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Scanning Electron Microscopic Examination of Rainbow Trout Gastrointestinal Mucosa

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Abstract

Aim to study: The aim of this study was to determine the morphological characteristics of the gastrointestinal mucosa of the rainbow trout.
Material and methods: This study was carried out on 10 rainbow trout's stomach and intestinal tissues using scanning electron microscope. In the study, 2.5% glutaraldehyde fixation and routine scanning electron microscopy procedures were applied.
Results: The surface architecture of the gastrointestinal tract was examined in detail. It was observed that the mucosal folds in the stomach increased in number towards the pyloric region and were arranged in a configuration that allowed food to be directed to the intestine. The columnar cells of the digestive tract mucosa and the mucosal openings that allow mucus secretion were shown in detail.
Conclusion: The gastrointestinal tract of trout, a carnivorous species, was studied in detail. The structure of the columnar cells, gastric glands and mucus openings of this system was revealed in three dimensions. The detailed anatomy of the mucosal surface, which is rapidly affected by food variation or pathological changes in fish, was revealed. This study will shed light on studies affecting the morphology of the digestive system.
Keywords: Intestine, rainbow trout, scanning electron microscopy, stomach.

Gökkuşağı Alabalığı Gastrointestinal Mukozasının Taramalı Elektron Mikroskobik İncelemesi

Öz

Çalışmanın amacı: Bu çalışmada gökkuşağı alabalığının gastrointestinal mukozasının morfolojik özelliklerinin incelenmesi amaçlanmıştır.
Materyal ve yöntemler: Bu çalışma 10 gökkuşağı alabalığının mide ve bağırsak dokuları üzerinde gerçekleştirilmiştir. Dokular taramalı elektron mikroskobu ile incelenmiştir. Çalışmada %2,5 gluteraldehit fiksasyon ve rutin taramalı elektron mikroskobu prosedürü uygulanmıştır.
Bulgular: Gastrointestinal sistemin yüzey mimarisi ayrıntılı olarak incelenmiştir. Midedeki mukozal kıvrımların pilorik bölgeye doğru sayıca arttığı ve gıdanın bağırsağa yönlendirilmesini sağlayacak bir konfigürasyonda düzenlendiği gözlendi. Sindirim sistemi mukozasının kolumnar hücreleri ve mukus salgılanmasını sağlayan mukozal açıklıklar ayrıntılı olarak gösterildi.
Sonuç: Etçil bir tür olan alabalığın gastrointestinal sistemi ayrıntılı olarak incelendi. Bu sistemin kolumnar hücrelerinin, mide bezlerinin ve mukus açıklıklarının yapısı üç boyutlu olarak ortaya kondu. Balıklarda besin çeşitliliği veya patolojik değişikliklerden hızla etkilenen mukozal yüzeyin detaylı anatomisi ortaya çıkarıldı. Bu çalışma sindirim sistemi morfolojisini etkileyen çalışmalara ışık tutacaktır.
Anahtar kelimeler: Bağırsak, gökkuşağı alabalığı, taramalı elektron mikroskobu, mide.

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Introduction

Rainbow trout has an extensive distribution in freshwater around the world and is one of the most widely cultivated species in aquaculture due to its many advantages, such as adaptability and productivity (Crawford & Muir, 2008). Although ever-increasing costs and sustainability are influential in the search for the most suitable aquafeed, the main focus should be on the knowledge of the function of gut structures and the effects of the feeds used on the gut (Barker et al., 2012). Since the beginning of the digestive system, dental structures, oral mucosa, and oropharyngeal structures have been studied in detail and associated with dietary preferences (Abbate et al., 2006; El Bakary, 2011; Abbate et al., 2012a; 2012b; Guerrera et al., 2015). Food ingestion and food preference are linked to taste buds located on the oral mucosa. Moreover, teeth play a crucial role in masticating food within the mouth and transforming it into manageable bites. In particular, molar-like and capped teeth are present in species that prefer crusted foods and facilitate food crushing (Whitehead, 1977; Bond, 1979).

Microridge structures on the oral mucosa are associated with mucus fluidity. Microridges are also thought to provide resistance to the oral mucosa (Ezeasor, 1982). Microridge structures are found in the esophageal mucosa as well as the oral mucosa of the digestive tract (Mahmoud et al., 2016). The mucus facilitates the passage of food in the digestive tract and protects the epithelial layer from mechanical influences. Mucus cells responsible for mucus production are found throughout the entire mucosa of the digestive tract and distributed throughout the oral cavity, esophagus, and digestive tract (Harabawy et al., 2008; Baoom, 2012; Guerrera et al., 2015). Stosik et al., (2023) mentioned that changes in the anatomical and histological structures of different intestinal sections in teleost fishes, which have different characteristics from mammals, are related to the immune system. Intestinal mucosal immunity in teleost fish, where lymphoid tissue is absent, is a controversial and current topic.

Intestinal morphology in fish is rapidly affected by diet and adapted to external factors. Dietary ingredients have a direct proportional effect on intestinal morphology in terms of digestibility and feed utilization. Fish intestinal morphology changes in a very short period to provide maximum benefit from feeds (Demirci et al., 2021). Extensive studies of intestinal morphology have been carried out in different livestock species (Elia et al., 2018), such as poultry and cattle (Verdile et al., 2019). However, the knowledge about the morphology of the internal structure of the gut wall in fish is still limited. There is a wide variability in gut characteristics among Teleostei species according to their feed preferences (Fagundes et al., 2016). The digestive tract in fish can also adapt rapidly and reversibly to the environment, depending on their physiological requirements. Several factors, such as taxonomy, feeding habitats, food type, and feeding frequency, affect the morphology of the digestive tract. Previous studies have shown that changes in rainbow trout intestinal morphology are directly related to fish diet. Research on fish intestinal morphology is mostly based on histopathological studies such as changes in villus length, thickening of the villus lamina propria, and lymphocyte infiltration (Li et al., 2019). The morphology of the fish digestive tract varies considerably in the proximal and distal parts of the intestine. Although the proximal parts have been studied in recent years, there are no descriptive morphological studies on the distal parts (Abbate et al., 2006; El Bakary, 2011; Abbate et al., 2012a; 2012b; Guerrera et al., 2015). This situation is related to the inadequacy of analysis due to the complex structure, especially in the distal intestinal parts (Ray & Ringø, 2014). Therefore, in order to establish

accurate reference values in rainbow trout, individuals between 220-280 g were studied. The aim of this study was also to determine the intestinal morphology of rainbow trout and to reveal the three-dimensional structure of the intestinal mucosa by scanning with scanning electron microscopy (SEM).

Material and Methods

In this study, tissues obtained from ten rainbow trout (average weight 220-280 g; n = 10) were used. The digestive tract tissues of the fish were fixed in 2.5% glutaraldehyde solution and postfixed in 1% osmium tetroxide for 1 hour for electron microscopy. Samples were serially dehydrated in graded acetone and dried in a critical point dryer (Quorum Technologies, E3100) and then coated with gold-palladium in a coater for SEM (Cressington, Sputter Coater 108 Auto). After tissue preparation, the samples were examined by SEM (FEI, Quanta FEG 250) under Everhart–Thornley detector-2.00Kv.

Results

Macroscopically, the gastrointestinal parts of rainbow trout were examined as stomach, pyloric caeca, small intestine, and large intestine. It was observed that the stomach was shaped like a hollow J character and its pyloric part was short. The cardiac part was separated with a circular thickening from the esophagus, and its inner surface was observed to have longitudinal mucosal folds. Immediately posterior to the pyloric part of the stomach, the pyloric caeca was observed with numerous bluntly terminated finger-like projections. The small intestine behind the pyloric caeca also had several single rows of finger-like projections. Although the transition of the small intestine to the large intestine was unclear, the caudal part of the intestine was wider.

SEM examination

Stomach

Scanning electron micrographs revealed that the mucosal folds of rainbow trout's cardiac part of the stomach were irregular. The gastric mucosa surface epithelium was observed close to each other like a flower bouquet (Figure 1A). There are no micro ridge structures on gastric mucosa epithelial cells. Mucosal folds were very dense and caudally oriented in the pyloric region (Figure 1C). Also, there were irregular-shaped polygonal areas with columnar cells in the pyloric caeca and fundus.

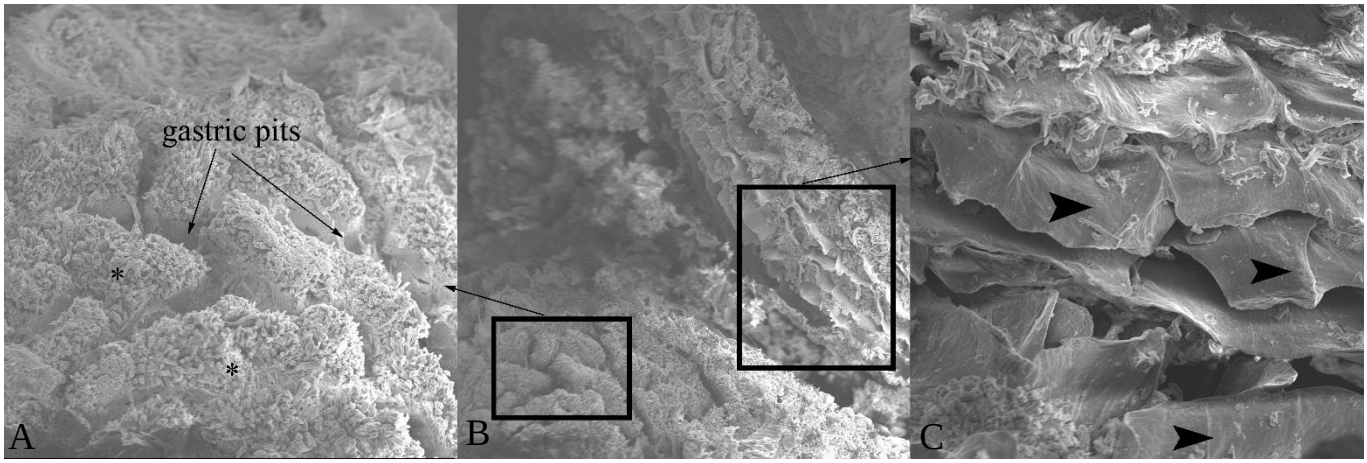

Figure 1. Scanning electron micrograph of stomach pyloric part surface. A) Left oblong area in B. B) Pyloric part surface. C) Right oblong area in B. *: Columnar cells. Arrowhead: Mucosal fold.

Pyloric caeca

The pyloric ceaca had mucosal folds in the inner surface (Figure 2A, B). The mucosal folds had an interconnected honeycomb appearance on the inner surface of the pyloric caeca (Figure 2A). Also, there were micro folds at the ends of the mucosal folds facing the lumen (Figure 2C). In places, the mucosa overlapped each other. On the transversal section of the finger-like blind processes, mucosal folds extending to the inner surface were observed; the inner surface was covered with columnar epithelium, and mucus openings were dense (Figure 2D).

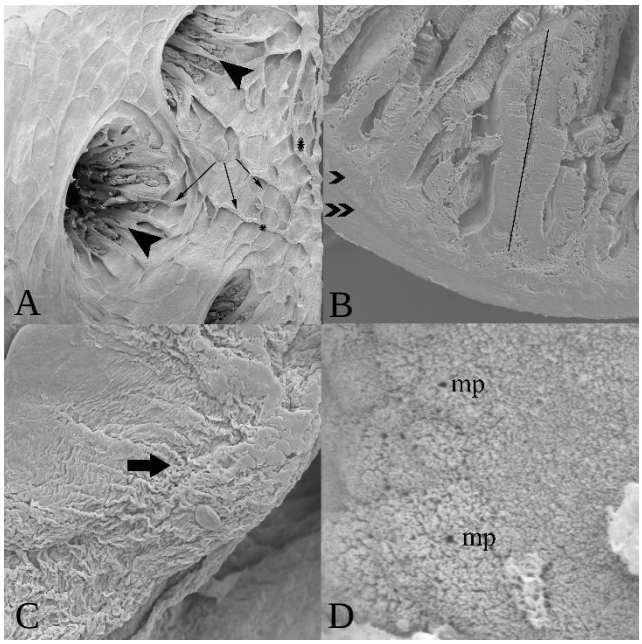

Figure 2. A) Scanning electron micrograph of pyloric caeca surface. B) Transversal slice area of the pyloric caeca. C) close-up view of mucosal fold in A. D) Columnar epithelial surface of the pyloric caeca. Thin arrows: mucosal folds, star: main mucosal fold, thick arrowhead: cecum of pyloric caeca, thin arrowhead: circular muscular layer, double thin arrowhead: longitudinal muscular layer, line: mucosal fold, thick arrow: micro folds, mp: mucosal pore.

Intestine

Scanning electron microscopy observations revealed that the luminal mucosa in the anterior regions of rainbow trout intestine exhibited thin, longitudinal mucosal folds. Above these mucosal folds, transverse and short band-shaped folds formed pouches on the mucosal folds (Figure 3A). Mucous openings were observed on the mucosa of the intestinal canal (Figure 3B, E). The mucosal folds of the large intestine were thicker than the small intestine's (Figure 3C), and mucosal folds had micro folds at the end of the facing the lumen (Figure 3D).

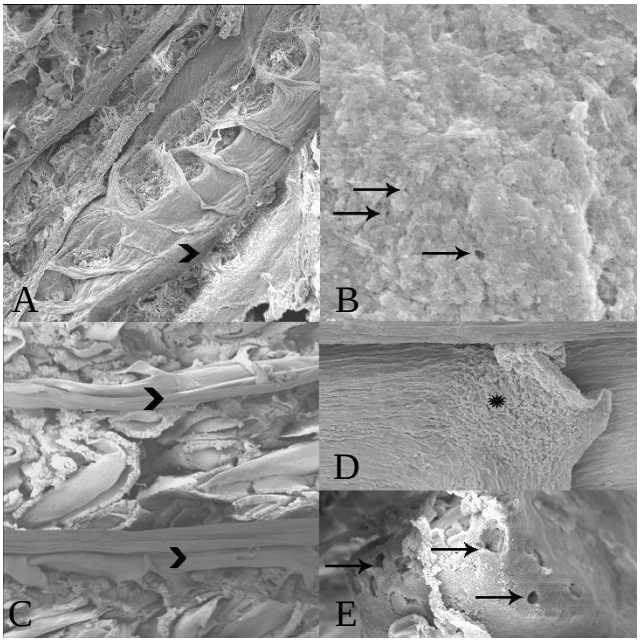

Figure 3. A) Scanning electron micrograph of small intestine surface. B) Close-up view of the small intestine surface. C) Scanning electron micrograph of large intestine surface. D) Close-up view of the mucosal folds surface of the large intestine. E) Close-up view of the small intestine surface. Arrowhead: mucosal folds, arrow: mucosal pores, * micro folds on the mucosal folds of the large intestine.

Discussion

The stomach varies considerably among fish species and is absent in some teleost species. The stomach is divided into three regions: cardia, fundus, and pylorus (Wilson & Castro, 2010). It is easy to define the stomach in the gastrointestinal system. However, in some species, such as Cyprinidae, Gobiidae, and Blennidae, the stomach appears to be an enlarged part of the gastrointestinal tract. Therefore, it is difficult to distinguish the stomach macroscopically. These species have adaptations such as glands in the esophagus and thickening of the muscular layer (Stevens & Hume, 2004; Flores et al., 2020). Depending on the feeding

habits, the stomach is present in many fish species and has different shapes, such as straight, U, J, and Y. The U-shaped stomach is found in herbivores, while the Y-shaped stomach is found in carnivores (Johnson & Clements, 2022). It has been reported that the stomach is j-shaped in trout (De Felice et al., 2021). In our study, the rainbow trout's stomach was J-shaped, similar to previously reported (Wilson & Castro, 2010; De Felice et al., 2021).

In fish, the stomach also releases hydrochloric acid (HCl) for food storage and modulation of digestion. Parietal cells from the gastric glands secrete HCl and create the acidic environment essential for the activation of digestion, which is the task of the stomach (Okuthe & Bhomela, 2021). These cells are more numerous in the cranial parts of the stomach than in the caudal parts. Therefore, the caudal part of the stomach is thought to have a food storage function rather than digestion. The presence of columnar cells in the gastric mucosa protects the gastric mucosa against the low pH of the stomach (Alves et al., 2021; Johnson & Clements, 2022). It is argued that mucus in the stomach enhances digestive activity and forms a barrier against physical and pathological factors (Pedini et al., 2005; Sharba et al., 2022). In the detailed SEM examination of the gastric surface in the study, parietal cells were seen between polygonal-shaped mucosal folds, columnar cells covering the gastric surface, and gastric glands were shown in detail (Figure 4).

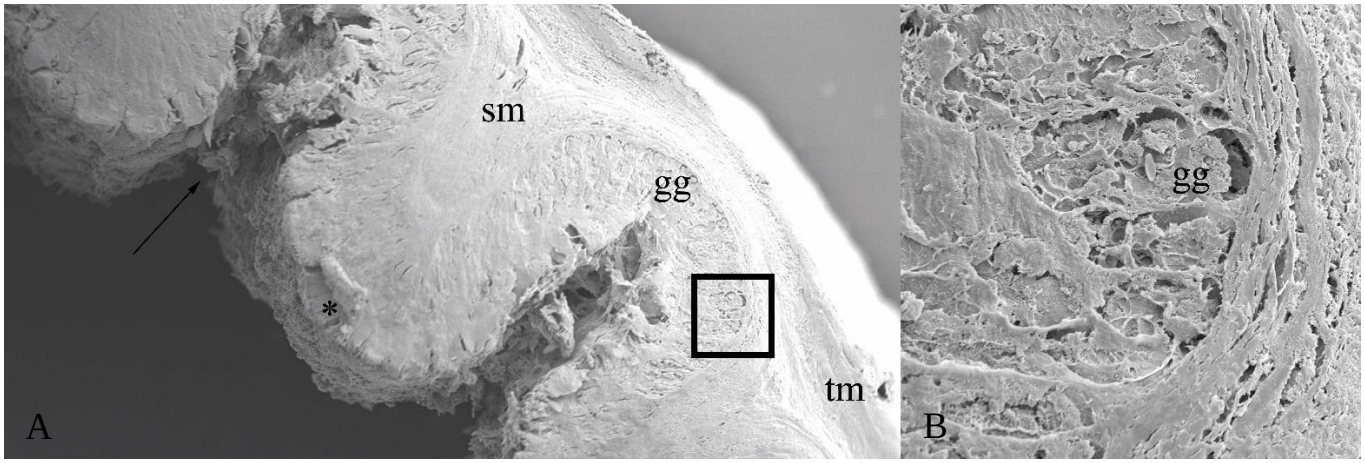

Figure 4. A) Scanning electron micrograph of transversal slice area of the stomach. B) Close-up view of oblong area in A. Arrow: gastric pit, sm: submucosa, gg: gastric glands, tm: muscular layer

The cranial parts of the intestines have finger-like pyloric caeca. Pyloric caecae are thought to shape the continuation of digestion in the stomach and increase the intestinal absorption surface without fermentation. The number and size of pyloric cecae vary according to animal species and the nature of the food (Farrag et al., 2020; Demirci et al., 2021). Our study observed that the columnar epithelium, a continuation of the gastric mucosa, was on the surface of the pyloric caeca, and there were mucosal folds. Furthermore, the honeycomb-shaped mucosal folds on the inner surface of the pyloric caeca may provide resistance to the mucosa during the digestive activity of the pyloric caeca.

Conclusion

In conclusion, the gastrointestinal tract of trout was analyzed in detail and ultrastructurally by SEM. The structure of columnar cells, gastric glands, and mucus openings on the gastric mucosal surface were revealed. The detailed anatomy of the mucosal surface affected by food diversity or pathological changes was revealed.

This study will shed light on studies affecting the morphology of the digestive system.

Financial Support

This study did not receive a grant by any financial institution/sector.

Ethical Statement

The study was conducted in accordance with the guidelines of the Kastamonu University Experimental Animals Local Ethics Committee (Decision no: 2023-9/39).

Author Contributions

Investigation: B.D.; Material and Methodology: B.D.; Supervision: B.D.; Visualization: B.D.; Writing-Original Draft: B.D.; Writing- review & Editing: B.D.

Conflict of Interest

The author declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Investigation of Oxytetracycline and Enrofloxacin Residue in Beef Collected from Hatay Province

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Abstract
Aim to study: This study purposed to investigate the residues of oxytetracycline and enrofloxacin in beef samples collected from different districts of Hatay province.
Material and methods: Fifty beef samples, each weighing 100 grams, were randomly acquired from butchers and markets. High-performance liquid chromatography was utilized for sample analysis.
Results: The maximum residue limits for red meat in the European Union and the Turkish Food Codex is 100 µg/kg for enrofloxacin, ciprofloxacin and oxytetracycline, while according to the Food and Agriculture Organization it is 200 µg/kg. Residues of oxytetracycline, enrofloxacin, and its metabolite ciprofloxacin were found below the maximum residue limits determined by the Turkish Food Codex and Food and Agriculture Organization in 5 (10%) out of 50 beef samples. In 90% of the samples, no residues of enrofloxacin, oxytetracycline, and ciprofloxacin were detected. However, enrofloxacin residues were found in 2 muscle samples (4%) at concentration of 47 and 57 µg/kg, and ciprofloxacin residues of 60 µg/kg. Additionally, oxytetracycline residues were detected in 2 samples (4%) at concentrations of 88 and 95 µg/kg.
Conclusion: It was observed that oxytetracycline and enrofloxacin are used in fattening in Hatay province and pre-slaughter waiting periods are adhered to.
Keywords: Beef, ciprofloxacin, enrofloxacin, HPLC-UV, oxytetracycline.

Hatay İlinden Toplanan Sığır Etlerinde Oksitetrasiklin ve Enrofloksasin Kalıntısının Araştırılması

Öz
Çalışmanın amacı: Bu çalışma, Hatay ilinin farklı ilçelerinden toplanan sığır eti örneklerinde oksitetrasiklin ve enrofloksasin kalıntısının araştırılması amacıyla yapılmıştır.
Materyal ve yöntemler: Çalışma materyali, kasap ve marketlerden rastgele 50 adet sığır eti, her biri 100 gr olacak şekilde toplanmıştır. Numuneleri analiz etmek için yüksek performanslı bir sıvı kromatografi yöntemi kullanılmıştır.
Bulgular: Avrupa Birliği ve Türk Gıda Kodeksi'nde kırmızı et için maksimum rezidüel limit enrofloksasin, siprofloksasin ve oksitetrasiklin için 100 µg/kg iken Gıda ve Tarım Örgütü göre 200 µg/kg'dir. Oksitetrasiklin, enrofloksasin ve metaboliti olan siprofloksasin kalıntıları, 50 sığır örneğinin 5'inde (%10) Türk Gıda Kodeksi ile Gıda ve Tarım Örgütü tarafından belirlenen maksimum kalıntı limiti altında bulundu. Örneklerin %90'ında herhangi bir konsantrasyonda enrofloksasin, oksitetrasiklin ve siprofloksasin kalıntılarına rastlanmamıştır. Kas numunelerinin ikisinde (%4) 47 ve 57 µg/kg konsantrasyonlarda enrofloksasin kalıntısı, birinde (%2) 60 µg/kg konsantrasyonda siprofloksasin kalıntısına rastlanmıştır. Ayrıca iki numunede (%4) 88 ve 95 µg/kg konsantrasyonlarında oksitetrasiklin kalıntısı tespit edildi.
Sonuç: Hatay ilinde sığır yetiştiriciliğinde oksitetrasiklin ve enrofloksasin kullanıldığı ve kesim öncesi bekleme sürelerine uyulduğu belirlendi.
Anahtar kelimeler: Sığır eti, siprofloksasin, enrofloksasin, HPLC-UV, oksitetrasiklin.

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Introduction

Access to healthy food is crucial for consumers. Medications administered to farm animals can enter the human body through the food chain, potentially causing adverse effects for consumers (Jayalakshmi, 2017). Antibiotics represent one of the most extensively used drug groups in both human and animal context. According to the World Health Organization (WHO), approximately half of the antibiotics produced in the world are used for non-human applications (WHO, 2022). In veterinary medicine, antibiotics serve therapeutic purposes for treating sick animals, prophylactic purposes to prevent infections, and are utilized as feed additives at sub-therapeutic levels to enhance growth (Tadesse & Tadesse, 2017).

Antibiotics can be administered to food-producing animals in various forms including oral, parenteral, muscular, and topical applications. The elimination time of different antibiotics from the animal body varies, influenced by factors such as dosage form, antibiotic type, and method of administration (Bou-Mitri et al., 2019). While some antibiotics become harmless and ineffective by decomposing to inactive metabolites, some accumulate in the animal body and transfer into the milk, meat, or egg. For this reason, some health problems may occur in humans who consume foodstuff of animal origin, which is exposed to improperly used antibiotics (Ortelli et al., 2018). Many studies show that antibiotics accumulate in the liver, kidney, muscle, and bone tissues of livestock animals, which exceeds acceptable limits determined by authorities (Sarker et al., 2018; Amagon et al., 2017). The most commonly used antimicrobials in food-producing animals are tetracyclines and fluoroquinolone (Lee et al., 2001).

Tetracyclines are broad-spectrum antibiotics that are widely used in humans and animals against both Gram-positive and Gram-negative bacteria, as well as bacteria such as *Mycoplasma*, *Rickettsia*, and *Chlamydia* (Cinquina et al., 2003). Tetracyclines can be administered to animals orally with food or drinking water, parenterally, or by intramammary infusion. Due to enterohepatic circulation, tetracycline antibiotic residues may remain in the body long after administration (Botsoglou, 2001). Enrofloxacin is a third-generation fluoroquinolone antibiotic with a very broad spectrum used in the treatment of respiratory tract infections, digestive, urinary, joint, genital, mammary, and dermal infections in all animals (Cinquina et al., 2003; CVMP, 2007). Enrofloxacin is known to be partially metabolized to ciprofloxacin in cattle, with the concentration of ciprofloxacin in blood being 25 to 35% of the parent drug (Pyun et al., 2008). Ciprofloxacin is effective against microorganisms resistant to other antimicrobial agents such as aminoglycosides, tetracyclines, macrolides, and β-lactams (Sultan, 2014).

While some antibiotics, such as penicillins, are easily degraded, antibiotics such as fluoroquinolones and tetracyclines are more persistent, remain in the environment longer, spread more, and reach higher concentrations, so their residues can be found in the environment (Li et al., 2008; Sim et al., 2011). Antibiotic residues present in animal foods pose a significant risk to human health, manifesting in various ways such as gastrointestinal disorders (Sarmah et al., 2006), allergic reactions, toxic effects (Fabrega et al., 2008), and the transmission of antibiotic-resistant bacteria to humans (Nisha, 2008). Additionally, tetracyclines may cause staining of young children's teeth and poor fetal development (Botsoglou et al., 2001). Some countries and national organizations set limits for residue levels of veterinary drugs using risk-based assessments (EEC, 1990; Tollefson & Miller, 2000) to ensure that consumers are not exposed to high levels of residues in foods of animal origin. In the

European Union (EU) and our country, the Ministry of Agriculture and Rural Affairs has determined maximum residue limits (MRLs) for antibiotics in foods and the use of antibiotics as feed additives for growth promotion purposes is prohibited (EEC, 1996; Resmi Gazete, 2017). In the EU and Turkish Food Codex (TFC), the MRL for red meat is 100 µg/kg for enrofloxacin and its metabolite ciprofloxacin and 100 µg/kg for tetracycline.

High levels of tetracycline and quinolone occurrence in red meat have been reported (Kimera et al., 2015; Omotoso & Omojola, 2015). There are a few studies about the residues of enrofloxacin and oxytetracycline in beef samples in Türkiye (Erdoğdu et al., 2009; Er et al., 2013). To the best of our knowledge, there is no existing study in the literature that has investigated enrofloxacin and oxytetracycline residues specifically in beef sold in Hatay province so far. This study aims to investigate oxytetracycline and enrofloxacin residues in beef samples collected from Hatay province by using the high performance liquid chromatography (HPLC) method to determine the residue level of enrofloxacin and oxytetracycline, which are antibiotics used in livestock, and to increase food safety.

Material and Methods

This study was conducted on 50 edible beef muscle tissues obtained from local butchers and markets randomly in Hatay province Antakya, Samandağ, İskenderun, Arsuz, Belen, Defne, Yayladağı, Reyhanlı, Altınözü, Kırıkhan, Kumlu districts in February 2021. Beef muscle sample was collected at 100 g each and brought to the laboratory. Samples were frozen at -20°C till the day of analysis.

High performance liquid chromatography analysis

HPLC system (Shimadzu, Tokyo, Japan) CBM-20A system-controlled pump (LC-20AT), degasser (DGU-20A), autosampler (SIL-20A) column oven (CTO-10A) and ultraviolet detector (SPD-20A UV-VIS) was built. For ciprofloxacin, oxytetracycline, and enrofloxacin, the wavelength was adjusted to 280 nm. For chromatographic separation, a Gemini TM C18 column (250 × 4.6 mm; internal diameter, 5 µm; Phenomenex, Torrance, CA) was utilized. Temperatures for the column and autosampler were 40°C and 24°C, respectively. With the aid of a pump equipped with a low-pressure gradient system, the mobile phase, which consisted of aqueous solution 88% (0.4% orthophosphoric acid, and 0.4% triethylamine, and 12% acetonitrile) was supplied to HPLC at a flow rate of 1 mL/min. Data analysis was done using LC solution software that was managed by an Asus PC.

Preparation and analysis of beef samples

Analysis of enrofloxacin and its active metabolite ciprofloxacin and oxytetracycline from muscle samples were performed using HPLC-UV using previous methods (Corum et al., 2019; Tekeli et al., 2020; Uney et al., 2021; Corum et al., 2023). Beef muscle tissues were thawed at room temperature and then weighed as 1 gram and homogenized at 10000 rpm for 45 seconds (Heidolph Silent Crusher M, Germany). The homogenized muscle tissue was added to the microcentrifuge tubes as 100 µg. Then, 200 µL of acetonitrile for enrofloxacin and ciprofloxacin analysis and 200 µL of methanol (0.1% trifluoroacetic acid) for oxytetracycline analysis were added to the muscle tissue. The mixture was vortexed for 45 seconds and then centrifuged at 10000 x g for 10 minutes. For analysis of enrofloxacin and ciprofloxacin, 100 µL of water was added to 100 µL of supernatant and vortexed

for 5 seconds. All samples were transported in autosampler vials and 25 µL of them were injected into the HPLC system.

Method validation

The chromatographic procedure was established following the European Medicines Agency (EMA, 2011) recommendations. Oxytetracycline stock solution was prepared in distilled water, while enrofloxacin and ciprofloxacin stock solutions were prepared in 0.01 M NaOH to be concentration of 1mg/ml and all solutions stored at -80°C in the freezer. Enrofloxacin, ciprofloxacin, and oxytetracycline standard solutions were added to blank samples of beef muscle to provide quality control samples and calibration standards (0, 0.04, 0.1, 0.2, 0.4, 1, 2, 4 and 10 µg/g). To detect the recovery, precision, and accuracy, the quality control samples of enrofloxacin, ciprofloxacin, and oxytetracycline at low, medium, and high concentrations (0.1, 1 and 10 µg/g) were used. The peak areas of matter, measured on plasma samples and analyzed in the same manner as any other sample, were compared to the peak areas of the standards to calculate recovery. Recovery was >90.46% for enrofloxacin and ciprofloxacin and >87.24% for oxytetracycline. To ascertain the limit of detection (LOD) and limit of quantitation (LOQ), blank plasma samples were loaded with the lowest standard solutions of enrofloxacin, ciprofloxacin, and oxytetracycline (0.01-0.1 g/mL). A concentration with an signal to noise ratio of 3 was designated LOD and a concentration with an S/G ratio of 6 was designated LOQ on the chromatogram. For enrofloxacin, ciprofloxacin, and oxytetracycline, the LOD value was 0.02 µg/ mL and the LOQ value was 0.04 µg/mL. Precision was determined using the repeatability of the intra-assays and inter-assays. Six iterations of analyses were carried out on six separate days for each level of quality control samples at low, moderate, and high concentrations (0.4, 4 and 40 g/mL) to determine intra-assay and inter-assay variations. The concentration was measured for each sample and the concentration in the enriched plasma samples was used to calculate the percentage of coefficients of variation. The intra-assay and inter-assay coefficients of variation were determined as ≤4.72% and ≤5.80% for enrofloxacin and ciprofloxacin, and ≤5.64% and ≤7.24% for oxytetracycline, respectively.

Results

As a result of HPLC analysis of beef collected from butchers and markets in the districts of Hatay, 5 (10%) of 50 beef samples were detected as positive for oxytetracycline, enrofloxacin, and ciprofloxacin residues. All 5 of the samples were under the determined by the TFC and FAO. Oxytetracycline, enrofloxacin, and its metabolite ciprofloxacin residues were not found in 90% of the samples. Enrofloxacin residues were detected at 47 and 57 µg/kg concentrations in 4% of the 50 samples. Ciprofloxacin residue was found at 60 µg/kg concentration in only 2% of the samples. Also, oxytetracycline residues were detected in 88 and 95 µg/kg concentrations in 4% of the samples.

Discussion

Antibiotics have been used for decades to treat and prevent bacterial infections and stimulate growth in animals. However, non-compliance with the withdrawal periods after antibiotic use causes residues in products obtained from animals and may threaten human health as a result (Olatoye & Ehinmowo, 2010; Turk & Oguz, 2016). FAO and WHO recommend MRLs for veterinary drugs in edible tissues of animal origin to prevent the residue concern. The maximum oxytetracycline residue limits in beef muscle tissues have been determined as 200 µg/kg by FAO (2015) and 100 µg/kg by EMA (2002), the TFC (2017). The maximum enrofloxacin and

ciprofloxacin residue limits were determined as 100 µg/kg for beef muscle tissues by EMA (2002) and the TFC (2017). In this study, 10% of samples were found positive for the oxytetracycline, enrofloxacin, and ciprofloxacin residues under the TFC, FAO and EMA maximum residue limit standards.

Kimera et al. (2015) determined a total of 60 cattle muscle samples in Tanzania and found the mean of oxytetracycline residue in muscle samples was 2604.1±703.7 µg/kg. Also, 71.1% of the samples included oxytetracycline residue, and 68.3% of the samples were above acceptable limits. When this study was compared with the current study, the number and concentration of residue samples in Tanzania were higher than the results in the study. Abbasi et al. (2012) investigated the level of oxytetracycline residues in 22 cattle muscles in Iran. The mean oxytetracycline level of muscle tissues was found between 154.2±79.2 µg/kg. It was determined that 16.6% of the muscle samples exceeded the 100 µg/kg limit recommended by the EMA.

Muriuki et al. (2001) detected oxytetracycline residues in 110 (44%) and chlortetracycline residues in 4 (1.6%) out of 250 samples, which included muscle, liver, and kidney tissues from beef carcasses in a study conducted in Kenya. Beef muscle tissue accounted 7.6% of these residues, with mean oxytetracycline residues in muscles ranging from 524 to 1060 µg/kg. These results were determined to be higher than 100 µg/kg, which is the MRL in edible muscle tissues determined by EMA (Muriuki et al., 2001). In Hatay province, oxytetracycline reside levels in two samples were lower than those found in Kenya, with a detection rate of 4% in muscle tissues, compared to 7.6% in Kenya. Olatoye & Ehinmowo (2010) investigated oxytetracycline residues in 60 cattle muscles in their study in Nigeria. The mean residual level of muscle tissues was determined as 51.80±90.53 µg/kg, and the lowest and highest value range was determined as 0-220 µg/kg. oxytetracycline residue was found above the acceptable limits in 11.62% of the samples in the study in Nierya. In the study in Hatay, it was found below the acceptable limits in 4% (Olatoye & Ehinmowo, 2010).

Baghani et al. (2019) investigated tetracycline and ciprofloxacin residues in 41 cattle muscles in a study conducted in Iran. In cattle samples, tetracycline residue was found positive in 31 samples, in the range of 0-1.78 µg/kg. It was observed that none of the samples exceeded the MRL of 100 µg/kg. Besides, ciprofloxacin residue was present in all 41 samples, residue amounts were in the range of 0.1-43.2 µg/kg and were below the residue limit set by EMA, 100 µg/kg. The results of this study show similarity to our study, while the levels of residues found are significantly lower than those found in the samples from Hatay. In Tehran, it can be said that the cattle are sent to slaughter after complying with the legal withdrawal periods of antibiotics.

Ramatla et al. (2017) determined the levels of tetracycline in 20 beef and ciprofloxacin in 15 beef in Mafikeng in the Northwest province of South Africa. The mean concentration in bovine muscle tissues was 110.3±9.4 µg/kg for ciprofloxacin and 48.6±30.2 µg/kg for tetracycline. Five of the beef muscle tissues exceeded the ciprofloxacin MRL, while no muscle tissue exceeded the maximum tetracycline residue level. When this study was compared with the study conducted in Hatay, tetracycline residue levels were similar and ciprofloxacin residues were higher.

Aliu & Sulaj (2014) reported quinolone residue in 14 (15.7%) of the 89 beef samples at the 28.22±1.11 µg/kg mean concentration, in Kosova. Enrofloxacin and ciprofloxacin residue was detected in 6.7% and 3.35% of the samples respectively. This study was compared with the

study conducted in Hatay, tetracycline residue levels were similar. In their study in Iran, Mashak et al. (2017) found quinolone residues in 79 of 162 beef obtained from local meat markets. The mean residue level was 5.51±1.17 µg/kg. It has been observed that beef meat does not exceed 100-120 µg/kg, which is the national residue limit standard set by the state of Iran. Considering the province of Hatay in terms of quinolones, residue levels exceeding MRL (100 µg/kg) were not found in both studies.

Türksever & Öner (2021) analyzed meat samples taken from 20 different places in Van province with the CHARM II test for tetracycline group antibiotics. As a result of the analysis of the samples, they determined that there was no detectable level of tetracycline group antibiotics.

Erdoğdu et al. (2009) examined 250 cattle and 25 sheep meat samples in Izmir for the residue of tetracycline-derived antibiotics. Oxytetracycline residue was found in 11 (4.4%) of the samples. The mean of oxytetracycline residue in cattle samples was 906.6 µg/kg in the range of 275-2540 µg/kg which is above the acceptable limits Oxytetracycline residue levels found in this study were higher than the current study. Er et al. (2013) analyzed fluoroquinolone residue in 104 beef muscle meat samples collected randomly from markets in Ankara. As a result of the analysis, 57.7% of the beef samples were positive and the mean residue concentration was 6.64±0.14 µg/kg. When compared with our current study, both were found below the maximum residue limits. However, while it was positive in 4% of the samples in the current study, it was positive in 57% of the samples in this study.

Conclusion

Public health is severely threatened by residue in foods. Antimicrobial agents, commonly used in livestock animals can cause important health issues such as antibiotic resistance and toxic reactions in humans through residues. For this reason, the withdrawal period of the products of animal origin should be followed, and care should be taken not to exceed the MRL in foods such as meat, milk, and eggs. In this study, it was observed that 10% of the beef offered for sale in Hatay under the maximum concentration limit of pharmacological active substance residues allowed in animal foods determined by the TFC and FAO. To eliminate the residue problem in foods of animal origin, the awareness of the animal producers and sellers about residues should be increased by organizing training.

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Ethical Statement

This study was approved by the Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (2021/01-10).

Author Contributions

Ö.Ç.D. and E.T. contributed to the study design and study material collection. D.D.Ç. contributed to the data analysis. All authors participated in writing the manuscript, and collectively reviewed and approved the final version.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Possible Unilateral Iris Melanoma in a Cat

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Abstract
A tumor known as feline iris melanoma has a high chance of metastasizing and is characterized by multifocal, golden yellow to brown pigmented patches in the iris. Despite the fact that cats of any age can develop this tumor, there is no breed or sex-specific susceptibility. The case material was a female Russian blue cat that was neutered at the age of 5, weighed 4.5 kg, and was taken to the surgical clinic of the faculty of veterinary medicine at the University of Siirt. According to the patient's medical history, a brown spot-like pigmented region had developed on the right eye's iris two months prior, and up until the cat was brought to the clinic, the pigmented patches in the iris grew in number and size. As a result of routine clinical examination, tonometry, haematological and biochemical examinations, uveal cysts and iris freckles with similar clinical symptoms were eliminated and it was concluded that the pathological condition was iris melanoma. In addition, it was determined that melanoma foci were involved only on the iris surface in line with the available possibilities and did not metastasise to other tissues and organs of the eye. Since the disease was in the initial stage, the general condition of the patient was good after clinical examinations and the owner of the patient did not accept enucleation, it was decided not to perform any intervention. The owner of the patient was instructed that during the follow-up phase, the foci should be checked and the patient should continue to be under supervision. When the patient was summoned back for a follow-up examination three months later, it was decided to undertake enucleation in case any potential melanoma foci or metastases had grown in other eye tissues or organs.
Keywords: Cat, iris, melanoma.

Bir Kedide Olası Tek Taraflı İris Melanomu

Öz
Kedilerde iris melanomu; iriste multifokal, altın sarısı renginden kahverengiye kadar değişik pigmente alanlar ile karakterize, yüksek metastaz riskine sahip bir neoplazidir. Bu neoplazi her yaştaki kedilerde görülebilmesine karşın ırk ve cinsiyet predispozisyonu göstermez. Olgu materyalini Siirt Üniversitesi Veteriner Fakültesi Hayvan Hastanesi Cerrahi kliniğine getirilen 5 yaşlı, 4,5 kg ağırlığında, dişi, Russian Blue ırkı, kısırlaştırılmış bir kedi oluşturdu. Hastanın anamnezinde, sağ gözde iris üzerinde 2 ay önce kahverengi nokta şeklinde bir pigmente alanın oluştuğu ve kliniğe getirilene kadar geçen bu süreç içerisinde iristeki pigmente alanların sayıca ve büyüklük olarak arttığı söylendi. Hastada yapılan rutin klinik muayene, tonometri, hematolojik ve biyokimyasal muayeneler sonucunda benzer klinik semptomlara sahip üveal kistler ve iris çilleri elimine edilerek patolojik durumun iris melanomu olduğu kanısına varıldı ve melanom odaklarının eldeki imkanlar doğrultusunda sadece iris yüzeyinde tutulum gösterdiği, gözün diğer dokularına ve organlara metastaz yapmadığı tespit edildi. Hastalığın henüz başlangıç evresinde olması, klinik muayeneler sonucu hastanın genel durumunun iyi olması ve hasta sahibinin enükleasyonu kabul etmemesi nedeniyle herhangi bir müdahalede bulunulmamasına karar verildi. Hasta sahibine ilerleyen süreçte hastanın gözlem altında tutulması ve odakların takip edilmesi gerektiği bilgisi verildi. Üç ay sonra tekrar kontrole çağırılan hastada olası melanom odaklarında bir artış veya gözün diğer dokularında ve organlarda bir metastaz tespit edilmesi durumunda enükleasyon uygulanmasına karar verildi.
Anahtar kelimeler: Kedi, iris, melanom.

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Introduction

Numerous conditions that affect the structures in the orbit, eye, and adnexa are included in the diseases that fall under the purview of veterinary ophthalmology. Neoplasms, which can be benign or malignant and ruin a tissue's homeostatic regulatory mechanisms, account for about 39% of ocular diseases in small animals. The tolerance limit for diseases is low due to the anatomy and function of the eye, though; even tiny, non-invasive, and slowly growing tumors can result in serious illness, color changes, and vision loss (Guerra Guimarães et al., 2021).

The iris, corpus ciliare, and choroid make up the uvea, the bulbus oculi's middle layer (Gelatt, 2008). The iris, which gives the eye its color, is a vascular area that is situated behind the cornea layer in the anterior section of the eye. Melanomas are malignant or benign tumors of the melanoblast and the melanocyte (Siripoonsub et al., 2016). According to Payen et al. (2008), melanocytic tumors can form in the skin, mucous membranes, eyes, eyelids, central nervous system, or eyes. Iridial melanomas in cats grow in the anterior uvea. According to Ionaşcu et al. (2012), anterior uveal melanoma is a pathologic condition that often develops unilaterally in cats and is not influenced by age or breed.

The first clinical sign in a patient affected by neoplasia is asymmetric hyperpigmentation of the iris. The hyperpigmented areas may persist for months or years until irregular iris masses develop. As the pigmented areas in the iris grow and coalesce, the iris becomes thicker and less mobile, resulting in iris involvement (Kalishman et al., 1998). Iris involvement is characterized by the presence of pigmented foci that gradually coalesce (over months to years) into larger and thicker pigmented areas and may eventually range from one or more golden yellow to dark brown spots (Gelatt, 2008). Sometimes there is peripheral spread to the choroid, irido-corneal angle, ciliary body, and scleral venous flexure. This can lead to secondary glaucoma. The tumor that reaches the sclera often extends to the orbit and metastasizes to distant organs by spreading to the cranial cavity via the optic nerve (Siripoonsub et al., 2016). Although tumor metastases are most common in the liver, they can also occur in the lung, kidney, spleen, lymph nodes, brain, and bone tissue (Kayes & Blacklock, 2022). In some cases, melanoma may remain stationary in the iris or develop slowly over time, resulting only in an aesthetic change in the iris (Duncan & Peiffer, 1991).

Malignant melanomas are the most common primary intraocular tumor in cats (Planellas et al., 2010). Reported metastasis rates in cats vary widely, ranging from 19% to 63% (Kayes & Blacklock, 2022). The diagnosis of iris melanoma is definitively made by histopathologic examination after a complete clinical and ophthalmologic examination. Ophthalmoscopy, tonometry, and ocular ultrasound are used to assess thickening of the iris root and ciliary body, determine the shape of the tumor and extent of local invasion, and detect secondary complications such as glaucoma, corneal edema, hyphema, and anterior uveitis (Ionașcu et al., 2012). The differential diagnosis should consider iris freckles, pigmented choroidal cysts, inflammation-related iridial discoloration, and other choroidal neoplasms (Boydell & Enache, 2012). Advanced cases typically have simple diagnoses, but this reduces the range of available treatments. Currently available treatment options include surgery, cryotherapy, radiotherapy, photodynamic therapy (PDT), and laser, but in the absence of clinical recommendations, the selection of a course of action is largely based on the facilities that are available, the preferences and experiences of the clinicians, and the owner's financial situation (Guerra Guimarães et al., 2021). We present here a case of possible

unilateral iris melanoma in the initial stage that did not metastasize to any tissue or organs.

Case Description

A female Russian Blue cat, 5 years old and weighing 4.5 kg, was brought to the Siirt University Veterinary Faculty Animal Hospital Surgery clinic. In the anamnesis, it was reported that a brown spot-shaped focus had developed in the right eye of the cat two months prior, and that over time, it had grown in number and volume without impairing the patient's vision. Regular general examination results, including those of the mucous membranes, lymph nodes, body temperature, heart rate, respiratory rate, peripheral pulse quality, capillary refill time (CRT), thoracic auscultation, and belly palpation, were normal. Inspection of the right eye revealed that there were approximately 15 brown pigment foci on the iris, irregularly distributed and more intense at 11-1 o'clock (Figure 1).

Figure 1. Brown pigmented areas irregularly distributed on the surface of the iris in the right eye.

The pupillary light reflex, assessed by shining light into the patient's right eye, was normal. Direct ophthalmoscopic examination of the right eye revealed that there was no pathology of the ciliary body, vitreous humor, optic disc, and other structures, and only brown pigment spots were observed on the surface of the iris (Figure 2). Intraocular pressure was measured three times with a rebound tonometer (Icare ic100, Hasvet, Türkiye) and the mean value was obtained. The mean results (right eye 21.33 mm/Hg, left eye 19 mm/Hg) (reference range 15- 30 mm/Hg, Von Spiessen et al., 2015) were within the reference range.

Figure 2. Brown pigmented foci in the iris of the right eye.

Table 1. Haemogram findings

Parameter	Result	Reference Ranges
WBC (x 10^3/uL)	5.8	5.5-19.5
Lenf (x 10^3/uL)	2.5	1.5-7.0
Mon (x 10^3/uL)	0.2	0-0.85
Gran (%)	3.1	–
Lenf (%)	42.2	20-55
Mon (%)	4.7	1-4
RBC (x 10^6/uL)	10.17	5.0-10.0
HGB (g/dL)	17.0	8.0-15.0
HCT (%)	51.4	24-45
MCV (Fl)	50.6	39.0-55.0
MCH (pg)	16.7	12.5-17.5
MCHC (g/dL)	33.0	30.0-36.0
RDW (%)	14.7	13.8-21.1
PLT	206	–
Eos (%)	2.8	2-12

WBC: White Blood Cell, Lenf: Lenfosit, Mon: Monosit, Gran: Granulocyte, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PLT: Platelet, Eos: Eosinophil, Reference Range: Yılmaz, 2022.

Clinical and direct ophthalmoscopic examination of the left eye revealed no pathologic findings. In the complete blood count, red blood cell (RBC), haemoglobin (HGB) and hematocrit (HCT) values were observed above the reference values

due to insufficient fluid intake of the animal before the examination (Table 1). In serum biochemical analyses, albumin (ALB), blood urea nitrogen (BUN) and amylase (AMLY) values, which were higher than reference values, and lactate dehydrogenase (LDH) value, which was lower than reference values, were not found to be significant considering their clinical reflections (Table 2).

Table 2. Biochemistry findings

Parameters	Results	Reference Ranges
AST (IU/L)	21.0	10-80
ALT (IU/L)	78.0	10-80
GGT (IU/L)	1.0	1-10
TP (g/dL)	7.1	5.4-7.8
ALB (g/dL)	4.7	2.1-3.9
ALP (IU/L)	21.0	10-80
BUN (mg/dL)	53.6	10-30
CRE (mg/dL)	1.43	0.8-1.8
TBIL (mg/dL)	0.20	0.1-0.6
DBİL (mg/dL)	0.02	0-0.3
AMYL (IU/L)	2251.0	500-1800
LIPAZ (IU/L)	36.0	23-375
HDL (mg/dL)	144.3	–
LDL (mg/dL)	18	–
TRIG (mg/dL)	64.0	10-114
CHOL (mg/dL)	204.0	90-205
CK (mg/dL)	67.0	50-450
CK-MB (mg/dL)	26	–
Mg (mEq/L)	2.27	1.5-3.5
Cl (mEq/L)	120.0	117-123
Fe (µg/L)	100.0	68-215
Na (mEq/L)	156.0	147-156
K (mEq/L)	4.71	3.8-4.5
CRP (mg/L)	1.5	–
LDH (IU/L)	71.0	75-490
P (mg/dL)	4.45	1.8-6.4

AST: Aspartate Aminotransferase, ALT: Alanine Transaminase, GGT: Gama-Glutamyl Transferase, TP: Total Protein, ALB: Albumin, ALP: Alkalen Phosphatase, BUN: Blood Urea Nitrogen, CRE: Creatinin, TBIL: Total Bilirubin, DBIL: Direct Bilirubin, AMYL:Amilase, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoproteins, TRIG: Triglyceride, CHOL: Cholesterol, CK: Creatin Kinase, CK-MB: Creatine Kinase-MB, Mg: Magnesium, Cl: Clor, Fe: Iron, Na: Sodium, K: Potassium, CRP: C-Reactive Protein, LDH: Lactate Dehydrogenase, P: Phosphorus, Reference Range: Turgut, 2000.

The differential diagnosis was made by the fact that iris freckles with similar clinical symptoms are congenital and iris cysts have a darker pigmentation. According to the clinical, hematological and biochemical test results, it was determined that the disease was still in the early stage and the melanoma foci had not yet developed, so it was decided to keep the patient under observation and follow up the foci. After three months, the patient was called in for a follow-up appointment, and it was determined to undertake enucleation in case the potential melanoma foci increased or metastasized to other eye tissues or organs.

Discussion

While intraocular melanomas can develop in adult cats of any age, they are most common in animals nine years of age and older, are typically unilateral, and lack race or gender predisposition (Boydell & Enache, 2012). Although the patient in this case was an adult (5 years old), the melanoma formed unilaterally despite the fact that he was not quite nine years old, in contrast to what is stated in the literature. The most prevalent intraocular neoplasia in cats is uveal melanomas. This neoplasia often develops as multifocal hyperpigmented regions with substantial local invasion of the anterior uvea on the anterior aspect of the iris. On the iris in this case, there were numerous, unevenly spaced brown hyperpigmented areas. These hyperpigmented regions, which only affected the front surface of the iris, were discovered to be denser around 11 and 1 o'clock.

Neoplastic infiltration along the ciliary body and sclera results from infiltration into the iridocorneal angle as the neoplasia advances. Secondary problems such corneal edema, hyphema, anterior uveitis, and glaucoma may emerge as a result of these metastatic occurrences. According to Kalishman et al. (1998), secondary glaucoma is a sign of diffuse melanoma with a bad prognosis and shortened survival. The patient did not have secondary glaucoma, as evidenced

by the intraocular pressure measurements in this case (right eye 21.33 mm/Hg, left eye 19 mm/Hg), which were both within the reference range. We also concluded that the patient had a good prognosis because direct ophthalmoscopy showed that other problems such corneal edema, hyphema, and anterior uveitis did not manifest.

Despite having a longer development period than dermal melanoma in cats, intraocular melanoma is typically thought to have a higher malignant potential (Patnaik & Mooney, 1988). The reported rates of metastasis for this neoplasm range greatly, from 19% to 63%. The liver is the site of tumor metastasis most frequently, although it can also happen there as well as in the lungs, kidneys, spleen, lymph nodes, brain, and bone. It is possible for melanoma to remain stable in the iris or to grow slowly over time, leaving the iris simply cosmetically altered in some circumstances (Kayes & Blacklock, 2022). According to the research, hyperpigmented areas involved in the iris surface in our case did not spread to other eye tissues or organs and instead just altered the iris' appearance.

Conclusion

The patient owner was therefore notified that the patient needed to be kept under observation and that the foci needed to be watched over going forward. When the patient was brought back for follow-up three months later, it was agreed to conduct enucleation in case there was an increase in potential melanoma foci or metastasis to other eye tissues and organs. Additionally, it has been determined that other veterinarians working in small animal clinics will benefit from learning about this uncommon feline pathology.

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Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: S.K.; M.B.; Material and Methodology: G.A.; M.B.A.; Supervision: M.B.A.; G.A.; Visualization: S.K.; M.B.; Writing-Original Draft: S.K.; M.B.; Writing-review & Editing: M.B.A.; G.A.; S.K.; M.B.;

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Case of Toxocariasis in a Cat Presented to a Private Veterinary Clinic

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Abstract
In this study, a case of zoonotic toxocariasis seen in a cat brought to a private veterinary clinic is presented. The case data were obtained from a 2.5 kg male tabby cat, aged 6 months, who was presented to a private veterinary clinic. Following the examination of the cat, vomiting was observed as a side effect of the anaesthetic administered during the eye removal surgery. Macroscopic parasites were detected in the stomach contents. In order to identify the species, the parasite was placed in a tube filled with 70% alcohol. The *Toxocara cati* was later identified microscopically at the Parasitology laboratory of Selçuk University Faculty of Veterinary Medicine. Our case report contains useful information since toxocariasis infection in cats is rarely seen in the stomach contents and has zoonotic importance. This case is presented with the hope of raising awareness, especially among veterinarians working in animal hospitals and small animal clinics.
Keywords: Cat, toxocariasis, veterinary clinic, zoonosis.

Özel Bir Veteriner Kliniğine Getirilen Kedide Toksokariazis Vakası

Öz
Bu çalışmada özel bir veteriner kliniğine getirilen kedide görülen zoonoz karakterli toksokariazis vakası sunulmuştur. Olgu materyalini özel bir veteriner kliniğine getirilen 6 aylık ve 2,5 kg ağırlığında olan tekir ırkı bir erkek kedi oluşturmuştur. Kedide muayene sonrası göz ekstirpasyonu ameliyatı esnasında verilen anestezi ilacının yan etkisiyle kusma şekillenmiştir. Mide içeriğinde parazitler makroskobik olarak görülmüştür. Tür teşhisinin yapılması için parazit %70 alkol içerikli tüpe konulmuştur. Selçuk Üniversitesi Veteriner Fakültesi Parazitoloji laboratuvarında yapılan mikroskobik inceleme sonucunda *Toxocara cati* tespit edilmiştir. Vaka sunumumuz kedilerde toksokariazis enfeksiyonunun mide içeriğinde nadir olarak görülmesi ve zoonotik öneme sahip olduğundan yararlı bilgiler içermektedir. Bu vaka özellikle hayvan hastaneleri ve küçük hayvan kliniklerinde görev yapan veteriner hekimlerde farkındalık yaratması umuduyla takdim edilmiştir.
Anahtar kelimeler: Kedi, toksokariazis, veteriner kliniği, zoonoz.

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Introduction

Toxocariasis is a zoonotic infection in cats caused by *Toxocara cati, Toxascaris leonina*, and *Toxocara malaysiensis* species (Hanedan & Bilgili, 2021). Although toxocariasis is a common infection worldwide, those living in areas with poor sanitation are at a higher risk of infection (Despommier, 2003).

People become infected when the eggs of the second-stage larvae of *Toxocara* spp. are accidentally swallowed by humans. Children stand out as the most vulnerable social group to infection due to their intense contact with soil (Martınez-Barbabosa et al., 2003).

Toxocara cati (*T. cati)* are cream-coloured nematodes with females 4-10 cm long and males 3-6 cm long. The cervical wings of these parasites are arrowhead-shaped. Their eggs are 65-75 µm in diameter, brown, round and thick-shelled, and their shells are serrated. *Toxocara cati* are parasites that live in the small intestines of domestic and wild cats. These parasites develop directly and do not require intermediate hosts. However, certain creatures, including rodents, certain insects, and earthworms, may act as paratenic hosts in the life cycle of *T. cati* has a complex life cycle and can develop in cats in different ways (Tınar, 2011; Doğanay, 2021). Infection usually occurs via galactogen or by ingestion of infected paratenic hosts. Therefore, the larvae do not experience lung-tracheal migration, so the kittens are less affected by tracheal migration-related symptoms because they are older when the parasites mature. Although lesions are only observable within the intestines, infected animals exhibit a range of symptoms, including abdominal bloating, dehydration, diarrhea, dull coat, respiratory complications, and growth retardation (Taylor et al., 2007). Adult ascarids diagnosis is determined by identifying the characteristic eggs via stool examination with the flotation technique. Furthermore, during anaesthesia, there is a chance that ascarids may appear in the vomit sample (Burgu & Sarımehmetoğlu, 2005; Aydenizöz, 2013).

Toxocariasis is a disease which can be asymptomatic in many cases. Disease symptoms may vary depending on the parasite load to which the creature is exposed and the location of these parasites in the internal organs of the creature. When clinical cases are evaluated, two different disease conditions are taken into consideration: Visceral Larva Migrans (VLM) and Ocular Larva Migrans (OLM). VLM, which is usually seen in children, presents with symptoms such as fever, abdominal pain, and cough. Eosinophilia and leukocytosis are usually observed in patients. Pica is a condition frequently encountered in patients' stories. If the patient does not reinfect, recovery may occur within a few weeks. In the case of re-infection, symptoms such as lymphadenopathy, hepatomegaly, urticaria, interstitial pneumonia, in which other organs and systems are also affected, and meningoencephalitis, when the central nervous system is affected, may be observed. If the parasites settle in the central nervous system, lungs, and heart tissues, the patient may die. In OLM cases, the age of the patient does not matter much. Even a single larva can unilaterally cause OLM. Visual acuity, visual clarity, visual acumen, visual performance, etc. may deteriorate. It manifests itself with symptoms such as endophthalmitis, uveitis, chorioretinitis, and granulomas in the retina. During eye examination, sometimes a moving larva can be seen inside the eye. In OLM cases, eosinophilia and leukocytosis may not always be seen in the patient's blood parameters (Anğ et al., 2011).

This research aims to provide information about the zoonotic toxocariasis case seen in a cat presenting to a private veterinary clinic.

Case Description

A 6-month-old male tabby cat weighing 2.5 kg was presented to a private veterinary clinic. The cat's owner reported an initial increase in the tear discharge, which eventually led to complete eye closure. It was revealed during the cat's anamnesis assessment that it had a prior calicivirus infection during its kitten period. After a comprehensive examination by the veterinary practitioner, it was concluded that the cat had experienced loss of vision in its right eye. The surgical procedure for eye removal is illustrated in Figure A. The cat experienced vomiting shortly after the administration of anesthesia. Visible and mobile parasites in the stomach contents are shown in Figure B. To identify the species, the parasite was placed in a tube containing 70% alcohol. The diseased right eye was sutured subsequent to the removal of the eyeball. After the patient wakes up after the operation, it is seemed appropriate to use the commercially prepared antibiotic clavulanic acid and amoxicillin trihydrate (Synulox®, Zoetis, Italy) subcutaneous injection at a daily dose of 8.75 mg/kg for 5 days and use 2 tablets of the commercially prepared antiparasitic drug fenbendazole, pyrantel pamoate and praziquantel (Caniverm®175 mg, Bioveta, Czech Republic) for 1 day. The patient was discharged after a 7-days stay in the hospitalization unit of the clinic (Figure C). The ascarid placed in the tube was examined under an Olympus Cx31 trinocular microscope in the Parasitology Laboratory of Selçuk University Faculty of Veterinary Medicine. Tınar (2011) was used as the identification key for species identification of the parasite. As a result of the examination, it was determined to be a *T. cati* as shown in Figure D.

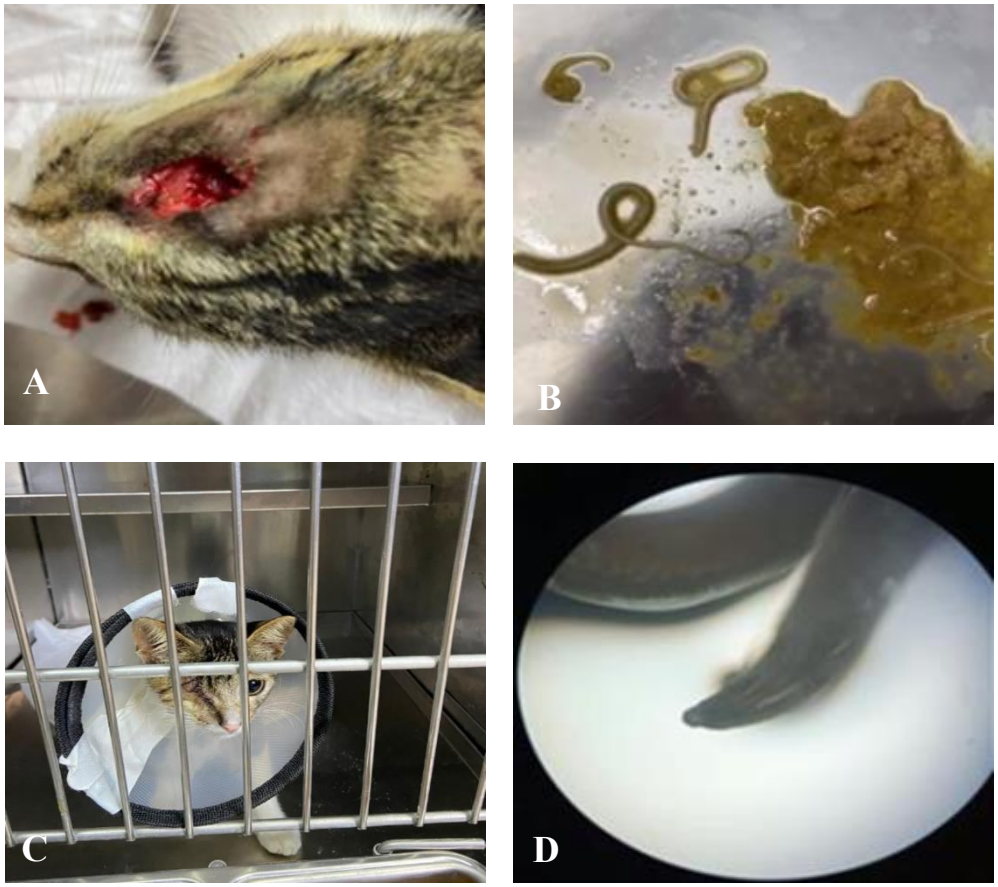

Figure. A) Macroscopic visual observation of parasites, B) Eye extirpation during surgery, C) Hospitalization of the patient after the operation, D) Detection of the parasite under the microscope.

Discussion

Toxocara spp. prevalence studies are present in Türkiye. These studies indicate the presence of *Toxocara* spp. in soil samples. The presence of their eggs has been detected at different rates in different regions. For instance, Şimşek et al. (2005) found that the presence of the substance was identified at a rate of 4.16% in Konya, 30.6% in Ankara, and 25.97% in Van. However, it was only detected in one out of the 744 samples collected from Elazığ. In 2015, a study was carried out in Samsun which involved the analysis of 187 samples of cat faeces gathered from the streets using the flotation method. The results showed that 32.1% of these samples were found to be infected (Gürler et al., 2015). A study conducted in Kırıkkale on 100 cats' fecal examination results revealed that 48.9% of the cats had *Toxocara* spp. The study reports that eggs of the causative agents were found (Korkmaz et al., 2016). Similarly, another examination method was used to examine the fecal samples of 465 stray cats in Izmir in 2021. The study identified that 16 of these cats had at least one *Toxocara* spp., *Hymenolepis* spp., and were found to be infected with *Dipylidium caninum* (Karakavuk et al., 2021).

Upon review of existing literature, it is reported that toxocariasis infection in cats has a global prevalence of roughly 17% (Rostami et al., 2020).

In recent years, it has been determined that toxocariasis infections in cats continue to be important. During this study, *T. cati* was found in the stomach contents as a result of anesthesia-induced vomiting, which is similar to previous observations of this species in the literature but differs from its appearance in stool samples in studies.

Conclusion

As a result, it is necessary to take a series of precautions to prevent Toxocara infections, which are of zoonotic importance. Regular parasitological testing of owned cats, as well as cats within shelter systems, is recommended to be carried out by a veterinarian. These tests should occur at established intervals or in certified facilities when appropriate. It should be known that the faeces of stray cats are a source of infection. It should be collected regularly by municipalities. Parks and gardens should be surrounded not only to define their boundaries but also to prevent the entry of cats. The stray cats population needs to be controlled. In the long term, ear tags or microchips should be applied to monitor animals after neutering and interventions should be carried out if necessary. As can be seen, there are certain responsibilities among individuals, educators, media, local governments, and general governments in solving this problem, which has educational, cultural, and economic aspects. Monitoring both stray and domestic cat populations worldwide are important in terms of toxocariasis infections, a zoonotic disease.

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This study did not receive a grant by any financial institution/sector.

Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: B.Ş.; H.Ö.; Material and Methodology: B.Ş.; U.U.; H.Ö.; Supervision: B.Ş.; P.Ş.; U.U.; Visualization: B.Ş.; H.Ö.; Writing-Original Draft: B.Ş.; P.Ş.; U.U.; Writing-review & Editing: B.Ş.; P.Ş.; U.U.; H.Ö.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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A Case Report of Hepatosplenomegaly Due to Chronic Salmonellosis in Holstein Calves

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Abstract

In this case report, our aim was to present the clinical, macroscopic, bacteriological, and antibiogram results of a chronic salmonellosis case in calves on a dairy farm. A total of 12 Holstein calves weaned at 75-90 days of age on a dairy farm with clinical signs were presented in the case report. The calves displayed intermittent fever, diarrhea, and pneumonia that did not respond to treatment. The most notable finding at necropsy was hepatosplenomegaly. The diagnosis of *Salmonella* spp. was confirmed from the samples taken from the calves, and antibiograms revealed the resistance to many antibiotics. The disease did not recur following attention to management practices and application of protective hyperimmune serum. The presented case report emphasizes that chronic *Salmonella* spp. infection may lead to severe losses in calves, highlighting pathological findings, antibiotic resistance, and preventive and management practices.

Keywords: Calf, chronic salmonellosis, hepatosplenomegaly.

Holstein Buzağılarda Kronik Salmonellozis'e Bağlı Hepatosplenomegali Vaka Raporu

Öz

Bu vaka raporunda bir süt işletmesinde buzağılarda karşılaşılan kronik salmonellozis olgularının klinik, makroskobik, bakteriyolojik ve antibiyogram sonuçlarını sunmayı amaçladık. Süt işletmesinde 75-90 günlük yaş aralığında olan, sütten kesilmiş ve klinik bulgu gösteren toplam 12 adet Holstein ırkı buzağı vaka raporuna dahil edildi. Buzağılarda tedaviye yanıt vermeyen aralıklı ateş, ishal ve pnömoni bulguları mevcuttu. Yapılan nekropside en dikkat çekici bulgu hepatosplenomegali olarak belirlendi. Buzağılardan alınan örneklerde *Salmonella* spp. tespit edildi ve antibiyogramda birçok antibiyotiğe dirençli olduğu görüldü. Yönetim uygulamalarına dikkat edilmesi ve koruyucu hiperimmun serum uygulaması sonrası hastalık bir daha görülmedi. Sunulan vaka raporunda kronik *Salmonella* spp. enfeksiyonunun buzağılarda ciddi kayıplara neden olabileceğine ve aynı zamanda enfeksiyonun patolojik bulguları, antibiyotik direnci, koruyucu ve yönetim uygulamalarına dikkat çekilmiştir.

Anahtar kelimeler: Buzağı, kronik salmonellozis, hepatosplenomegali.

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Introduction

Salmonella (as genus) spp. is a Gram-negative bacterium commonly found in cattle. It causes enteric and septicemic infections in humans and many animal species (Mohler et al., 2009). Most of the *Salmonella* spp. important for veterinary medicine belong to the subspecies *Salmonella enterica* (*S. enterica)*. Within this subspecies, *Salmonella typhimurium* (*S. typhimurium)* and *Salmonella dublin* (*S. dublin)* are the most common serotypes in cattle (Smith, 2009). The most common mode of transmission is the fecal-oral route. Salmonellosis causes fever, loss of appetite, bloody and mucous feces in calves (Wray & Davies, 2000). Salmonellosis is usually seen in subclinical and acute clinical forms and acute forms of salmonellosis are represented as the tip of the iceberg. Infection can be seen in epidemic as a result of management mistakes and exposure of calves to stress (Hadimli et al., 2011). Although pathologists usually associate salmonellosis with enteric lesions such as diphtheritic membranes (Wray & Davies, 2000), highly variable necropsy findings have been identified in various literature data (de Aguiar et al., 2021; Molossi et al., 2021; Casaux et al., 2023).

Acknowledging the limited quantity of case reports on *Salmonella* spp. in the literature, this case report presents the clinical, macroscopic, bacteriological and antibiogram results of a chronic *Salmonella* spp. outbreak observed in calves aged 75-90 days on a dairy cattle farm. The purpose of this case report is to aid veterinarians in the diagnosis, control and prevention of similar incidents.

Case Description

On a farm in the Central Anatolian region, clinical signs were observed in 12 Holstein calves from a group of 60 weaned calves aged 75-90 days. The calves on the farm received routine vaccinations against respiratory diseases with a double dose at 30 and 60 days of age. The calves on the farm were systematically weaned at the age of 60 days. After weaning, the calves are fed with a diet consisting of flaked maize, alfalfa and calf starter feed. Before weaning, the calves were fed with milk, alfalfa and calf starter feed. No clinical signs were observed in non-weaned calves. However, it was determined that all animals on the farm utilized the same water source.

Each of the 12 calves examined in the study exhibited clinical manifestations, including intermittent high fever, pneumonia, diarrhea, anorexia, and dehydration. Arthritis, fibrinous and hemorrhagic feces were detected in only two calves. The fecal samples from the 12 calves in the study demonstrated a watery and voluminous consistency (Figure 1). The feces of 8 of the 12 calves with diarrhea were analyzed for rotavirus, coronavirus, *Cryptosporidium parvum*, *Giardia* and *Escherichia coli* with the rapid antigen test kit (BoviD-5 Ag, Bionote, Inc. Korea) and no causative agent was detected. Blood samples taken from 12 calves showing clinical signs were polymerase chain reaction (PCR) tested for Bovine viral diarrhea virus in a private laboratory and negative results were obtained.

Figure 1. Diarrhea in a calf infected with *Salmonella* spp.

Sulfamethoxazole/trimethoprim was used to all calves with clinical signs by the farm veterinarian.

When there was no response to the treatment, florfenicol was applied. Meanwhile, the calves were also given supportive treatment with nonsteroidal anti-inflammatory drugs, including flunixin meglumine and meloxicam. Nine out of the 12 calves that showed clinical signs while treated died within seven days. Necropsy was not performed on these calves. The remaining three calves had no response to treatment, were cachectic, and died within 21-24 days. Necropsy of the three calves that died later was performed in detail by the farm veterinarian immediately after death. Samples taken from the internal organs (liver, spleen, lung, intestine) and fecal samples taken from the intestinal contents of the calves were stored at +4 °C and sent to a specialized laboratory for cultivation.

In all calves necropsied under farm conditions, notable findings included pulmonary congestion, hyperemia in the heart and intestines, enlargement of mesenteric lymph nodes, thickening of intestinal walls, hepatosplenomegaly and a yellow-orange colored liver. Diffuse red consolidated areas were observed in the lungs. Examination of one calf revealed mild yellow discoloration of the omentum, serosal surfaces of the forestomachs and adipose tissue (Figure 2).

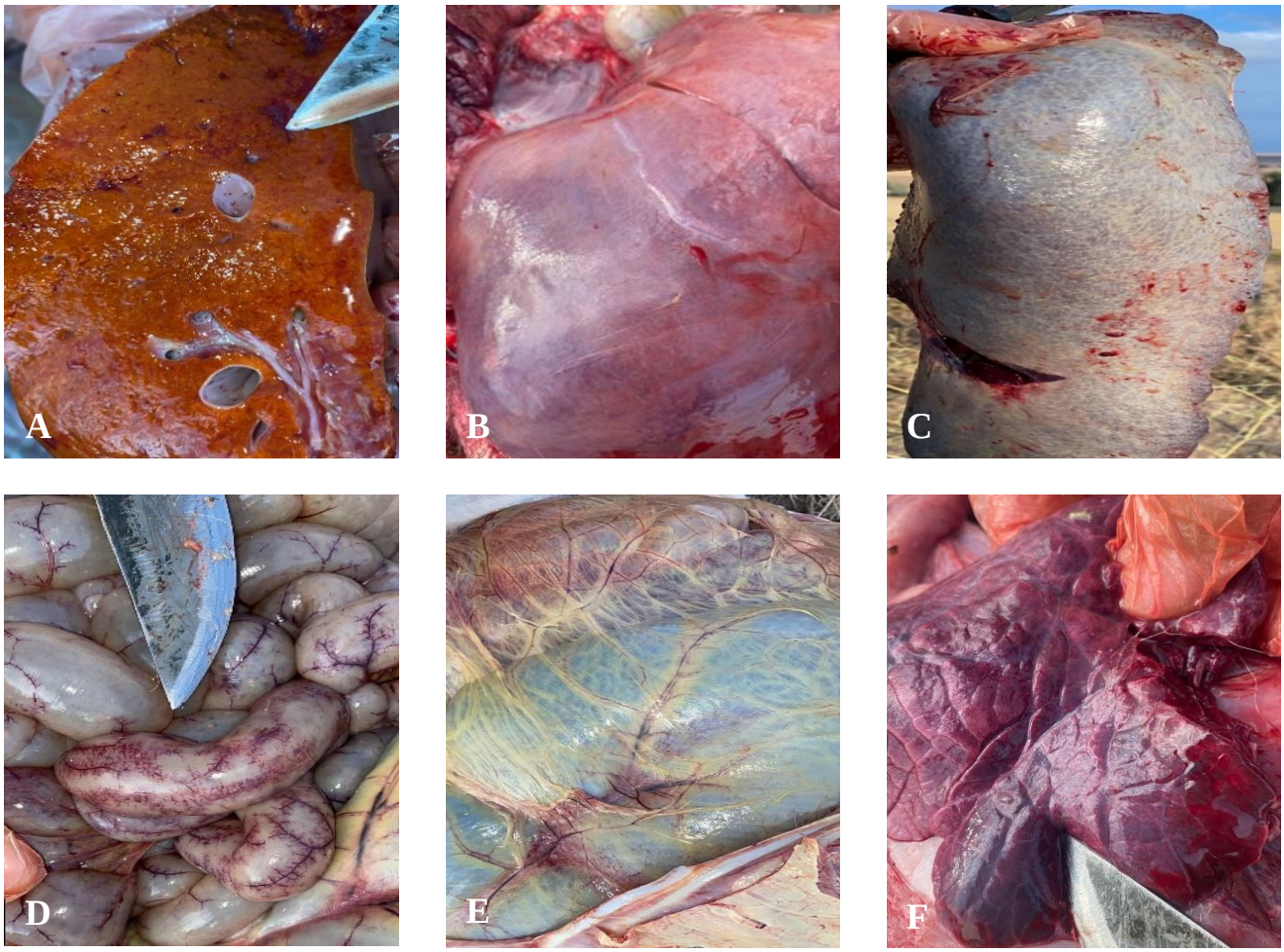

Figure 2. Macroscopic findings in calves with salmonellosis. A) The liver has light orange discoloration. B) Enlargement of the liver. C) Enlargement of the spleen is evident from the rounded borders and slight capsular tension. D) Intestinal hyperemia. E) The omentum, serosal surfaces of the forestomachs and adipose tissue are yellowed. F) The lung has a smooth, bright and reddened surface.

In the bacteriological evaluation, Gram-negative, lactose-negative, sucrose-glucose-negative, hydrogen sulphide-positive, lysine decarboxylation-positive, o-nitrophenyl-beta-D-galactoside-positive, urea-negative agents were identified as *Salmonella* spp. and isolated from all samples. Antibiogram results of the isolated *Salmonella* spp. are presented (Table). Bacteriological cultivation and antibiogram analyses were assessed in a specialized laboratory.

Table. Antibiogram results of the isolated *Salmonella* spp.

Antibiotics Used	Results
Amoxicillin-clavulanic acid	Resistant
Ampicillin	Resistant
Carbenicillin	Resistant
Cefoperazone	Resistant
Cephalexin	Resistant
Cloxacillin	Resistant
Erythromycine	Resistant
Florfenicol	Resistant
Lincomycin	Resistant
Oxytetracycline	Resistant
Penicillin G	Resistant
Spectinomycin	Resistant
Sulphamethoxazole/trimethoprim	Resistant
Cefquinome	Sensitive
Danofloxacin	Sensitive
Enrofloxacin	Sensitive
Doxycycline	Sensitive
Gentamicin	Sensitive
Kanamycin	Sensitive
Marbofloxacin	Sensitive
Neomycin	Sensitive

In the presented case report, the diagnosis of salmonellosis was confirmed based on clinical examination, macroscopic findings, and laboratory results. Following the diagnosis, detailed investigations at the farm revealed that the flaked maize was heavily contaminated with dog feces, suggesting that this was the probable source of the outbreak. All newborn calves in the herd were administered a protective dose of 10 mL subcutaneous antiserum (Multisera Combined, Atafen®) at one-week intervals, repeated three times. Additionally, stray dogs were removed from the farm. In the close follow-up of the farm for one year, it was determined that the infection did not recur, and deaths related to this disease ceased.

Discussion

Salmonellosis is a common infection in farms. *Salmonella* spp. typically affect cattle between one and three months of age, and this age group accounts for 80% of cattle cases. In the present case, the age range of the calves diagnosed with *Salmonella* spp. is consistent with the literature data (Hadimli et al., 2011; Pecoraro et al., 2017; Casaux et al., 2023). Salmonellosis can be diagnosed through clinical findings, macroscopic, microbiological and PCR methods (Costa et al., 2018). In addition to identifying the causative agent, clinical examination and macroscopic findings are important diagnostic methods for the disease (Pecoraro et al., 2017; Costa et al., 2018). The diversity of *Salmonella* spp. serotypes and the potential for different serovars to have distinct virulence factors require broad prophylactic strategies (Hadimli et al., 2011).

Clinical findings such as intermittent high fever, pneumonia, arthritis, diarrhea, etc. in calves are consistent with literature data (Hadimli et al., 2011; Pecoraro et al., 2017). Enteritis and respiratory clinical findings may help in the differential diagnosis of salmonellosis (Pecoraro et al., 2017). The high mortality observed in the presented case report is consistent with the literature data (Casaux et al., 2023).

Salmonella spp. observed in calves may partially benefit from antibiotic treatment (de Aguiar et al., 2021), but generally do not respond to antimicrobial agents due to resistant *Salmonella* spp. strains (Molossi et al., 2021). Especially in calves with septicemic, aggressive antibiotic treatment in the early period of infection and treatment according to the antibiogram result is recommended (Wray & Davies, 2000). In the presented case report, although antibiotic

treatment was applied to all sick calves, no response to treatment was obtained. It is stated that antibiotic-resistant *Salmonella* spp. species will cause severe epidemics in animals and humans due to unconscious antibiotic use (Varma et al., 2006). In the presented case report, *Salmonella* spp. resistant to many antibiotics were detected in the antibiogram analysis of the samples obtained from the farm. Even if animals infected with *Salmonella* spp. are treated, they remain as carriers and continue to be a source of infection. Therefore, the primary strategy for salmonellosis is vaccination and preventive measures (Mohler et al., 2009; Hadimli et al., 2011).

While *Salmonella* spp. is a major cause of diarrhea, serovar and host-associated factors can lead to severe systemic consequences. Hyperemia in the heart and intestine, enlarged mesenteric lymph nodes, thickening of the intestinal wall and hepatosplenomegaly observed at necropsy are consistent with the literature data (de Aguiar et al., 2021; Molossi et al., 2021; Casaux et al., 2023). At necropsy, hepatosplenomegaly was remarkable in the calves. The extraintestinal route of *Salmonella* spp. can cause of systemic inflammatory infections such as splenomegaly. Although *Salmonella* spp. is a mucosal pathogen, the main anatomical sites of bacterial replication are the spleen and liver (Jones & Falkow, 1996). In an experimental study, splenomegaly was determined in mice infected with *Salmonella* spp. It has been reported that increases in spleen cells, phagocytes and neutrophils may cause this condition and that these increases peak especially in the chronic phase of the disease. The spleen may enlarge more than ten times (Jackson et al., 2010; Rosche et al., 2015). In human medicine, hepatomegaly is reported in almost all cases infected with *Salmonella* spp. (Ramachandran et al., 1974). Hepatosplenomegaly may have been caused by the calves being in the chronic stage of the disease and the spread of infection by haematogenous route. Mild jaundice detected in only one calf is consistent with the literature data (Molossi et al., 2021; Casaux et al., 2023).

The occurrence of the disease in weaned calves and the detection of feed contaminated with the feces of stray dogs; environmental factors, management errors and exposure of calves to stress are consistent with the increase in clinical cases (Mohler et al., 2009; Hadimli et al., 2011). The absence of clinical signs in dairy cows and suckling calves suggests that the source of infection is not dairy cows. All calves were fed from mixed milk in the tank. Additionally, the absence of animal entry from outside and the detection of feed contaminated with dog feces suggest that dogs are the source of infection. Wildlife is reported to play an important role in the transmission of *Salmonella* spp. infections (Evans & Davies, 1996).

Conclusion

It was determined that intermittent fever, diarrhea, and pneumonia, which did not respond to antibiotics, could be observed in calves with chronic *Salmonella* spp. The most striking macroscopic finding was hepatosplenomegaly. It is emphasized that hyperimmune serum used for prophylaxis can stop the outbreak and the importance of management practices.

Financial Support

This study did not receive a grant by any financial institution/sector.

Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: A.E., S.Ü; Material and Methodology: A.E., S.Ü; Supervision: A.E.;

Visualization: A.E., S.Ü; Writing-Original Draft: A.E.; Writing – Review & Editing: A.E.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Current Semen Extenders for Bulls

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Abstract
Artificial insemination is the most widely used biotechnological application for animal breeding in cattle breeding. It is crucial to properly store the sperm obtained from the breeding bulls while maintaining their spermatological characteristic using suitable methods. During both long or short-term storage of spermatozoa, an ideal storage medium must be employed. For this purpose, diluents have been developed to meet the needs of spermatozoa. An ideal semen diluent contains ingredients that spermatozoa need, such as energy substances, protective agents against cold shock, buffering solutions that protect against pH changes, cryoprotectants to reduce damage to spermatozoa during freezing and antibiotics against microbial contamination. Semen dilution also allows for increasing the available semen volume to obtain more straws. Maintaining spermatological parameters at the best possible level during semen storage has important economic implications in this industry. For this reason, scientists continue to develop new diluents to achieve the optimum benefits from semen diluents. This review is aims to provide information about semen diluents used in bulls.
Keywords: Bull, extender, semen, storage.

Boğalarda Kullanılan Mevcut Sperma Sulandırıcıları

Öz
Suni tohumlama, sığır yetiştiriciliğinde hayvan ıslahı için en yaygın kullanılan biyoteknolojik uygulamadır. Damızlık boğalardan elde edilen spermlerin uygun yöntemler kullanılarak spermatolojik özelliklerini koruyarak uygun şekilde saklanması çok önemlidir. Spermatozoanın gerek uzun gerekse kısa süreli saklanması sırasında ideal bir saklama ortamı kullanılmalıdır. Bu amaçla, spermatozoanın ihtiyaçlarını karşılamak için seyrelticiler geliştirilmiştir. İdeal bir semen seyreltici; enerji maddeleri, soğuk şokuna karşı koruyucu ajanlar, pH değişikliklerine karşı koruma sağlayan tampon çözeltiler, dondurma sırasında spermatozoanın zarar görmesini azaltan kriyoprotektanlar ve mikrobiyal kontaminasyona karşı antibiyotikler gibi spermatozoanın ihtiyaç duyduğu bileşenleri içerir. Semen dilüsyonu ayrıca daha fazla payet elde etmek için mevcut semen hacminin artırılmasına da olanak tanır. Spermanın saklanması sırasında spermatolojik parametrelerin mümkün olan en iyi seviyede tutulması, bu sektörde önemli ekonomik etkilere sahiptir. Bu nedenle, bilim insanları sperma sulandırıcılarından optimum faydayı elde etmek için yeni sulandırıcılar geliştirmeye devam etmektedir. Bu derleme, boğalarda kullanılan sperma sulandırıcıları hakkında bilgi vermeyi amaçlamaktadır.
Anahtar kelimeler: Boğa, sulandırıcı, sperma, saklama.

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Introduction

The bull holds the important economic value in the cattle breeding (Foote, 2003). A single ejaculate from a bull typically contains much more spermatozoa than is required for impregnantion. Therefore, it is important to dilute the semen use in insemination of multiple cattle is possible (Arthur et al., 1996; Hinsch et al., 1997). For example, 6 ml of semen contains a sufficient number of spermatozoon cells to inseminate 200-300 cows. However such a volume of semen cannot be actually divided into 200-300 parts. In addition, it has been determined that spermatozoa in undiluted sperm live for a short time and many spermatozoa die even if they are cooled down to 5 °C very slowly. Consequently, scientists started to work on how to store spermatozoa for a long time without losing their life span. They tried to dilute the semen using various diluents. They used natural fruit juices such as tomato juice and coconut milk to dilute the semen. Later, they started to develop semen diluents prepared with various organic and non-organic chemicals that not to harm the spermatozoa (Sönmez, 2015).

Various diluents are used both for short-term storage of semen at +4˚C and when frozen at -196˚C. These diluents can be made in vitro environments by adding substances such as egg yolk, milk, and milk powder (Filho et al., 2018). Since sperm extenders contain energy-providing substances such as simple sugars, they help spermatozoa maintain their vitality for a long time (Sevinç & Hafs, 1961). In addition, antioxidants added to semen during dilution protect the proteins in the plasma membrane of spermatozoa, allowing spermatozoa to survive longer (Stout et al., 2009). Since the spermatozoon membrane in mammals is rich in polyunsaturated fatty acids, membrane structures can be damaged due to lipid peroxidation, protein denaturation and loss of function in cases where reactive oxygen species increase. As a result, DNA damage, apoptotic changes in spermatozoa and deprotamination may occur. For the aforementioned reasons, the addition of various antioxidants to sperm diluents in order to protect the semen from damage caused by reactive oxygen species during long or short-term storage leads to the prevention or reduction of this damage (Gupta et al., 2022).

Table. Characteristics of an ideal semen extender (Merdan, 2017).

1	Semen extenders should be isotonic
2	Semen extenders must contain the essential mineral substances required to maintain the viability of spermatozoa
3	Semen extenders should contain energy substances such as glucose and fructose
4	Semen extenders should contain cyroprotective agents [such as glycerol, dimethyl sulfoxide (DMSO), dimethyl acetamit (DMA) ethylene glycol]
5	Semen extenders should contain incorporate substances that can eliminate metabolic residues of spermatozoa (buffer, tris, etc.)
6	Semen extenders should be able to preserve both the plasma membrane integrity and the acrosome membrane integrity and structure of spermatozoa
7	Semen extenders should include antibiotics to control of bacterial contamination (penicillin, streptomycin, etc)

Substances Added to the Semen Extenders

Buffer Solutions

The main role of buffer agents added to semen extender is to prevent pH changes resulting from the metabolic activities of sperm cells. These agents also provide isotonic pressure needed for sperm cell. On the other hand this agents should not be toxic to the sperm cells (Sönmez, 2015).

Phosphate Buffer Solutions*:* Phosphate buffer solution is prepared by adding 2 g of Na_2PHO_4 and 0.2 g of K_2PHO_4 to 100 ml of distilled water. Although this solution is sufficient to buffer the pH, when combined with egg yolk, it clouds the environment, making it difficult to observe spermatozoa (Sönmez, 2015).

Sodium Citrate Buffer Solutions: Sodium citrate buffer solution is prepared by adding 2.9 g of $Na_3C_6H_5O_7.2H_2O$ to 100 ml of distilled water. The other modified solution is prepared by adding 2.12 g of $Na_3C_6H_5O_7.2H_2O$ and 0.183 g of citric acid to 100 ml of distilled water (Sönmez, 2015).

Tris Buffer Solutions: It is reported that the concentration of Tris in semen diluent, ranging between 10-50 mM, does not adversely affect spermatozoon motility and metabolism. The desired pH level and osmotic pressure of the Tris solution are achieved by adding citric acid to the extender (Sönmez, 2015).

Egg Yolk

Cold shock during the cooling of semen adversely affects sperm motility. Egg yolk, which contains phospholipids help protect the sperm cell membrane and acrosome against cryogenic injury. The protective effect of egg yolk against cold shock is due to its low concentration of substances such as lecithin and B lipovitellin during the cooling storage and freezing process. On the other hand, the high antioxidant content in egg yolks has been reported to reduce the rate of lipid peroxidation in the spermatozoon membrane. In the extenders prepared for bull semen, egg yolk is added at a rate of 20% to various diluents prepared in general (Sönmez, 2015; Bustani et al., 2021).

Soybean Lecithin

Soybean lecithin can be used as an alternative to egg yolk. Like egg yolk, soy lecithin contains palmitic acid, oleic acid, stearic acid, and phosphotidylcholine. Also, it has advantages over egg yolk by avoiding contamination from animal sources. Soy lecithin has been successfully used to freezing bull semen as a replacement for an egg yolk (Kumar et al., 2015; Gamal et al., 2016; Migule-Jimenez et al., 2020).

Cryoprotectants

To prevent damage to spermatozoa by the temperature changes during the freezing and thawing processes, certain chemical substances must be added to the extenders. These substances which reduce the formation of ice crystals during freezing, are called as cryoprotectants. Cryoprotectants can be divided into two classes: those that act by entering the cell (internal cryoprotectants) and those that act from outside the cell (external cryoprotectants). The most commonly used internal cryoprotectant is glycerol. Glycerol penetrates into the cell, allowing some of the water inside the cell to escape and freezing the water inside to form smaller ice crystals. Glycerol is usually added 3-10% to semen diluents. Dimethisulfoxide, DMA, ethylene glycol and propylene glycol are also internal cryoprotectants (Akçay, 2023). However some researchers suggested that of 5-15% cryoprotectant in extender yields better results in protecting against cold shock (Bhattacharya, 2018). Also, disaccharides are considered impermeable substances to cells. These sugars interact with phospholipids of the plasma

membrane, increasing sperm survival after cryopreservation (El-Sheshtawy et al., 2015, Akçay, 2023).

In addition to glycerol, DMSO can also be used for the protection of spermatozoa against cold shock. Also, ethylene glycol and propylene glycol too can be used as a cryoprotectant. Various carbohydartes such as sucros, trehalose, raffinose, and lactose can serve as cryoprotectant. However, glycerol remains the most succcesful cryoprotectant.

Sugars

Sugars, especially fructose, are the main energy source of spermatozoon cells. Sperm cells also can metabolize glucose, galactose, sucrose, maltose, xylose, raffinose, and mannose. The sugars added to the diluent also increase the level of protection spermatozoa against cold shock (Raheja et al., 2018; Bustani et al., 2021).

Antibiotics

The microbial load of semen is highly variable. The bacterial load of semen may increase, especially if the prepuce is not adequately cleaned during semen collection or if equipment hygiene is not maintained. Although the vast majority of the bacteria here are not pathogenic, they consume energy substances that are essential for spermatozoa. Many antibiotic agents like penicillin and streptomycin, ceftiofur, apramycin, and aminoglycosides or linco-spectin + tylosin + gentamycin are used to inhibit the growth of bacteria that can infect the semen (Sönmez, 2015; Raheja et al. 2018).

Other Additives

In semen diluents, many substances of plant and animal origin are also being experimented to achieve better results. In studies conducted by various researchers, plant-based additives such as pomegranate juice, green tea, strawberry, and coconut oil were added to the diluent. In a study, it was found that the addition of 1-5% strawberry juice to the Tris diluent was beneficial for the preservation of spermatological parameters during the cooling of semen and 3-6% during the freezing of semen (El-Sheshtawy et al., 2018). In another study, it was found that the addition of 1% green tea extract to tris-citric acid diluent improved in vivo and in vitro fertilization results and reduced lipid peroxidation (Ahmed et al., 2020). Tarig et al. (2017) reported that 2% coconut oil did not improve spermatological parameters in their study.

Honey is rich in sugar and antioxidants. Malik (2019) reported in his study that the addition of honey to the diluent significantly affected the motility values before the freezing and reduced sperm anomalies after freezing and thawing. In a scientific study, it was reported that the addition of 2.5% honey to tris diluent gave optimum results compared to BioXcell® diluent during the freezing of spermatozoa (Yimer et al., 2015). In another study, it was revealed that the addition of 1% honey to BioXcell® diluent in the storage of bull semen produce more effective results than the group without honey (El-Nattat et al., 2016).

Another substance of animal origin added to semen is fish oil. Malik et al. (2018) reported in their study that the diluent prepared as include 150 mg/100 ml fish oil in the reconstituent improved sperm quality after the freezing/thawing of buffalo sperm.

In a study, it was reported that the addition of nano selenium particles to tris-egg yolk-fructose semen diluent at a dose of 1.0 μg/ml improved sperm quality after freezing and thawing (Khalil et al., 2019).

Hu et al. (2009) reported that the addition of vitamin B_{12} to semen diluent at a dose of 2.5 mg/mL improved semen quality after thawing.

Gupta et al. (2022) reported that addition of curcumin (at the dose of 10 µM) in tris-egg yolk extender heled the protection of spermatological quality in post-thawing period in *Hariana* bull semen.

Currently Used Semen Extenders

Sodium Citrate-Egg Yolk Extender

Sodium citrate-egg yolk extender is prepared by adding 20% egg yolk and glucose (0.5%) to sodium citrate solution. Also, antibiotic such as pencilllin and streptomycin are added to the extender (Sönmez, 2015). This extender prepared by adding egg yolk to a 2.9% Sodium citrate solution. Nebel et al. (1985) reported that sodium citrate diluents containing 10% and 15% egg yolk provided much better preservation than sodium citrate diluents containing 5% and 0% egg yolk used in the freezing process.

Tris-Egg Yolk Extender

Researchers have developed a Tris yolk diluent that has better buffering capacity than phosphate buffer and sodium citrate buffer solutions and has less toxic effects on sperm cells. This extender yields significantly better results in the freezing compared to other extenders (Sönmez, 2015). In a study conducted by Prastiya et al. (2023), sodium citrate–egg yolk extender was prepared as Tris aminomethane 1.6%, citric acid 0.9%, lactose 1.4%, distilled water 80%, raffinose 2.5%, egg yolk 20%, penicillin 100000 IU/100 mL, streptomycin 0.1 g/100 mL, and 13% glycerin. They also add greeen tea extract to the tris- egg yolk extender according to their results green tea extract added at 0.15 mg/mL provided better preservation of cell membrane integrity, sperm motility and viability during cryopreservation of semen from Bali bulls.

Homogenized Milk And Skim Milk Extenders

Fat or skim milk alone fulfills many of the characteristics of a good semen diluent. However, if milk is to be used as a diluent, it must initially neutralize a substance called lactenin, which is harmful to sperm cells. For this purpose, the milk should be heated to 95ºC and kept at this temperature for 10 minutes. Fat and lipoproteins in the milk diluent help protect sperm cells from pH changes and cold shock. Glycerol and antibiotics are also added when preparing the milk diluent to be used in the freezing process (Sönmez, 2015; Bustani et al., 2021).

Commercial Extenders

There are ready-made diluents prepared by commercial companies to be used for long and short-term storage of semen in bulls. Egg yolk and glycerol are also added to these diluents. These powdered diluents can be dissolved in distilled water and made ready for use. Laiciphos®, Biociphos-plus®, BioXcell®, and OptiXcell® (IMV, LAigle, France), Andromed® and Triladyl® (Minitube, Tiefenbach, Germany), Optidyl® and Triladyl® (Biovet, France) are commercial semen extenders prepared for bull semen storage.

Many researchers have conducted comparative studies on which commercial diluent works best for long or short-term storage of bull semen. In a comparative study, OptiXcell® extender preserved sperm motility much better than Andromed® and BioXcell® extender during short-term storage in the refrigerator (at the +5ºC degree) (Fernandez- Novo et al., 2021). In another study conducted by Muiño et al. (2007), they reported that the use of Biladyl diluent containing egg yolk resulted in a significantly higher rate and longer survival of spermatozoa compared to Andromed® and Biociphos-plus® diluents without egg yolk in post-thawing period. Pieper et al. (2023) stated that Triladyl®, OptiXcell® and

BioXcell® diluents may show different results in the preservation of sperm motility, membrane integrity and acrosomal degradation according to differences in the duration of the equilibration process during freezing of bull semen.

Conclusion

In conclusion, studies are ongoing on the development of semen diluents to eliminate or minimize the damage that may occur during short or long term storage of bull semen.

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Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: E.H.A.; Supervision: E.H.A.; Visualization: E.H.A.; Writing-Original Draft: E.H.A.; Writing- review & Editing: E.H.A.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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