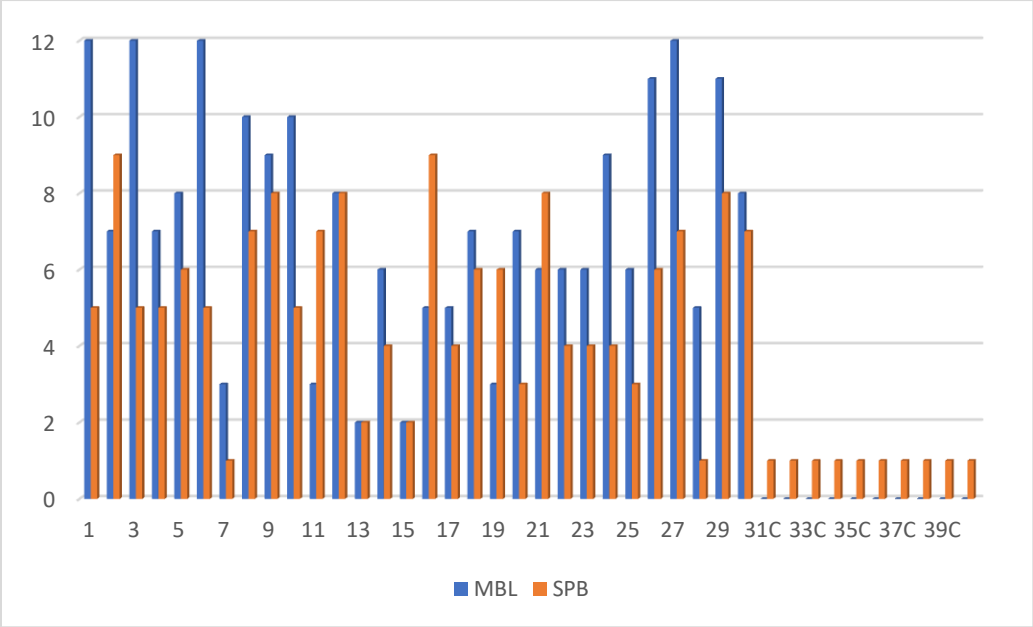
The semi-quantitative analysis results of pathological lesions in the tissues were summed from left to right and top to bottom. The scores were calculated horizontally (TLS) with values above the arithmetic mean marked as high and those below marked as low. Based on this, total lesion scores ≥ 13,7 was considered as severe while lower scores were considered as mild. For immunohistochemical data, total lesion scores of MBL were ≥ 7,26 and SPB ≥ 5,3 were considered as severe while lower scores were considered as mild. Lesions and immunoreactivity values are presented in Figure 3 and 4.

Figure 3. Indicate the severity of lesions in lung tissues (TLS).



Horyzontal plane: Case number, Vertical plane: Severity of lesions, C: Control group

Figure 4. Values of MBL and SPB expressions in lung tissues.



Horyzontal plane: Case number, Vertical plane: Severity of lesions, C: Control group

Semi-quantitative analyse shows that the animals with the highest disease severity were typically around three months old and had possible underdeveloped immune systems. Most of these animals were small ruminants, but there was no significant correlation with sex. Another notable finding was that severe infections occurred predominantly in small ruminants.

When the immunohistochemical reactions were analysed using semi-quantitative methods, it was observed that the regions with the most intense positivity for Anti-MBL were, in order, alveolar lumens, interstitium, and bronchial/bronchiolar lumens, whereas Anti-SP-B protein showed the following order: alveolar lumens, bronchial/bronchiolar lumens, syncytial cell formations, and interstitium.

Statistical Analysis

In order to determine the relationship between Mannose-Binding Lectin (MBL) and Surfactant Protein B (SP-B) levels in animals diagnosed with pneumonia, a Pearson correlation analysis was performed. Correlation coefficients (r) were interpreted according to Mukaka (2012): 0–0.3 (insignificant), 0.3–0.5 (low), 0.5–0.7 (moderate), 0.7–0.9 (high), and 0.9–1.0 (very high). Prior to the analysis, the Shapiro–Wilk normality test was conducted to confirm that the data were normally distributed. Subsequently, a paired sample t-test was used to compare MBL and SPB levels between the control group and pneumonic animals. The statistical method was determined based on the study of Dr. Selvi (2024). All statistical analyses were performed using Jamovi (Version 2.6; The jamovi project, 2024) and R software (Version 4.4; R Core Team, 2024).

Pearson correlation analysis revealed a significant positive correlation between MBL and SPB ($r = 0.385$, $p < 0.05$), suggesting that higher MBL levels were associated with higher SPB levels in pneumonic animals.

Furthermore, when comparing the control and pneumonic groups, both MBL and SPB levels differed significantly ($p < 0.001$), indicating that pneumonia was associated with elevated levels of these biomarkers. The detailed results of the paired sample t-test are presented in Table 2.

Table 2. Paired T test results

		p	**Mean**	**SE**
SPB	SPB Control	<.001	4.60	0.686
MBL	MBL Control	<.001	9.00	0.907

SE: Standart error

DISCUSSION

Parainfluenza 3 (PI3) infections, which play a significant role in viral pneumonias, have been extensively studied in research on respiratory diseases. Numerous studies conducted in Türkiye have investigated the seroprevalence of the disease and reported a notably high prevalence within respiratory system diseases in farm animals (Alpay et al., 2014; Avcı et al., 2014; Ataseven et al., 2010; Yavru et al., 2005; Yesilbag and Gunlukgor, 2008). While analyses conducted using blood or nasal swabs provide some insights, it is known that not every PI3 virus entering the body causes infection, making it difficult to reach definitive conclusions regarding the accuracy of the results (Alpay et al., 2014; Avcı et al., 2014; Yavru et al., 2005). In this study, 30 lung tissue samples from ruminants diagnosed with pneumonia of suspected viral origin, archived in the Department of Pathology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, between 2017 and 2020, were examined using immunohistochemical methods. PI3 positivity was identified in 15 of these samples (50%), providing limited but valuable insights into the significance of the virus among viral pneumonias in the region.

The pathological changes in tissue sections were classified into acute destructive and chronic proliferative phases. Among the cases examined, 26 were found to be in the acute destructive phase, while 4 were in the chronic proliferative phase. This observation revealed that the majority of deaths caused by respiratory failure associated with PI3 infection occurred during the acute destructive phase, which progressed severely and ultimately resulted in death.

The severity and distribution of immunohistochemical anti-PI3 reactions in tissues have been previously described by Ceribası et al. (2014). In this study, intense immunopositive areas were observed on the luminal surfaces of bronchial, bronchiolar, and alveolar epithelia, as well as in cellular debris and inflammatory cells within the lumen. Moderate immunopositive reactions were also detected in the interstitium, both freely and within the cytoplasm of macrophages. These findings were consistent with those reported by Ceribası et al. (2014).

MBL (Mannose-Binding Lectin) is a polysaccharide-based protein expressed by the liver during inflammation (Eddie et al, 2009; Erken, 2013; Garcia-Laorden et al, 2008; Gunesaçar et al, 2011; Jack et al, 2001). Recent studies have investigated its efficacy against infectious agents, including bacteria, viruses and fungi, via the lectin pathway. The primary function of MBL is to bind to infectious agents, facilitating their recognition by inflammatory cells. This supports the activation of the complement system and enhances cellular and humoral responses via the lectin pathway (Degn et al, 2007; Fujita et al, 2004; Mu et al, 2019; Worthley et al, 2005). In this study, the intensity of MBL in areas of anti-PI3 positivity in ruminant lungs during the acute pneumonia phase highlights the role of MBL in acute inflammation. In contrast, the mild immunopositive areas observed during the chronic proliferative phase suggest that MBL secretion decreases proportionally with the severity of the inflammation.

Surfactant protein B (SP-B), expressed by type II pneumocytes, is a component of surfactant that forms a film layer in the alveolar lumen. This layer acts as a barrier to pathogens and prevents alveolar collapse during respiration (Mulugeta et al, 2006; Whitsett et al, 2002). SP-B exists in two forms: immature and mature. Immature forms are expressed during the foetal period and mature postnatally. Deficiency of immature or proSP-B proteins can lead to a condition known as respiratory distress syndrome (Hazıroglu et al., 1998; Whitsett et al., 2002). Type II pneumocytes are regenerative cells that secrete mature SP-B proteins. Therefore, in the acute destructive phase, anti-SP-B immunopositive responses are mild to moderate, whereas in the chronic regenerative phase, they are enhanced due to repair efforts in remaining tissue (Ozyıldız et al., 2017; Whitsett et al., 2002). In this study, mild to moderate immunopositive reactions were observed in bronchial, bronchiolar and alveolar lumens and epithelia during the acute destructive phase. In the chronic proliferative phase, more intense positivity was observed in intact alveolar epithelia and lumens, consistent with the findings of Ozyıldız et al. (2017).

Previous studies suggest that SP-B is not directly associated with the induction of inflammation, which is why it does not exhibit high expression during acute inflammation (Mulugeta et al., 2006; Ozyıldız et al., 2017; Whitsett et al., 2002). However, in this study, the anti-SP-B reaction was found to be intense in alveolar lumens, while relatively less pronounced in other areas. This may be attributed to the phagocytosis of damaged surfactant by inflammatory cells during acute inflammation. Therefore, it appears to be more related to the removal of damaged tissues than to the induction of inflammation. Additionally, the reduced positivity in interalveolar septa compared to the lumens may be due to the partial expulsion of the damaged film layer through coughing or its absorption via lymphatic vessels.

When comparing the expression levels of MBL and SP-B proteins by semi-quantitative analysis, a positive correlation was identified. However, MBL secretion increased in acute events and decreased in chronic events, whereas SP-B protein secretion was low in acute events but high in chronic events. This finding is in agreement with the study by Ozyıldız et al. (2017), which suggests that SP-B protein secretion reflects the body's ongoing repair efforts during chronic events.

Ethical Statement

This study" Examination of Lung Lesions in Parainfluenza-3 Infections in Ruminants with Pathological and Immunohistochemical Methods" was conducted in compliance with all relevant ethical standards. Since this research did not involve any live animal subjects, an ethics committee approval was not required. The data used in this study were obtained from archived paraffin blocks, ensuring no harm or distress to any live animals during the course of this research.

Any version of this article (whether developed or partially modified) has not been presented orally at any symposium, nor has it been published as a full text or abstract.

Ethics Committee Approval

Ethics Committee Approval is not necessary. The data used in this study were obtained from archived paraffin blocks, ensuring no harm or distress to any live animals during the course of this research.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) – Author 2 (%50)
Data Collection (CRediT 2) Author 1 (%60) – Author 2 (%40)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%60) – Author 2 (%40)
Writing the Article (CRediT 12-13) Author 1 (%50) – Author 2 (%20) – Author 3 (%30)
Revision and Improvement of the Text (CRediT 14) Author 1 (%50) – Author 2 (%20) – Author 3 (%30)

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Conflict of Interest

Authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being
12 Responsible Consumption and Production

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Determination of *Toxoplasma gondii* in Beef, Chicken Meat and Offal Sold in Kayseri, Türkiye

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ABSTRACT

Toxoplasma gondii is a globally distributed protozoan capable of infecting humans as well as numerous warm-blooded hosts, such as poultry, and livestock. One of the primary ways it is transmitted to humans is through the consumption of raw or insufficiently cooked meat containing the parasite's tissue cysts. This study investigated the existence of *T. gondii* DNA in retail beef, chicken meat and offal sold in Kayseri, Türkiye. A total of 100 samples were collected from local butchers and delicatessens, comprising 25 samples each of chicken meat, chicken liver, cubed beef and beef liver. Genomic DNA was extracted from these samples and analyzed using TaqMan-based real-time PCR targeting the B1 gene, which is a highly conserved and sensitive marker for *T. gondii*. All samples tested negative, with no DNA amplification observed within the assay's threshold (Ct ≤ 35). These results indicate a low level of, or absence of, *T. gondii* contamination in the meat products tested in this region during the sampling period. However, when interpreting the results, factors such as limited sample size, seasonal variation, regional animal husbandry practices, and low parasitic load must be considered. Additionally, the heterogeneous distribution of tissue cysts and the possible degradation of parasitic DNA may influence detection. Given the public health significance of toxoplasmosis, further studies involving larger sample sizes, diverse geographical locations and complementary diagnostic techniques are recommended. Continuous surveillance, the implementation of good agricultural and hygienic practices, and raising public awareness of proper meat handling, preparation, freezing and cooking are crucial to minimising the risk of *T. gondii* transmission through foodborne pathways.

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INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a foodborne protozoan parasite with a worldwide distribution and an obligatory intracellular lifestyle, exhibiting a broad host range that includes humans, domestic animals, and birds (FAO/WHO, 2014; Khan et al., 2025; Naeem et al., 2025). The parasite exhibits three infectious forms: tachyzoites (both intracellular and extracellular), bradyzoites that reside within tissue cysts, and sporozoites enclosed in oocysts. (Culbasan et al., 2023; Opsteegh et al., 2024). Cats, the definitive hosts, shed oocysts in their feces, which may contaminate soil, water, and food. These oocysts, once ingested, can infect numerous animal species. Additionally, the parasite invades the muscles and brain of intermediate hosts, and ingestion of these tissue cysts can cause human infection. (Pinto-Ferreira et al., 2019; Almuzaini, 2023; Ahmad et al., 2024; Naeem et al., 2025).

The majority of *T. gondii* infections are asymptomatic or present with mild influenza-like illness. In contrast, severe complications may occur when the parasite establishes tissue cysts in the brain, ocular structures, or other organs, particularly in pregnant women and immunocompromised individuals. (Wang et al., 2017; Bağcı et al., 2022; Goh et al., 2023). Maternal infection during pregnancy poses a major risk for congenital transmission, whereas reactivation of latent toxoplasmosis can lead to life-threatening disease in patients with HIV, organ transplant recipients, or other immunosuppressed populations (Weiss and Dubey, 2009; Bălălău et al., 2020). According to a World Health Organization (WHO) report, *T. gondii* ranked fourth among 24 important foodborne parasites worldwide and second in Europe (WHO, 2015; Bouwknegt et al., 2018; Opsteegh et al., 2024). Epidemiological data indicate that about one-third of the global population carries serological evidence of *T. gondii* infection (Tenter et al., 2000). Human toxoplasmosis can be transmitted through blood transfusion, organ transplantation, by ingestion via the oral route, or vertical transmission from mother to fetus. Numerous studies have identified foodborne transmission as a significant contributor to human toxoplasmosis (Almería and Dubey, 2021; Marín-García et al., 2022; Culbasan et al., 2023; Opsteegh et al., 2024). Oocysts excreted in feline faeces can contaminate fruit and vegetables, while unpasteurised milk products containing tachyzoites and undercooked meat harbouring tissue cysts or tachyzoites are considered major sources of oral transmission (Marín-García et al., 2022; Culbasan et al., 2023). In livestock, toxoplasmosis results in substantial economic losses due to mortality, abortion, neonatal deaths and decreased production (Ahmed et al., 2014; Culbasan et al., 2023). Food-producing animals pose a potential public health risk due to their ability to harbour varying concentrations of *T. gondii* tissue cysts in their edible tissues (Yang et al., 2024). Although chickens are susceptible to *T. gondii* infection, they generally remain asymptomatic. Viable tissue cysts have been detected in various organs and tissues (eg, brain, heart, and skeletal muscles), representing the primary sites of transmission risk (Opsteegh et al., 2016). The role of cattle in *T. gondii* epidemiology is unclear, but undercooked beef and raw milk are potential sources of transmission (Belluco et al., 2018). From a food safety perspective, beef, chicken and their internal organs are potential risk factors for *T. gondii* transmission (Ducrocq et al., 2021; Symeonidou et al., 2023). The objective of this study was therefore to determine the presence of *T. gondii* in beef, chicken and their internal organs consumed in Kayseri, Türkiye, and to assess the potential risks to public health.

MATERIAL and METHODS

Samples

For this study, samples of chicken meat, chicken liver, cubed beef meat, and beef liver were obtained from various butchers and delicatessens in Kayseri, Türkiye. Between November and December 2021, a total of 100 samples were collected from five different sales points to examine the

presence of *T. gondii*. From each of the 100 samples, five isolates were obtained, resulting in a total of 500 isolates for further analysis. This comprised 25 randomly selected samples each of chicken meat, chicken liver, cubed beef meat, and beef liver. All materials were transported under cold chain conditions and processed in the laboratory on the day of collection.

DNA Extraction

Tissues were minced, pooled, and homogenized in 90 mL distilled water using a Stomacher Lab Blender for 2 min. A 10 mL aliquot was centrifuged at 5,400 rpm for 20 min at 4 °C, and the resulting pellet was further centrifuged at 12,000 rpm for 3 min. Pellets were rinsed sequentially with 200 µL of buffer (1 mM EDTA, 10 mM Tris-HCl) and 300 µL of 0.5 M EDTA (pH 8.0), followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The final pellet was resuspended in 200 µL PBS and mechanically disrupted with 0.5 mm glass beads in a TissueLyser LT for 5 min. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the supplier's instructions. DNA quality was verified on a 1% agarose gel, and concentration was quantified with the Qubit dsDNA HS Assay Kit and Qubit Fluorometer 3.0 (Thermo Fisher Scientific, USA). Genomic DNA was stored at −20 °C until qPCR.

Real-Time PCR Analysis

Detection of *T. gondii* DNA was performed according to the procedure previously described by Culbasan et al. (2023). Real-time PCR analyses were performed on a CFX96 Touch Detection System (Bio-Rad, USA) using a TaqMan probe (carrying a 6-FAM reporter dye at the 5' end and a BHQ-1 quencher at the 3' end) assay designed to amplify a 529 bp region of the B1 gene. gDNA obtained from *T. gondii* tachyzoites was used as a positive control, while nuclease-free water served as a negative control. Each reaction mixture (total volume 25 µL) contained 1x Luminaris Color Probe High ROX qPCR Master Mix, 0.3 µM of each primer, 0.2 µM of the probe, and 2.5 µL (5-10 ng/µL) of template DNA. Samples with a threshold cycle (Ct) value of ≤35 were considered positive. Details of primer sequences and PCR cycle parameters are presented in Table 1.

Table 1. Primer and probe sequences and amplification conditions used for the detection of *T. gondii* by qPCR.

Primer/Probe Name	Sequence (5′–3′)	qPCR Amplification Conditions	Reference
ToxoRE_F	CACAGAAGGGACAGAAGTCGAA	50°C 2 min 95°C 10 min 95°C 15 s 60°C 30 s 72°C 30 s (40 cycle)	Kasper et al., 2009
ToxoRE_R	CAGTCCTGATATCTCTCCTCCAAGA		
TaqMan Probe	6-FAM-CTACAGACGCGATGCC-BHQ1		

Statistical analysis

The prevalence of *T. gondii* DNA among the analyzed samples was assessed using the chi-squared test implemented in PAST software (version 4.03). A significance level of $p \leq 0.05$ was adopted, corresponding to a 95% confidence interval. Descriptive statistics, including frequency distributions and percentage values, were employed to summarize the findings.

RESULTS

This study involved collecting a total of 500 tissue samples from 100 specimens, including chicken meat (n = 25), chicken liver (n = 25), cubed meat (n = 25) and beef liver (n = 25). The samples were analyzed using real-time PCR targeting the B1 gene of *T. gondii*, which is commonly used due to its high sensitivity and specificity. All samples yielded negative results for *T. gondii* as their threshold (Ct) values were above 35, indicating an absence of detectable parasitic DNA within the limits of sensitivity of the assay (Figure). As all of the samples tested negative for *T. gondii* DNA, it was not possible to conduct a statistical analysis to compare prevalence among the different sample types (Table 2).

Figure. Amplification plot showing *T. gondii* positive control (Ct = 28.85) and no amplification in tested samples

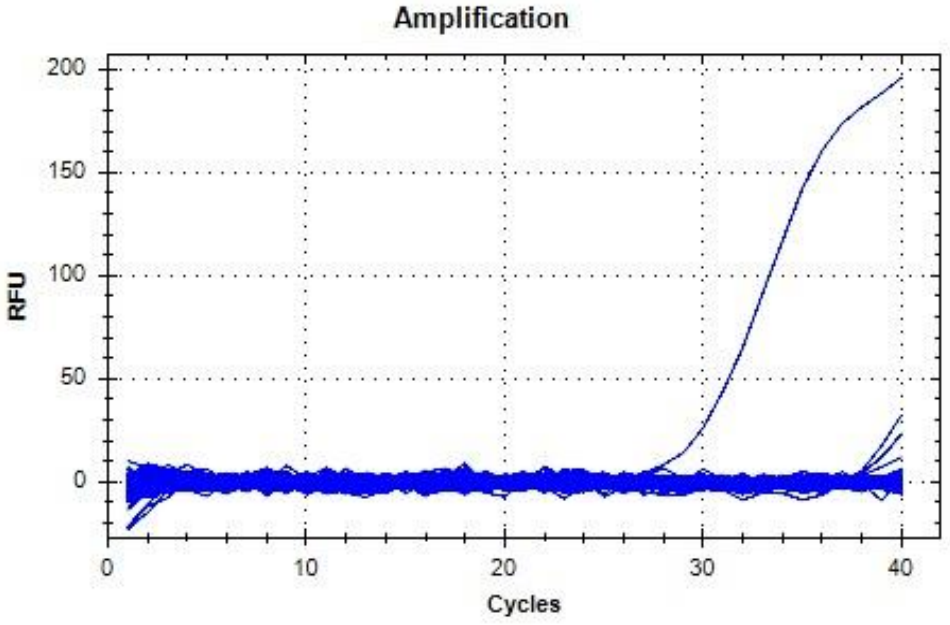

Table 2. Distribution of *T. gondii* in the samples analyzed in the study

Samples	Number of Samples	Positive Samples (n, %)	Negative Samples (n, %)
Chicken Meat	25	0	25 (100%)
Chicken Liver	25	0	25 (100%)
Cubed Beef	25	0	25 (100%)
Beef Liver	25	0	25 (100%)
Total	100	0	100 (100%)

DISCUSSION

The ingestion of raw or inadequately cooked meat continues to be recognized as a primary route of *T. gondii* transmission to humans. Therefore, epidemiological investigations through prevalence studies of food, evaluation of public health risks and development of targeted control measures are essential (Vilela et al., 2024). The prevalence of *T. gondii* in poultry changes significantly, linked to factors such as the serological techniques used, geographic location, sample size, animal species, and farming practices, particularly hygiene standards. In this study, qPCR analysis targeting the B1 gene revealed no detectable *T. gondii* DNA in any of the examined poultry or beef tissues, suggesting an absence of the parasite.

Although cattle are a major source of meat globally, the clinical signs of toxoplasmosis in this species tend to be mild and are less well recognized than in other livestock (Shariatzadeh et al., 2023). Tissue cysts are more susceptible to environmental conditions than oocysts; nevertheless, they can stay infectious for up to three weeks at standard refrigeration temperatures (1–4 °C), aligning with the typical shelf life of fresh meat. DNA of *T. gondii* has been identified in a range of meat products, including raw or smoked sausages, cured bacon, ham, and minced meat (dos Santos Silva et al., 2020; Marín-García et al., 2022). Previous studies on the detection of *T. gondii* in beef samples have reported prevalence rates ranging from 0.6% to 19.3% (Amdouni et al., 2017; Iqbal et al., 2018; Lafrance-Girard et al., 2018; Mahami-Oskouei et al., 2017; Rahdar et al., 2012; Mazuz et al., 2023). However, similar to our findings, Plaza et al. (2020) reported a prevalence of 0%. According to Burrells et al. (2018), the lower prevalence of *T. gondii* in cattle samples compared to chicken samples and its absence in our study may be due to cattle's greater biological resistance.

Chicken meat is widely consumed because it is inexpensive and quick to produce. As an alternative to red meat, consumers are turning to poultry, which is lower in fat and more affordable, to meet their protein needs (Yesilova, 2024). However, poor hygiene during production can result in poor poultry performance and meat quality (de Aquino et al., 2020; da Silva et al., 2024). Chickens are considered important intermediate hosts in the epidemiology of *T. gondii* due to their susceptibility to infection, ground-feeding behavior and access to outdoor environments (Chikweto et al., 2011; Rodrigues et al., 2019; Dubey et al., 2020). In our study, no *T. gondii* DNA was detected in chicken meat or offal samples. However, global studies report vast diversity in the prevalence of *T. gondii* in chicken meat (Mahami-Oskouei et al., 2017; Iqbal et al., 2018; Salinas et al., 2021; Zrelli et al., 2022a; 2022b). Meat from free-range chickens and pasture-raised livestock was found to pose a higher risk of *T. gondii* exposure than meat from captive-raised cattle and cage-raised chickens. These results indicate that rearing conditions and environmental exposure significantly impact the contamination of meat products with *T. gondii*. Free-range chickens and pasture-raised pigs are at a higher risk of infection due to their increased likelihood of meeting *T. gondii* oocysts in the environment (Guo et al., 2015). The absence of *T. gondii* DNA in all samples may be due to the animals being raised under closed or intensive farming conditions, which limits exposure to oocysts. Additionally, the discrepancy in prevalence observed in our study compared to previous findings may be attributed to variations in the quantity of oocysts shed by infected cats into the environment, differential susceptibility among breeds, distinct rearing conditions and climatic factors. Furthermore, *T. gondii* cysts are susceptible to low temperatures, with viability decreasing significantly below -12°C (Gencay et al., 2013). Furthermore, the absence of *T. gondii* detection in this study may be due to the small number of *T. gondii* tissue cysts and their uneven distribution within muscle tissue. To minimise this limitation, we pooled five different meat tissues per animal to increase the effective sample size, as suggested by Opsteegh et al. (2020). Despite this approach, no *T. gondii* B1 gene was detected in any of the pooled samples, suggesting a very low parasite burden or absence of infection.

CONCLUSION

Edible tissues of food animals may harbor latent *T. gondii* cysts, which represent an important reservoir for human infection, even though the animals themselves often show no clinical signs. In conclusion, the study's sampling does not encompass all meat products from naturally infected animals in Türkiye, highlighting the need for nationwide research to assess the presence and viability of *T. gondii* in dairy and meat, as well as to estimate the occurrence of foodborne toxoplasmosis in humans. To reduce the risk of infection, (i) undercooked meat and meat products should either be avoided or properly frozen, (ii) good agricultural and husbandry practices should be implemented at farm level, and (iii) health authorities should develop and enforce effective training programs focused on controlling and preventing *T. gondii* transmission.

Ethical Statement

This study has not been presented or published anywhere else before.

Ethics Committee Approval

This study does not require ethics committee approval.

Author Contributions

Research Design (CRediT 1) Author 2 (%40) – Author 6 (%60)
Data Collection (CRediT 2) Author 1 (%25) – Author 3 (%25) – Author 4 (%25) – Author 5 (%25)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%25) – Author 2 (%25) – Author 3 (%20) – Author 4 (%15) – Author 5 (%15)
Writing the Article (CRediT 12-13) Author 2 (%55) – Author 3 (%30) –Author 6 (%25)
Revision and Improvement of the Text (CRediT 14) Author 1 (%35) – Author 2 (%35) –Author 5 (%30)

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Conflict of Interest

The authors declare that there is no conflict of interest of interest associated with this manuscript.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being
12 Responsible Consumption and Production

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The Effect of Wharton's Jelly Mesenchymal Stem Cell Application on Haematological Parameters in Cows with Subclinical Mastitis

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ABSTRACT

This study aimed to investigate the effect of intramammary Wharton's Jelly mesenchymal stem cell (WJ-MSC) administration on haematological parameters in cows with subclinical mastitis. In the study, 20 Holstein cows kept under equal care and feeding conditions during the same lactation period were used. The California Mastitis Test (CMT) was applied to identify animals with subclinical mastitis. Animals were divided into 4 groups: control (n=4, 3 ml DMEM solution, intramammary, days 2 and 9), Wharton's Jelly mesenchymal stem cells (n=4, WJ-MSCs, 2.5 x 10^7 WJ-MSCs, intramammary, days 2 and 9), subclinical mastitis (n=6, SM, parenteral antibiotic treatment, days 2 and 9), and SM+WJ-MSCs (n=6, 2.5 x 10^7 WJ-MSCs, days 2 and 9). On the 1st and 30th days of the study, blood samples were taken directly from the jugular vein under aseptic conditions into EDTA tubes containing anticoagulant for the evaluation of haematological parameters. Parameters related to infection and inflammation were evaluated in blood count analyses. It was found that the reference ranges for the parameters leukocyte (WBC), erythrocyte (RBC), haemoglobin (HGB), haematocrit (HCT), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil percentage Neu (%), and lymphocyte percentage (Lym%) varied. The WBC value, which was above the reference range on day 1, was measured within the reference range on day 30. It was found that the Neu% value, which was above the reference value in the Day 1 measurement, and the Lym% value, which was below the reference value, were within the reference values in the post-treatment measurement. The results obtained indicate that the application of WJ-MSCs has no negative effect on haematological parameters and may even play a balancing role in the inflammatory response for some parameters due to its immunomodulatory effects.

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INTRODUCTION

Subclinical mastitis (SM) is one of the most important and common infectious diseases of the udder. Infection and an inflammatory reaction are present in the mammary gland tissue, however no clinical symptoms are observed (Ghai et al., 2022). Because it does not cause macroscopic changes in mammary tissue or milk, the disease progresses silently, making it difficult to implement treatment and control programs and facilitating the spread of infection within the herd (Hillerton and Berry, 2005; Kumari et al., 2018; Zeweld and Tarekegn, 2025). SM causes both direct and indirect losses, including reduced milk yield and altered composition, recurrent cases of mastitis, animal loses, and treatment costs. In dairy farming, maintaining animal health and high milk yield is one of the most fundamental elements ensuring the sustainability of profitability. Therefore, SM poses a globally critical problem not only in terms of animal health but also in terms of the efficiency of the dairy industry, the quality of dairy products, and food safety (Gonçalves et al., 2018; Gonçalves et al., 2020; Sarı et al., 2025).

Early and accurate diagnosis of SM is of great importance in minimising its negative effects. In the diagnosis of mastitis, somatic cell count (SCC) and pathogen isolation are considered the gold standard (Hristov et al., 2018). While direct cell counting can be used for SCC determination, cell presence can also be determined using indirect methods (Carvalho-Sombra et al., 2021). The California Mastitis Test (CMT), a semi-quantitative and indirect method for determining SCC, detect the presence of neutrophil granulocytes, mononuclear cells, and mammary epithelial cells in milk (Sadat et al., 2023). CMT offers a significant advantage in detecting SCC because it is a quick, inexpensive, and easy test using a four-compartment plastic container and CMT reagent (Zeweld and Tarekegn, 2025).

Wharton's Jelly mesenchymal stem cells (WJ-MSCs) are obtained from connective tissue located between the vessels of the umbilical cord and the amniotic epithelial layer (Cardoso et al., 2012; Lange-Consiglio et al., 2017). Fibroblast-like cells located in this region are primitive mesenchymal stem cells that migrated through the umbilical cord during embryogenesis (Taghizadeh et al., 2011; Wang et al., 2008). Additionally, WJ-MSCs stand out as a promising cell source for preclinical and clinical applications due to their potential to differentiate into various cell types (endothelial, cardiomyocyte, neurone, osteoblast, etc.) (Kim et al., 2013; Obtulowicz et al., 2016). The most important characteristics of WJ-MSCs include low immunogenicity, high proliferative capacity, immunomodulatory and anti-inflammatory properties. The low immunogenicity feature is characterized by low (MHC Class I) or no (MHC Class II) expression of the Major Histocompatibility Complex (MHC) on the cell surface (Cardoso et al., 2012; Ma et al., 2005). Given these advantageous properties, particularly their low immunogenicity and anti-inflammatory potential, the present study aims to evaluate the systemic safety profile of intramammary WJ-MSC administration in cows with subclinical mastitis by assessing peripheral haemogram parameters, in comparison with standard antibiotic therapy.

MATERIAL and METHODS

Animals and Experimental Design

This study was approved by the Selçuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Centre Ethics Committee (SÜVDAMEK) with Local Animal Ethics Committee Decision No. 2024/128 dated 05.09.2024. The 20 raw milk samples from dairy cows and breeding producers used in the study were selected from Holstein cows in the same lactation period

(second-lactation), kept under equal care and feeding conditions at the Atasancak Acıpayam Agricultural Enterprise, and not treated with mastitis vaccine or antibiotics, by performing the CMT (KerbaTest, Ref. No: 1514, Germany). CMT reaction results were scored as 0 (-), 1 (weak positive, ±), 2 (moderate positive, ++), 3 (strong positive, +++) and 4 (highly positive, ++++). The control and WJ-MSC groups were composed of animals that were CMT-negative for at least 3 consecutive tests, while the SM and SM+WJ-MSC groups were composed of animals that were CMT-positive for at least 3 consecutive tests. The selected cattle were divided into four groups: the control group (n:4), the WJ-MSC group (n:4), the SM group (n:6), and the SM+WJ-MSC group (n:6). The experimental study lasted 30 days, and the applications were performed on the 2nd and 9th days of the study (7 days apart, 2 applications). The control group received a 3 ml solution of DMEM (Dulbecco's Modified Minimal Essential Medium) applied to a randomly selected mammary quarter. The WJ-MSC suspension, at a concentration of 2.5 x 10^7 cells in 3 ml of DMEM solution, was administered to the animals in the WJ-MSC group into a randomly selected mammary quarter. In the SM group, 3 ml of DMEM solution was administered to each affected mammary quarter in addition to parenteral antibiotic therapy based on antibiogram test results. In the SM+WJ-MSC group, a suspension of 2.5 x 10^7 WJ-MSCs in 3 ml of DMEM solution was administered to each affected mammary quarter (Hatipoglu et al., 2025). On the 1st and 30th days of the study, venous blood samples were taken under aseptic conditions directly from the jugular vein into EDTA tubes containing anticoagulant for a hemogram analysis. These samples were then transported under cold chain to the laboratory where the analysis would be performed.

Complete Blood Cell Analysis

From blood samples, the levels of leukocyte (WBC), erythrocyte (RBC), haemoglobin (HGB), haematocrit (HCT), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil percentage Neu(%), and lymphocyte percentage (Lym%) counts were analysed using an automated blood cell counter (Abbott Cell Dyn 1800 Haematology Analyser, Germany).

Statistical Analysis

The numerical values obtained from the haematological analysis results of venous blood samples taken on days 1 and 30 of the study were analysed GraphPad Prism (Version 10.6.1, GraphPad Software, San Diego, CA, USA). Since the study involved repeated measures at two time points (Day 1 and Day 30) on the same animals, the primary analytical approach was determined as the Linear Mixed Model (LMM). For each haematological parameter, the model included Group (Control, WJ-MSCs, SM+Antibiotic, SM+WJ-MSCs), Time (Day 1, Day 30), and the Group × Time interaction as fixed effects. To account for between-subject variance and the repeated nature of the data, Animal ID was modelled as a random effect (random intercept). The Restricted Maximum Likelihood (REML) method was used for model estimation. Model assumptions were evaluated by examining the residuals rather than raw data. Normality of residuals was checked using Shapiro–Wilk tests and Q–Q plots, while homoscedasticity was assessed via residual-vs-predicted plots. As the residuals met the assumptions of normality and homoscedasticity, standard LMMs (Gaussian distribution) were applied. The significance level was set at $\alpha = 0.05$. Omnibus F-tests were reported for the general significance of fixed effects. In the presence of a significant interaction, 'simple effects' analyses were conducted to test Day 1 vs. Day 30 differences within each group, with family-wise error rate control provided by the Šidák/Bonferroni correction. If the interaction was not significant, interpretation focused on the main effects. Post-hoc tests (e.g., Duncan) were strictly avoided when the omnibus test was not significant. Descriptive statistics are presented as mean ± standard error (SE). To

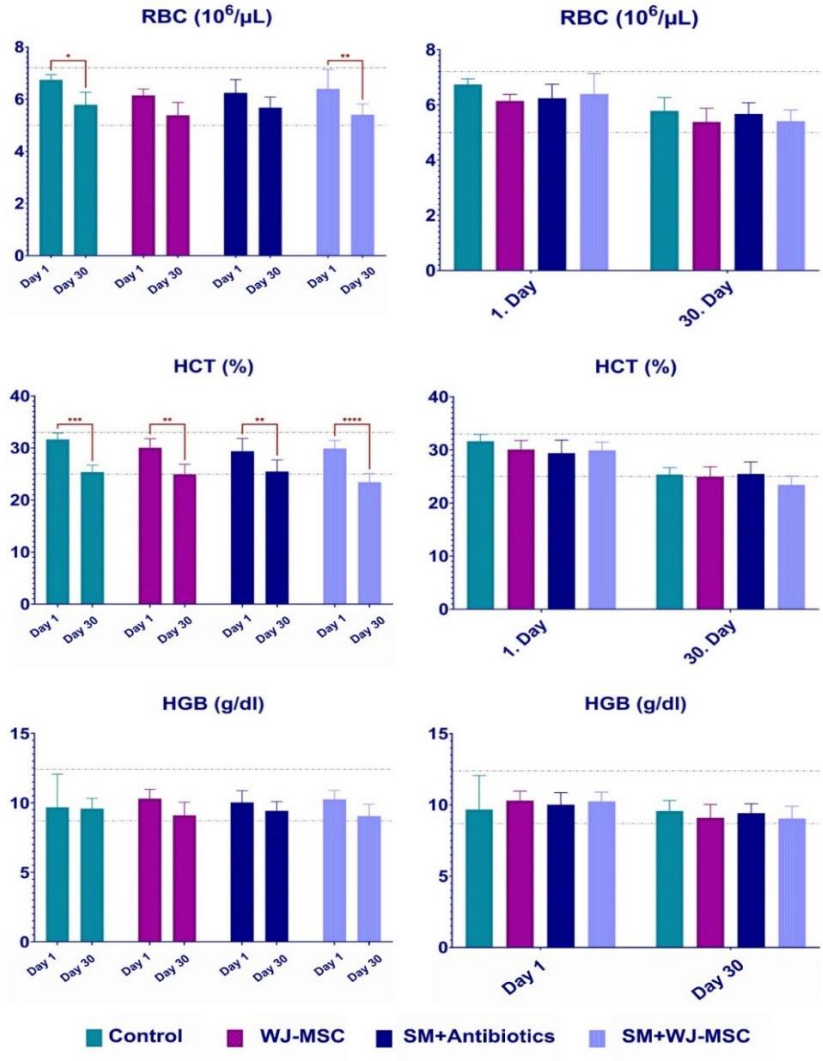
facilitate visual understanding of the time effect and potential interactions, line graphs (Mean ± SE) showing group means at each time point were generated. Effect size classifications were reported only for statistically significant effects to ensure robust interpretation.

RESULTS

The Linear Mixed Model (LMM) analysis revealed that the "Time" factor was the predominant determinant for the variations in haematological parameters. In most cases, the fixed effect of "Group" and the Group × Time interaction were not statistically significant (P>0.05). Furthermore, pairwise comparisons between experimental groups at either Day 1 or Day 30 yielded no significant differences after Bonferroni correction (P > 0.05). Consequently, the results are interpreted primarily based on temporal changes within groups, as illustrated in the figures.

A significant Time × Group interaction was observed for HCT (P = 0.0232), accompanied by a robust main effect of Time (F (1, 16) = 304.5, P <0.0001). Post-hoc analyses demonstrated a significant decrease in HCT levels across all experimental groups from Day 1 to Day 30 (Control: P = 0.0002; WJ-MSCs: P = 0.0019; SM+Antibiotic: P = 0.0037; SM+WJ-MSCs: P <0.0001) (Figure 1).

Figure 1. Evaluation of erythroid parameters before (Day 1) and after (Day 30) treatment.

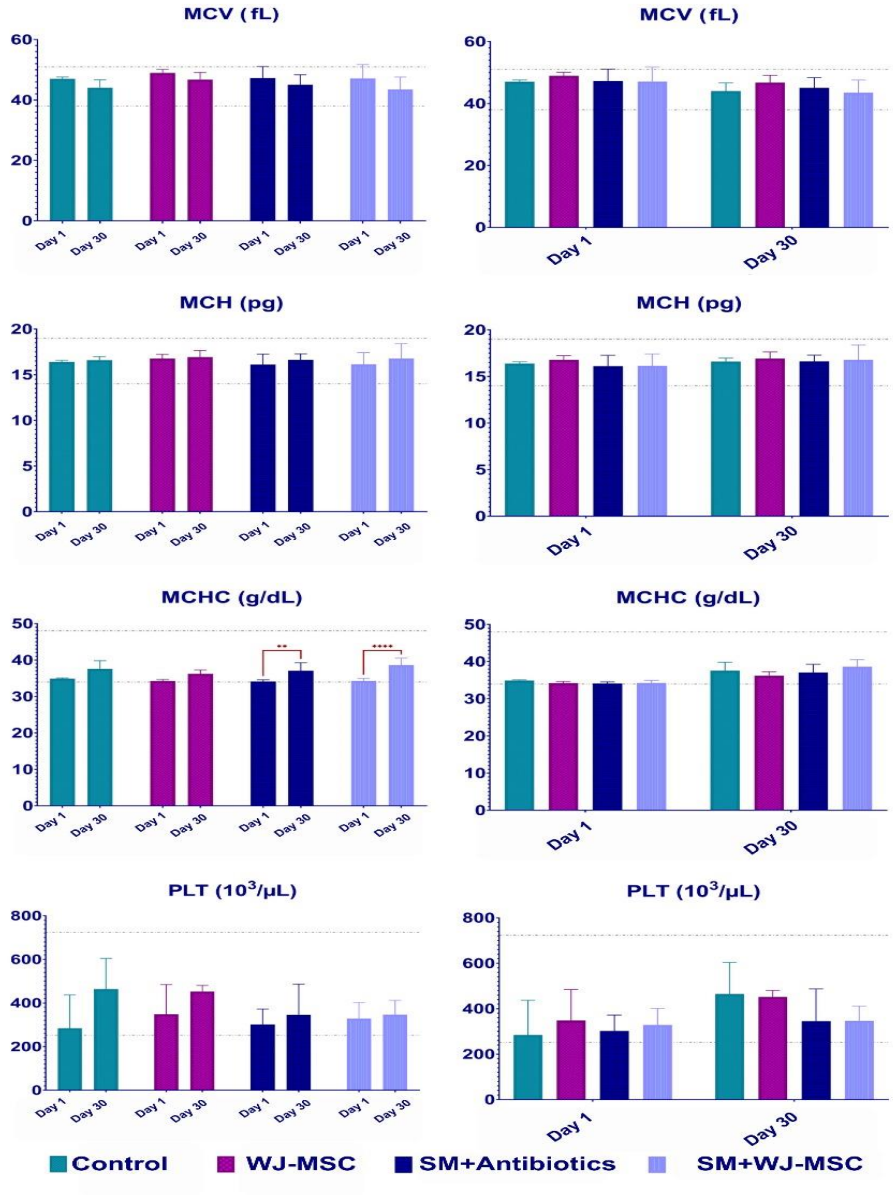


The left panels allow for direct within-group comparisons over time, and the right panels provide an overview of groups at each time point. Red brackets mark significant decreases in parameters on Day 30 compared to Day 1 (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001). A significant Time × Group interaction was found for HCT (P = 0.0232). Gray dashed lines represent the physiological reference intervals.

Similarly, RBC counts showed a significant main effect of Time ($F(1, 16) = 90.16$, $P < 0.0001$), with notable inter-individual variability ($P = 0.0007$ for subjects). Bonferroni-corrected within-group comparisons indicated significant reductions in RBC levels on Day 30 compared to Day 1 specifically in the Control ($P = 0.0345$) and SM+WJ-MSCs ($P = 0.0051$) groups, while other groups showed no significant pairwise changes (Figure 1).

Regarding MCHC, a significant main effect of Time was found ($P < 0.0001$). Within-group analyses revealed a significant increase in MCHC levels on Day 30 in the SM+Antibiotic ($P = 0.0052$) and SM+WJ-MSCs ($P < 0.0001$) groups (Figure 2). Although HGB levels showed a statistically significant main effect of time ($F (1, 16) = 6.601$, $P = 0.0206$), individual within-group comparisons did not reach statistical significance after correction ($P > 0.05$) (Figure 1).

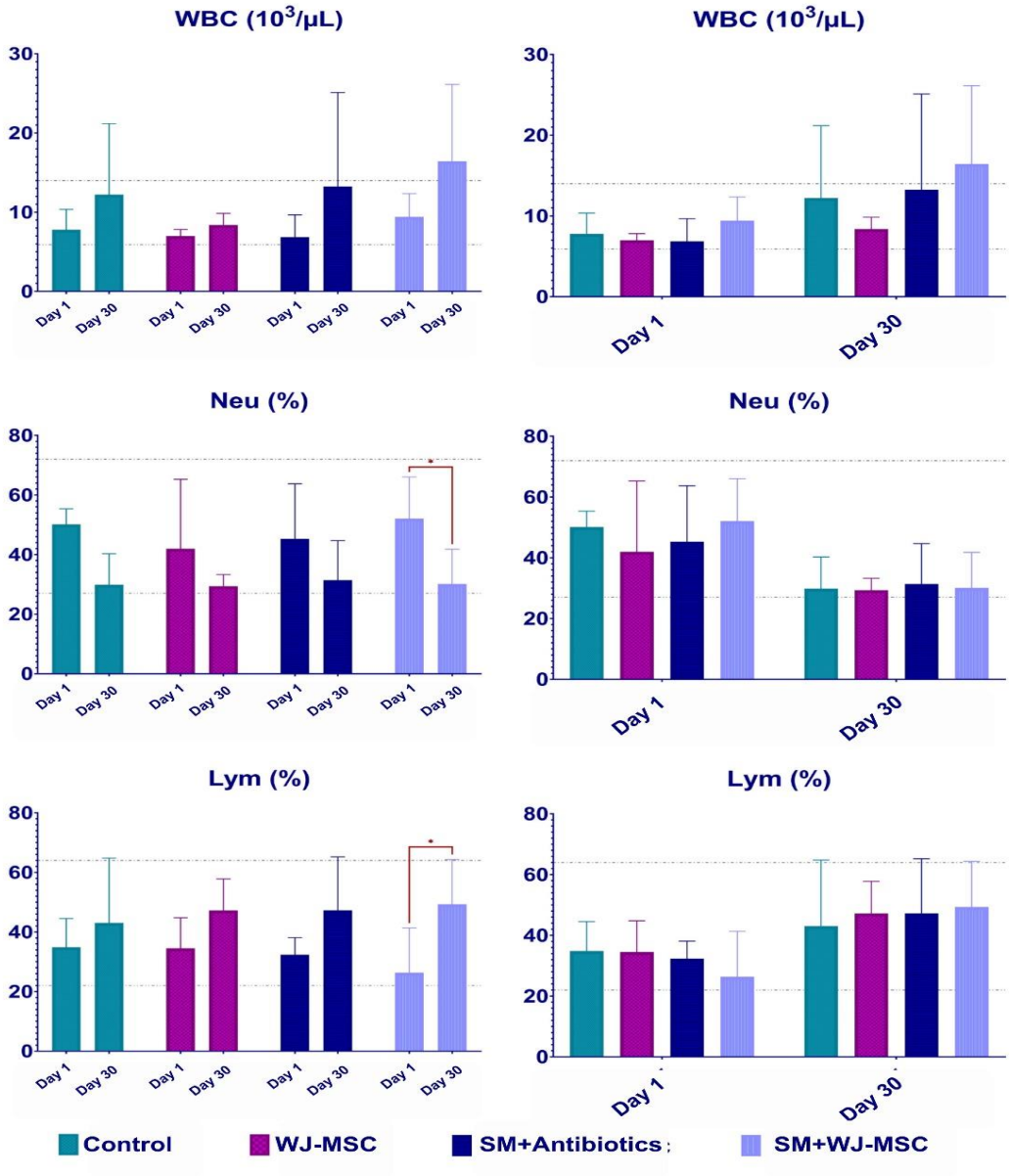
Figure 2. Comparative analysis of erythrocyte indices and platelet counts.



The left panels show changes from Day 1 to Day 30 within groups, and the right panels show comparisons between groups. Significant increases in MCHC levels on Day 30 are marked with red brackets (** $P < 0.01$, **** $P < 0.0001$). Other parameters did not show significant pairwise changes within groups after Bonferroni correction, although a general time effect was present. Gray dashed lines indicate the standard reference intervals.

For Neu%, a significant main effect of Time was detected (F (1, 16) = 15.47, P = 0.0012). Pairwise comparisons showed a significant decrease in Neu% solely in the SM+ WJ-MSCs group on Day 30 (P = 0.0424) (Figure 3). Conversely, Lym% exhibited a significant main effect of Time (F (1, 16) = 10.75, P = 0.0047), with a corresponding significant increase observed exclusively in the SM+ WJ-MSCs group (P = 0.0328) (Figure 3). No significant Group or Interaction effects were found for these parameters (Figure 3).

Figure 3. Temporal changes in leukocyte parameters across study groups.



The left panels display the comparison of Day 1 vs. Day 30 for each group side-by-side, while the right panels group the data by time points. Data represent Mean ± SE. Red brackets indicate statistically significant within-group differences between Day 1 and Day 30 based on Two-Way RM ANOVA with Bonferroni correction (* P < 0.05). Notably, significant alterations in Neu% and Lym% were observed in the SM+ WJ-MSCs group. Gray dashed lines denote the species-specific reference intervals (lower and upper bounds) obtained from the Cornell University Animal Health Diagnostic Center.

Total WBC counts showed a significant main effect of Time ($F(1, 16) = 6.707$, $P = 0.0197$); however, neither the Time × Group interaction ($P = 0.7235$) nor the Group effect ($P = 0.5434$) was significant. Despite the general time effect, Bonferroni-corrected comparisons did not reveal statistically significant changes within any individual group ($P > 0.05$) (Figure 3).

For other haematological indices (e.g., PLT, MCV, MCH), while isolated main effects of Time were occasionally observed, they did not translate into consistent group differentiation or significant pairwise differences after multiple comparison adjustments. These findings suggest general physiological variations over time rather than treatment-specific effects (Figure 2).

DISCUSSION

Haematological profiling is one of the standard diagnostic methods used to assess the physiological parameters of dairy cattle and diagnose systemic diseases. Although it rarely led to a correct and definitive diagnosis, it provides important information for monitoring diseases and predicting their outcomes (Kara et al., 2024; Roland et al., 2014). The present study was designed to evaluate, in cows with subclinical mastitis, the therapeutic response (efficacy) and systemic safety of intramammary WJ-MSCs administration, in comparison with standard antibiotic therapy, using peripheral haematological indicators. Overall, the dominant determinant of variability across most haematological variables was time, whereas the main effect of group was not significant for most parameters and the Time × Group interaction reached statistical significance only for HCT. Likewise, no meaningful separation between groups was detected at either sampling point (Day 1 or Day 30). Although statistically significant within-group temporal changes were noted for selected variables—particularly Neu% and Lym% in specific groups—these were not interpreted as treatment-specific divergence because the Time × Group interaction was not significant for these parameters. Collectively, these findings indicate that the observed changes primarily reflect within-animal temporal dynamics, and that a consistent, treatment-specific differentiation between antibiotic therapy and WJ-MSCs treatment was not evident. From a clinical and translational standpoint, the absence of systematic between-group differences supports the interpretation that intramammary WJ-MSCs administration did not elicit an overt adverse systemic haematological response under the conditions of this study.

Haematocrit (HCT), value is a fundamental parameter that indicates the percentage of red blood cells in the total blood volume. There is a strong relationship among RBC, HGB, and HCT, and it is observed that their values generally decrease or increase together (Turkson and Ganyo, 2015). In mastitis, particularly in clinical presentations, reductions in RBC, HGB, and HCT have been reported, whereas subclinical mastitis may be accompanied by smaller and more heterogeneous alterations (Lakshmi et al., 2024; Sadat et al., 2023). In the current study, HCT exhibited a pronounced main effect of time together with a statistically significant Time × Group interaction; however, the lack of consistent between-group differences at either Day 1 or Day 30 suggests that this interaction is best interpreted as reflecting differences in the magnitude of temporal change rather than clear separation between treatment groups. Importantly, when the direction and size of change are considered in a clinical context—particularly where values remain within reference intervals—the observed erythrogram shifts are more compatible with time-associated physiological variability (e.g., hydration/plasma volume dynamics, sampling-related factors, lactational adaptation, and handling-related stress) than with a treatment-emergent haematological toxicity signal (Paape et al., 1973; Sobolev et al., 2021).

Among erythrocyte indices, MCV, MCH, and MCHC are parameters that represent the average volume of erythrocytes, the average amount of haemoglobin found in an erythrocyte, and the haemoglobin concentration relative to erythrocyte volume, respectively. These indices are primarily used to characterise anaemia phenotypes; therefore, they are not directly applicable for defining mastitis per se (Mordak et al., 2024). Previous studies in sheep with CM or SM indicate that MCV, MCH and MCHC may fluctuate without consistent, statistically significant differences compared to healthy animals (Ah, 2016; AL-Hadithy and Suleiman, 2014; Etim et al., 2014, Mordak et al., 2024, Sarvesha et al., 2017). In the present study, time-related effects were detectable for selected indices and within-group changes were observed for MCHC in some groups; nevertheless, the overall pattern—absence of sustained between-group separation and values remaining within physiologically acceptable limits—supports interpretation as non-specific temporal variation rather than an intervention-related erythrocyte disorder. Accordingly, our haematological data do not suggest that intramammary WJ-MSCs administration induces systemic anaemia, polycythaemia, or clinically meaningful haematological dyscrasia. This interpretation is consistent with reports of perinatal/adult tissue-derived MSC approaches in mastitis models indicating stable haematological parameters within reference intervals following administration (Peralta et al., 2020; Pokorska et al., 2024; Ghai et al., 2022).

Importantly, Peralta et al. (2020) investigated an allogeneic adipose tissue–derived MSCs (AT-MSCs) intramammary therapy in an experimentally induced Staphylococcus aureus CM model and included a conventional antibiotic comparator group. Across sampling days, haematological variables—including erythrocyte and platelet counts and haemoglobin/PCV—remained within reference intervals and did not differ significantly, despite repeated intramammary MSC administrations. Collectively, these findings provide external support that intramammary MSC-based strategies can be evaluated alongside conventional antibiotic therapy while maintaining a reassuring systemic haematological safety profile.

Leukogram variables (WBC, Neu%, Lym%) are practical indicators for assessing systemic inflammatory status and immune cell distribution (Abdel-Hamied et al., 2020; Braun et al., 2021). In SM, systemic leukocyte responses are often limited and variable because inflammation may remain largely localised to mammary tissue, and peripheral blood indices may remain close to baseline depending on pathogen burden, chronicity, and host factors (De and Mukherjee, 2013; Carvalho-Sombra et al., 2021). By contrast, clinical mastitis more frequently produces clearer systemic haematobiochemical perturbations, including more consistent leukocyte alterations (Sarvesha et al., 2017). In the present study, the significant main effect of time for WBC suggests a temporal shift in leukocyte dynamics across the sampling interval; however, the absence of significant within-group Day 1–Day 30 differences after conservative correction implies that this temporal signal was not sufficiently strong and homogeneous within each group. With respect to differential counts, the decrease in Neu% and the increase in Lym% observed over time in the SM+WJ-MSCs group are clinically noteworthy and directionally compatible with a shift of the neutrophil-to-lymphocyte balance toward physiological ranges as inflammatory pressure diminishes (Çetinkaya et al., 2020). Nevertheless, because the Time × Group interaction was not significant for Neu% or Lym%, these changes should not be interpreted as definitive evidence of a treatment-specific immunomodulatory effect attributable to WJ-MSCs. Rather, they are best discussed as time-associated patterns that may accompany changes in subclinical inflammatory activity, a cautious interpretation that is consistent with studies reporting that MSC administration tends to maintain systemic inflammatory indicators within physiological limits rather than inducing overt pathological deviations (Ghai et al., 2022). Indeed, temporal fluctuations in peripheral leukocyte responses after MSC administration have been

described previously: Pokarska et al. (2024) observed an early rise in peripheral WBC and neutrophils within 24 h of BMSC/ADSC administration followed by a systematic decline at later time points (72 h and 7 days) toward baseline levels. Such kinetics support the plausibility of transient immune cell mobilization in the early post-administration period with subsequent return toward homeostasis (Peralta et al., 2020).

Platelets (PLTs) are fragments of megakaryocytes whose most important function is to ensure haemostasis. Most platelets reside in the spleen, with smaller amounts in the liver and bone marrow, and are released into circulation following hormonal stimulation (Benko et al., 2025; Boudreaux and Ebbe, 1998; Russell, 2010). No significant changes in PLT values have been reported in cattle with CM and SM. Thrombocytopenia is more typical of severe mastitis than SM (Hagiwara et al., 2014). In this study, although time-associated variability in PLT was observed, the lack of consistent between-group differentiation and the absence of robust corrected within-group changes do not support strong inferences regarding thrombopoietic or haemostatic effects attributable to WJ-MSCs. Therefore, platelet findings are most appropriately framed as non-specific temporal variation within physiological limits. In line with this view, Ghai et al. (2022) reported that following allogeneic umbilical cord blood-mesenchymal stem cells (UCB-MSC) administration, haemogram parameters—including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT—did not change significantly between sampling days and remained within reference intervals.

CONCLUSION

Today, antibiotic treatment is the most common method for controlling mammary gland infections. Antibiotic use negatively impacts public health and food safety due to the risk of antimicrobial resistance and residues in milk. Therefore, research is being conducted in the field of regenerative medicine to develop alternative treatment methods for bovine mastitis. This study aimed to compare intramammary WJ-MSCs administration with standard antibiotic therapy in cows with subclinical mastitis, to evaluate both therapeutic response and the systemic safety profile based on peripheral haemogram parameters. Overall, time was the primary determinant of variation in haematological measures, and no significant between-group separation was detected at either Day 1 or Day 30. The finding that the Time × Group interaction was significant only for HCT, together with the absence of consistent treatment-specific differentiation in the remaining variables, suggests that neither WJ-MSCs nor antibiotic administration produced a distinct or differential adverse effect on the systemic haemogram; rather, the observed changes largely reflect time-associated physiological dynamics. Nevertheless, to more clearly delineate potential effects and treatment-related biological responses, further studies incorporating additional time points and supported by acute-phase proteins, cytokine profiling, and mammary/milk parameters (e.g., SCC and bacterial load) are warranted.

Ethical Statement

This study is based on the doctoral thesis titled "Investigation of the Therapeutic Effects of Mesenchymal Stem Cells Isolated from Wharton's Jelly in Cows with Subclinical Mastitis Using Pathological and Molecular Methods," which was presented on 15.05.2024 under the supervision of Assoc. Prof. Dr. Mehmet Burak ATEŞ.

Ethics Committee Approval

05/09/2024 dated and numbered 2024/128 was given by Selcuk University, Faculty of Veterinary Medicine Experimental Animal Production and Research Centre Ethics Committee (SÜVDAMEK).

Author Contributions

Research Design (CRediT 1) Author 1 (%10) Author 4 (%10) - Author 5 (%10) - Author 6 (%10) - Author 7 (%10) - Author 8 (%10) - Author 9 (%10) - Author 10 (%10) - Author 11 (%20) -
Data Collection (CRediT 2) Author 1 (%20)- Author 2 (%10) Author 3 (%20)- Author 5 (%25) – Author 11 (%25)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%25)- Author 3 (%20)- Author 5 (%25) – Author 11 (%30)
Writing the Article (CRediT 12-13) Author 1 (%60) – Author 5 (%20) – Author 11 (%20)
Revision and Improvement of the Text (CRediT 14) Author 5 (%40) – Author 11 (%60)

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Conflict of Interest

The authors declare that they have no conflict of interest.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being
12 Responsible Consumption and Production

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Infectious Causes of Abortions and Stillbirths in Horse Breeding

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ABSTRACT

Horse breeding is a fundamental component of economic and social development in many countries. Maintaining high fertility rates and ensuring the birth of healthy foals is crucial for the sustainability of the equine industry. However, both infectious and non-infectious factors contribute significantly to reproductive losses, including miscarriages, stillbirths, and postpartum fetal deaths. Infectious agents such as bacteria, viruses, fungi, and parasites play a significant role, while non-infectious causes include stress, transportation, hormonal imbalances, and uterine torsion. Early and accurate diagnosis of these factors is critical for effective treatment and prevention. In this context, the implementation of comprehensive preventive strategies, such as routine vaccination (e.g., against equine herpesvirus), isolation of pregnant mares, regular veterinary checkups, ongoing monitoring, and appropriate nutritional management, is crucial. This review aims to inform breeders and veterinarians about the underlying causes of miscarriage, infertility, and foal deaths in horses and to emphasize the importance of preventive medicine in reducing these risks.

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INTRODUCTION

Following the process of domestication, the utilization of horses has undergone a series of changes over time. Initially employed as a food source during the paired periods, horses were subsequently raised for use in warfare and as part of the labour force during the following periods. The advent of mechanization and technological development has led to the current practice of breeding horses for recreational and sporting purposes. Horses possessing superior characteristics are selected as breeders, and these horses are subsequently utilized in flat running and obstacle jumping competitions (Paksoy and Ünal, 2019).

To achieve success in the field of equine breeding, it is essential to obtain foal that possess both high endurance and speed ability from mothers and fathers that demonstrate superior yield power. Achieving this objective is contingent upon factors such as an elevated fertility rate, uncomplicated pregnancy processes, and an augmentation in the number of healthy foals born. It is imperative to acknowledge the numerous infectious and non-infectious factors that can influence these processes. Infectious factors include bacteria, fungi, parasites and viruses, while non-infectious factors encompass transport, hormonal disorders, torsion and stress (Li et al., 2024).

This review examines the potential causes of litter failure and infertility problems in horses. It also aims to provide information to breeders and veterinarians for the solution of these problems with a holistic approach.

Pregnancy Process in Horses

Complications arising prior to and following parturition in equines have the potential to result in the termination of the pregnancy process or the failure of the foal to survive post-partum. It is therefore vital to be well-versed in the physiological and behavioural conditions of mares during pregnancy and parturition. The average gestation period in mares is 330-345 days. It is notable that births in mares tend to occur predominantly during nocturnal hours (Paksoy and Güngör, 2024). The birth process in horses is comprised of three distinct stages. In the first stage, the foal assumes the correct position within the uterus. During this period, the mare typically exhibits increased activity, often walking at a faster pace within the barn, and displays frequent defecation behaviour. It is during this stage that restless behaviour is observed, and labour is initiated. The second stage of labour is characterised by the rupture of the foetal membranes, the presence of water, and the eventual occurrence of birth. The final stage of labour is characterised by the continued contraction of the uterine muscles and the expulsion of the membranes (Smith, 2023). It is estimated that the incidence of difficult labour in mares is approximately 10%. As horses are seasonal polyestrous animals, it is imperative to adhere strictly to the vaccination schedule, and mating should be scheduled in accordance with the veterinarian's guidance. In mares where the optimal time for mating has been overlooked, there is a demonstrable decline in fertility, resulting in financial and moral losses in the context of horse breeding (MacMillan and Cockrem, 1986).

Infectious Agents Causing Abortions and Foal Mortality in Horses

The prevalence of equine abortions caused by infectious agents currently ranges from 18.7% to 53.1%. These infectious agents include bacteria, viruses, and pathogenic organisms such as fungi or parasites.

Bacteria

Bacterial infections have been identified as the primary cause of infectious abortions in mares. Moreover, it has been reported that such cases are more prevalent during the latter third of the gestation period. The most prevalent bacterial abortive and genital system agents observed in equines include.

Salmonella enterica subspecies *enterica* serovar abortus equi (*Salmonella abortus equi*), *Taylorella equigenitalis*, *Streptococcus equi* subsp. *zooepidemicus* (*Streptococcus zooepidemicus*), *Staphylococcus aureus, Escherichia coli, Klebsiella spp., Pseudomonas spp., Chlamydia abortus, Leptospira* and *Rhodococcus equi* (Yeni and Balevi, 2023; Yeni et al., 2024). *Streptococcus equi* subsp. *zooepidemicus* is the bacterium most frequently identified in cases of abortion. It is responsible for almost 28% of bacterial causes of abortion (Smith et al., 2003).

With the exception of *Leptospira* spp. and nocardioform infection, most bacterial placentitis in mares are the result of an ascending infection (Tibary and Pearson, 2015). This condition is characterised by early breast development, increased uteroplacental thickness at the level of the cervical star, and mucopurulent vaginal discharge. If left untreated, ascending placentitis can result in fetal death and expulsion (Leon et al., 2023). Bacterial placentitis generally leads to abortion during the sixth to ninth months of pregnancy (Laugier et al., 2011). Treatment of bacterial agents causing abortion includes tocolytic drugs to reduce increased uterine contractions, anti-inflammatory drugs to inhibit cytokine and prostaglandin production, and antimicrobial therapy to control bacterial growth. Antimicrobial therapy should be based on the results of antimicrobial susceptibility testing of bacteria isolated from vaginal discharge or cervical swabs (Tibary and Pearson, 2015).

Viruses

The most prevalent abortive agents reported worldwide are equine herpesvirus type 1 (EHV-1) and the rarer equine herpesvirus type 4 (EHV-4), in addition to equine arteritis virus (EAV), equine infectious anaemia virus and West Nile virus. (Laugier et al., 2011; Costa et al., 2022).

Members of the *Alphaherpesviridae* family, namely EHV-1 and EHV-4, have been associated with urinary tract infections and abortion in young horses. Although the lesions in abortions caused by EHV-1 and EHV-4 are similar, EHV-4 has been reported to cause abortions relatively more sporadically (Reed and Toribio, 2004). In cases of abortion caused by EHV-1 and EHV-4, the foetus exhibits signs of inflammation, including the presence of acidic and pleural fluid, as well as edematous and congested lungs. The liver is characterised by the presence of grey necrotic spots (Bryans et al., 1977; Ostlund, 1993). As indicated by studies conducted, the presence of EHV-1 has been documented in 15–26% of foetal losses (Laugier et al., 2011). EHV-1 can spread rapidly among horses and affect the entire population of pregnant mares (Damiani et al., 2014). A study conducted in Türkiye reported EHV-1 and EHV-4 seropositivity rates of 23.2% and 78%, respectively (Ataseven et al., 2010).

The equine arteritis virus (EAV), which belongs to the *Arteriviridae* family, has been demonstrated to be the causative agent of reproductive disorders and respiratory infections in equids (Timoney and McCollum, 1993). The pathophysiology of EAV-induced abortions in pregnant mares is characterised by panvasculitis, which results in oedema, bleeding and abortion (Del Piero, 2000). EAV infections have been observed to result in abortion in pregnant mares and have a deleterious effect on the genital system (Balasuriya, 2014; Damiani et al., 2014). In Türkiye, EAV seropositivity has been

reported as 6.5%-24.4% (Kırmızıgül et al., 2007; Gür et al., 2018). Equine infectious anemia (EIA) is a viral disease caused by the equine infectious anemia virus (EIAV), which belongs to the genus *Lentivirus*, family *Retroviridae* (Lupulovic et al., 2021). Infection is characterised by a persistent infection, which is typified by recurrent febrile episodes associated with viremia, fever, thrombocytopenia, and wasting symptoms (Lupulovic et al., 2021). Despite the global prevalence of the disease, numerous studies have demonstrated that enterprises within our nation are not affected by it (Akpınar et al., 2023). It has been reported that the virus can be transmitted to foals via vertical route and may cause abortions (Gregg and Polejaeva, 2009).

The West Nile virus (WNV) is a positive-sense, single-stranded RNA enveloped virus that belongs to the genus *Flavivirus* in the family *Flaviviridae* (Castillo-Olivares and Wood, 2004). The symptoms of the disease are most commonly characterised by the presence of encephalitis, ataxia, limb weakness, lying down and muscle fasciculation (Angenvoort et al., 2013). Transplacental transmission of WNV poses a risk of foaling in pregnant mares with severe neurologic WNV disease (Venter et al., 2011). There is no specific drug that is effective against viral diseases. However, good hygiene and management practices, together with the symptomatic treatment of infected horses, may help to prevent the spread of viral infections. Vaccines play an important role in preventing these infections (Bresgen, 2011). Current recommendations for the treatment of recumbent horses include providing supportive and nutritional care, rehydration, and frequent evacuation of the bladder and rectum to prevent colic. Reducing central nervous system (CNS) inflammation is also recommended (Laugier et al., 2011; Pusterla et al., 2022).

Fungal and Parasitic Infections

Several fungal agents can affected to horses however only some agents well described. The most common fungal agents seen in horses are *Aspergillus* spp., *Mucor* spp., *Candida* spp., *Histoplasma capsulatum* and *Cryptococcus* spp. Has been reported *Coccidioidomycosis* and *Histoplasma capsulatum* can lead abortion in horses (Giles et al., 1993; Laugier et al., 2011; Cafarchia et al., 2013).

Certain protozoan diseases have been observed to result in infertility and death in equines. Protozoans such as *Theileria equi, Babesia caballi, Neospora* spp. and *Toxoplasma gondii* have been identified as causative agents of various clinical symptoms, including abortion, icterus, anaemia, fever, neurological symptoms, liver and kidney failure (Erol et al., 2022). The present study was conducted for the purpose of investigating the prevalence of certain protozoa. The study revealed the prevalence rates of the protozoa *B. caballi, T. equi, T. gondii*, and *Neospora* spp. in horses to be 12.12%, 34.84%, 9.09%, and 10.6%, respectively. Furthermore, the molecular prevalence of the viral agents was found to be 3.03% for equine influenza virus and 6.06% for equine herpesvirus 5. The presence of the equine viral arteritis virus and other herpesviruses (types 1, 2 and 4) could not be detected in any of the samples examined (Baydar et al., 2023). Treatment for fungal and parasitic infections should use specific antifungal and antiparasitic drugs. For this purpose (Cafarchia et al., 2013). As drugs used to treat fungal diseases are expensive and not always effective, determining susceptibility to antifungal drugs is important (Voelter-Ratson et al., 2014). The first step in preventing parasitic infections in horses should be to reduce the transmission of parasites between animals. Although typical prevention programmes for these parasites are based on the use of antiprotozoal drugs, this can also be achieved through non-chemical means, such as frequently collecting pasture droppings or feeding grazing animals parasite-catching fungi (Lewis et al., 1999; Proudman and Matthews, 2000; Love, 2003).

Non-Infectious Agents Causing Abortions and Foal Mortality in Horses

Reproductive failure is the most significant cause of financial and moral losses in the field of horse breeding. Termination of pregnancy prior to 10 months of gestation is categorized as abortion, a process that poses significant challenges for equine enterprises. The etiology of miscarriage can be categorized into non-infectious and infectious causes. Non-infectious causes include multiples, poisoning, management disorders, umbilical cord torsion, prolonged transport, trauma, hormonal disorders, inadequate care and feeding. Early diagnosis and treatment of the problem are of paramount importance in order to prevent pregnancy loss (Li et al., 2024).

In the field of equine breeding, twins have been identified as a significant contributing factor to foal mortality. Depending on the lack of nutrition, this may result in termination of pregnancy in the last months of pregnancy or failure of the born foal to survive. In the case of one twin foal diagnosed during an early ultrasound examination, termination of the pregnancy is recommended. It is imperative that virgin mares, mares with foals, mares fed with foods of high nutritional value, and mares with a history of twin births are closely monitored (Alamaary and Ali, 2020; Peere et al., 2024).

Embryonic losses have been observed to be more prevalent in aged mares than in younger ones. The underlying cause of this phenomenon is attributed to the fact that the oviduct's environment, which is conducive to embryonic development, is not as conducive to the development of older mares. While fertility remains relatively unaffected by age, pregnancy loss is susceptible to the impact of age (Roach et al., 2021).

Hormonal disorders have been demonstrated to exert an influence on the process of pregnancy. The hormone progesterone, which is produced by the corpus luteum, is imperative for the continuation of pregnancy. Uterine cysts have been demonstrated to disrupt the functioning of the corpus luteum, thereby restricting progesterone production. The regular analysis of progesterone is therefore recommended to ensure the correct monitoring of the stages of pregnancy in mares. It is imperative to emphasize that close monitoring of hormone levels is paramount, particularly during the initial five months of pregnancy. Consequently, the analysis of progesterone, gonadotropin, and estrogen levels is imperative in the assessment of reproductive activities in mares (Hollinshead et al., 2022; Raghupathy and Szekeres-Bartho, 2022).

The pathological condition that occurs when the umbilical cord folds excessively is known as umbilical cord torsion. This condition can result in the obstruction of umbilical vessels, thereby impeding the infant's ability to feed. The length of the umbilical cord is influenced by several factors. The movements of the foetus and umbilical cord can be monitored by ultrasound examination (Li et al., 2024).

Mycotoxins, such as aflatoxins, are frequently identified as contaminants in animal foodstuffs. These mycotoxins have been demonstrated to induce reproductive and immune complications in equines. Diagnosis of mycotoxins is typically accomplished through the utilization of immunological analysis (Chiminelli et al., 2022; Xu et al., 2022).

Inadequate care and feeding, mechanical injuries, lack of veterinary care and poor farm management are among the factors that adversely affect pregnancy. Furthermore, foal deaths are frequently observed in horses subjected to extended travel. In mares exposed to stress factors, cortisol levels may increase and foal deaths may occur. To address these challenges, strategies have been developed to prevent foal losses and enhance reproductive performance in horse breeding. These

strategies involve the implementation of planned and programmed management procedures. The implementation of environmental modifications has been demonstrated to enhance equine welfare and to mitigate the occurrence of abortions. The implementation of a system in which a specialized veterinary surgeon oversees horse breeding can ensure the continuous monitoring of the mare, facilitating the early detection and rapid treatment of any issues that may arise (Li et al., 2024).

Testing and Diagnosis

Correct diagnosis of the underlying causes of abortions in horses facilitates treatment (Alamaary and Ali, 2020). In general, diagnostic tests for these disease agents include the following:

1. Sending the fetus and placenta to the diagnostic laboratory for examination
2. Physical examination
3. Blood and serological tests
4. Culture
5. Cytology and/or biopsy

Ways to Prevent Foal Deaths

There are many prevention and control methods to prevent pup mortality. In general, it is important to take these measures within the rules of preventive medicine. These should be determined as routine vaccination (equine herpes virus etc.), separation of pregnant mares from other horses and suspicious & sick horses (isolation), routine health checks, careful observation and developing a suitable diet under the guidance of an equine nutritionist (Ataseven et al., 2010; Bresgen, 2011).

CONCLUSION

Our study examines potential risk factors for pregnancy loss in horses and provides detailed guidance on prevention, diagnosis, treatment and management. The prevalence of equine abortions caused by infectious agents currently ranges from 18.7% to 53.1%. These infectious agents include bacteria, viruses, and pathogenic organisms such as fungi or parasites. Preventing infertility problems, primarily by adopting preventive medicine, is an essential component of managing litter loss. Strategies to prevent non-infectious and infectious pupping problems include appropriate nutritional recommendations, vaccination against infectious diseases and eradication methods. Adopting proper treatment and biosecurity strategies routinely will greatly facilitate the solution of problems.

Ethical Statement

This research article has not been published anywhere before.

Ethics Committee Approval

This study did not require ethics committee approval.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) – Author 2 (%50)
Data Collection (CRediT 2) Author 1 (%00) – Author 2 (%50)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%25) – Author 2 (%25) – Author 3 (%25) – Author 4 (%25)
Writing the Article (CRediT 12-13) Author 1 (%25) – Author 2 (%25) – Author 3 (%25) – Author 4 (%25)
Revision and Improvement of the Text (CRediT 14) Author 1 (%25) – Author 2 (%25)

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Conflict of Interest

The authors declared that there is no conflict of interest.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being

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