Bu-Ali Sina University

Avicenna Veterinary Research

Autumn 2025 | Volume 1 | Issue 4

Avicenna Veterinary Research
Vol. 1, No. 4, Winter 2025: 1-5
Bu-Ali Sina University

ORIGINAL ARTICLE

Evaluation of Hemoglobin and Oxyhemoglobin Dynamics Following Trifluralin Administration in Wistar Rats

Mohammad Baseri Azghandi[a], Saeid Nafisi[b,*], Mahsa Hallaj Salahi Pour[b]

[a] Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article info

Article history:
Received 2025-11-11
Received in revised form
2025-09-17
Accepted 2025-08-10

Keywords:
Trifluralin
Hematotoxicity
Oxyhemoglobin
Wistar rats
Hypoxia
Herbicide toxicity
Spectrophotometry

Abstract

Trifluralin is a widely used dinitroaniline herbicide known for its soil persistence and microtubule-disrupting activity. While its phytotoxic and genotoxic effects are well documented, its acute impact on hematological parameters-particularly oxygen transport biomarkers-remains poorly understood in veterinary models. This study aimed to evaluate the dose- and time-dependent effects of trifluralin on total hemoglobin and oxyhemoglobin concentrations in female Wistar rats following intraperitoneal administration. Rats were administered trifluralin at doses of 250, 500, and 750 mg/kg (24% concentration), and blood samples were collected at 30- and 60-minutes post-injection. Spectrophotometric analysis was used to quantify total hemoglobin and oxyhemoglobin levels. Statistical comparisons between control and treated groups were performed. Trifluralin administration led to dose- and time-dependent elevations in hemoglobin parameters. Significant increases in total hemoglobin were observed at 250 and 500 mg/kg within 30 minutes, with the highest oxyhemoglobin level recorded at 750 mg/kg. At 60 minutes, hemoglobin values in lower dose groups showed partial decline, while oxyhemoglobin remained elevated in the high-dose group. These findings suggest that trifluralin may induce transient hematological stimulation and modulate oxygen transport capacity, depending on dose and exposure duration. Further investigation is needed to clarify the underlying mechanisms and systemic implications.

1. Introduction

Trifluralin, a dinitroaniline herbicide chemically known as , , -trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine, is widely used for pre-emergent weed control in over 40 crop species including cotton, soybean, alfalfa, and various vegetables and fruit trees (Zhang et al., 2023). Commercial formulations such as Treflan and Elancolan are typically prepared as emulsifiable concentrates or granules. Due to its extremely low water solubility (<1 ppm), trifluralin exhibits high soil persistence and strong adsorption to soil particles, limiting its mobility but raising concerns about long-term accumulation in agricultural environments.

The herbicidal action of trifluralin is primarily mediated through inhibition of microtubule polymerization, which disrupts mitotic spindle formation and cell division in root meristems. While its phytotoxic and genotoxic effects are well documented, emerging evidence suggests that trifluralin may also exert systemic toxicity in non-target organisms, including mammals. In rodent models, sublethal exposure has been asso-

*Corresponding author: S. Nafisi (Professor)
E-mail address: dr.saeed.nafisi@gmail.com
http://dx.doi.org/10.22084/avr.2025.31480.1015

ciated with oxidative stress, hepatocellular damage, and hematological alterations (Fernandes *et al.*, 2013; EFSA, 2009).

In mammals, trifluralin has been associated with oxidative stress, hepatotoxicity, and hematological alterations. Acute oral toxicity studies in rodents report an LD_{50} of approximately 5000 mg/kg, classifying it as moderately toxic. Sublethal exposure may impair erythrocyte integrity and oxygen transport, potentially leading to hypoxia and systemic dysfunction (Fernandes et al. 2013).

Hemoglobin, the principal oxygen-carrying protein in red blood cells, plays a vital role in maintaining tissue oxygenation. It consists of four heme groups bound to globin chains, each capable of reversibly binding oxygen. Oxyhemoglobin, the oxygen-bound form, reflects the functional capacity of hemoglobin and serves as a sensitive biomarker for hypoxic stress. Disruption in hemoglobin synthesis or degradation can result in hematological disorders, including anemia and methemoglobinemia (Rifai, 2017).

Blood constitutes approximately 7–8% of total body weight, with plasma comprising about 55–60% of its volume. Plasma is primarily composed of water, electrolytes, and proteins such as albumin, fibrinogen, and globulins, which play essential roles in maintaining osmotic pressure, immune defense, and coagulation (Cohn and Shaz 2023). Erythrocytes, produced in the bone marrow, are specialized for oxygen transport and contain high concentrations of hemoglobin. Their biconcave shape and membrane flexibility enable efficient passage through narrow capillaries and facilitate optimal gas exchange (Basu and Kulkarni 2014).

Given the widespread use of trifluralin and its potential to interfere with oxygen transport mechanisms, this study aimed to evaluate dose- and time-dependent changes in total hemoglobin and oxyhemoglobin concentrations in female Wistar rats following intraperitoneal administration. By applying spectrophotometric analysis at two time points, the research provides insight into the acute hematotoxic and hypoxia-inducing potential of trifluralin in a controlled veterinary model.

2. Materials and Methods

2.1. Experimental Animals

A total of 18 adult female Wistar rats (mean body weight: 250±4 g) were obtained from the Laboratory Animal House of Urmia University, Faculty of Veterinary Medicine. All procedures involving animals were conducted in accordance with institutional guidelines and approved by the Ethics Committee of Urmia University. Animals were acclimatized for 24 hours under controlled conditions (temperature: 20±2°C; 12-hour light/dark cycle) with unrestricted access to food and water.

2.2. Toxin Preparation

Trifluralin (commercial name: Treflan), a dinitroaniline herbicide, was obtained as a 48% stock solution and diluted to 24% using double-distilled water. The compound is known to disrupt microtubule formation and has been used in rodent toxicity studies (Zhang, et al. 2023).

2.3. Hematological Evaluation

The 18 rats were randomly divided into three experimental groups (n=6 per group), each receiving a different dose of trifluralin (250, 500, or 750 mg/kg body weight) via intraperitoneal injection.

3. Blood Sampling Was Performed in three Stages

1. Baseline Sampling: Under ether anesthesia, blood was collected from the right retro-orbital sinus using microhematocrit tubes. Approximately 0.7 mL of blood was mixed with potassium EDTA and centrifuged at 2000 rpm for 10 minutes to separate plasma.
2. 30 Minute Post-Injection Sampling: Thirty minutes after trifluralin administration, blood was collected from the left retro-orbital sinus under anesthesia. Plasma was separated as described above.
3. 60 Minute Post-Injection Sampling: At 60 minutes post-injection, blood was collected via jugular vein incision. One milliliter of blood was mixed with EDTA and centrifuged to obtain plasma.
Plasma samples were analyzed for hemoglobin and oxyhemoglobin concentrations using a UV/Visible spectrophotometer based on Drabkin's method (Drabkin, 1946; Rifai, 2017).

4. Statistical Analysis

All statistical analyses were performed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Prior to hypothesis testing, the normality of data distribution was assessed using the Kolmogorov–Smirnov test. Variables with normal distribution were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

5. Results

5.1. Hematological and Oxygen-Carrying Parameters

The effects of intraperitoneal administration of trifluralin (24%) at doses of 250, 500, and 750 mg/kg were evaluated at 30- and 60-minutes post-injection.

Spectrophotometric analysis revealed dose- and time-dependent alterations in total hemoglobin (Total Hb) and oxyhemoglobin (Oxy Hb) concentrations.

- 250 mg/kg (30 min)

Total Hb levels increased significantly compared to controls ($p<0.05$), while Oxy Hb levels showed a mild, non-significant elevation (Fig. 1).

- 250 mg/kg (60 min)

Total Hb remained slightly elevated, though not statistically significant. Oxy Hb levels declined modestly, without reaching significance (Fig. 2).

- 500 mg/kg (30 min)

Total Hb levels increased significantly compared to controls ($p<0.05$). Oxy Hb levels showed a slight, non-significant increase. (Fig. 3).

- 500 mg/kg (60 min)

Both Total Hb and Oxy Hb levels showed mild decrease compared to controls, but neither reached statistical significance ($p<0.05$) (Fig. 4).

- 750 mg/kg (30 min)

A pronounced and statistically significant elevation in both Total Hb and Oxy Hb was observed ($p<0.05$), indicating a strong hematological response. (Fig. 5).

- 750 mg/kg (60 min)

Total Hb returned to near-control levels, while Oxy Hb remained significantly elevated ($p<0.05$), suggesting sustained oxygen transport stimulation (Fig. 6).

- Cumulative Comparison

At 30 minutes post-injection, total hemoglobin levels increased significantly across all treated groups. The highest concentration was observed at 500 mg/kg, followed by 250 and 750 mg/kg. This pattern suggests a non-linear, dose-dependent hematological response, with peak stimulation occurring at the intermediate dose (Fig. 7).

Oxyhemoglobin levels also rose at 30 minutes in all groups, with the most pronounced and statistically significant increase recorded at 750 mg/kg. The 250 and 500 mg/kg groups showed mild elevations that did not reach statistical significance. At 60 minutes, oxyhemoglobin levels declined in lower dose groups, while the 750 mg/kg group maintained elevated levels (Fig. 7).

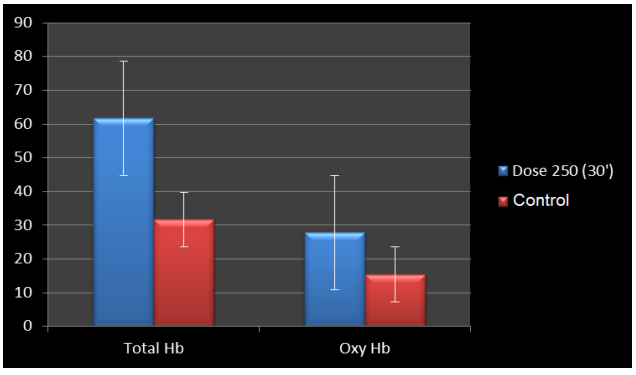

Fig. 1. Comparison of Total Hb and Oxy Hb between control and 250 mg/kg (30 min).

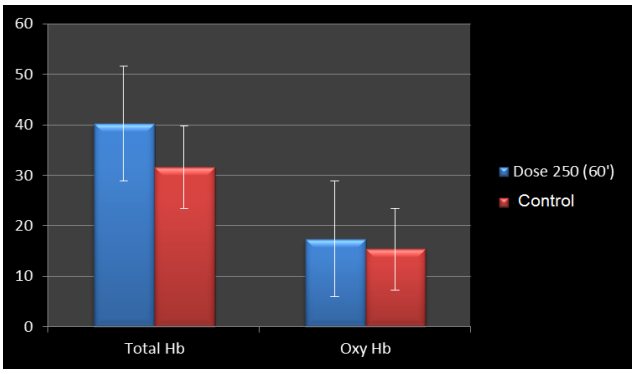

Fig. 2. Comparison of Total Hb and Oxy Hb between control and 250 mg/kg (60 min).

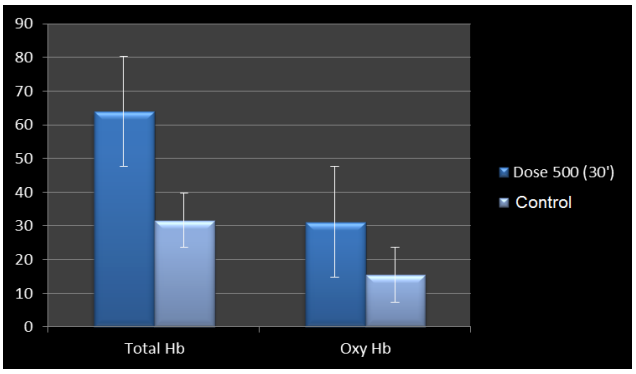

Fig. 3. Comparison of Total Hb and Oxy Hb between control and 500 mg/kg (30 min).

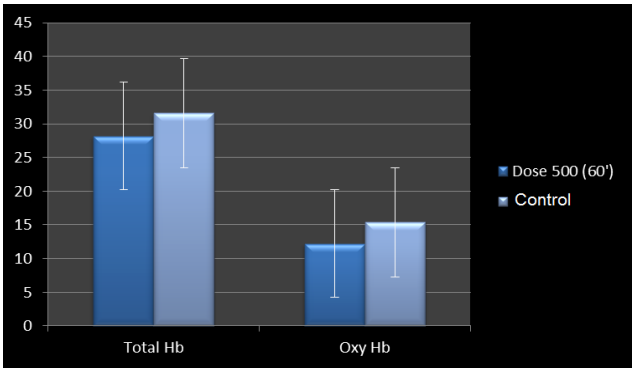

Fig. 4. Comparison of Total Hb and Oxy Hb between control and 500 mg/kg (60 min).

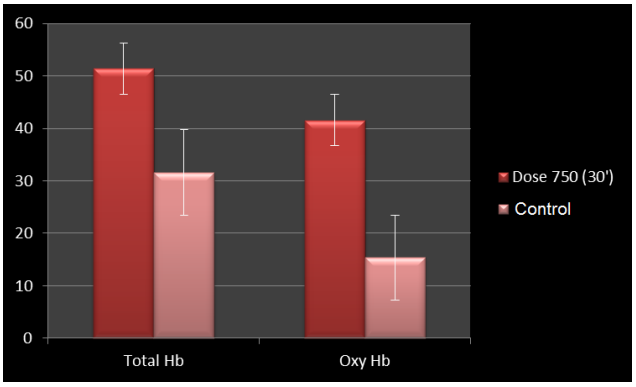

Fig. 5. Comparison of Total Hb and Oxy Hb between control and 750 mg/kg (30 min).

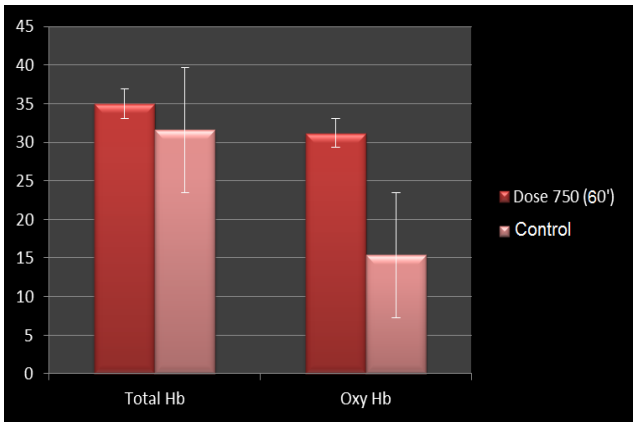

Fig. 6. Comparison of Total Hb and Oxy Hb between control and 750 mg/kg (60 min).

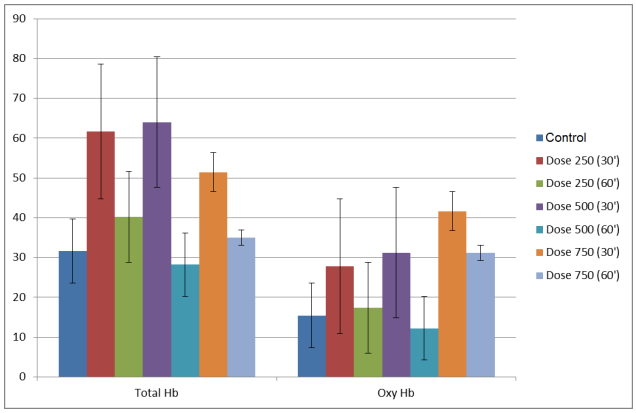

Fig. 7. Overall comparison of Total Hb and Oxy Hb across all treatment groups and time points.

6. Discussion

The present study investigated the acute hematological effects of trifluralin, a widely used dinitroaniline herbicide, in female Wistar rats. By quantifying total hemoglobin and oxyhemoglobin levels at two time points following intraperitoneal administration of three different doses, the results revealed a biphasic, dose- and time-dependent pattern of hematological response.

At 30 minutes post-injection, total hemoglobin levels increased significantly in all treated groups (250, 500, and 750 mg/kg), with the highest elevation observed at 500 mg/kg. This early rise may reflect hemoconcentration, splenic contraction, or redistribution of erythrocytes in response to chemical stress. Such compensatory mechanisms have been described in acute toxicological models and may serve to preserve oxygen transport under transient challenge (de Kort *et al.*, 2020; Rifai, 2017).

Oxyhemoglobin levels also rose at 30 minutes, with the most pronounced and statistically significant increase occurring at 750 mg/kg. This suggests a dose-dependent enhancement in oxygen-binding capacity, potentially reflecting early physiological adaptation. However, the response was not sustained: by 60 minutes, hemoglobin parameters in the lower dose groups declined toward baseline, while oxyhemoglobin at 750 mg/kg showed a significant reduction, indicating persistent hypoxic stress or oxidative disruption.

Previous studies have primarily focused on the genotoxic and hepatotoxic effects of trifluralin (Fernandes et al., 2013; Zhang et al., 2023), with limited attention to its hematological impact. The current findings expand this understanding by demonstrating acute modulation of hemoglobin dynamics, including transient stimulation and delayed suppression. The decline in oxyhemoglobin at high dose and later time point aligns with documented cases of methemoglobinemia and red blood cell deformation following exposure to nitroaromatic compounds (Iolascon *et al.*, 2021).

From a mechanistic perspective, hemoglobin undergoes continuous redox cycling, and its autoxidation generates reactive oxygen species such as superoxide and hydrogen peroxide, which can damage erythrocyte membranes and impair oxygen delivery (Daraghmeh & Karaman, 2024; Rifkind et al., 2003). Disruption of this balance may lead to oxidative stress, membrane instability, and impaired tissue oxygenation. The sustained suppression of oxyhemoglobin at 750 mg/kg supports this hypothesis and warrants further investigation into antioxidant defense systems and erythrocyte integrity (Rifkind *et al.*, 2015).

The use of Wistar rats provides translational relevance to veterinary toxicology, given their well-characterized hematological baselines and physiological similarity to larger mammals (Patel et al., 2024). Spectrophotometric analysis based on Drabkin's method enabled precise quantification of hemoglobin derivatives, offering a robust framework for assessing pesticide-induced hematotoxicity (Drabkin, 1946; Chinwuba *et al.*, 2024).

This study represents a novel contribution by quantifying oxyhemoglobin dynamics following acute trifluralin exposure. The results highlight the herbicide's potential to induce both transient stimulation and delayed hypoxia, establishing a foundation for future research into its systemic effects. These findings may inform regulatory guidelines and veterinary risk assessment protocols, particularly in contexts of frequent or unavoidable pesticide exposure.

Further studies are needed to explore subacute and chronic exposure scenarios, dose thresholds for irreversible damage, and the role of antioxidant supplementation or membrane stabilization strategies. Comparative investigations across species and developmental stages could enhance translational relevance and guide safer pesticide practices in both veterinary and agricultural settings.

7. Conclusion

Acute exposure to trifluralin significantly alters hemoglobin and oxyhemoglobin concentrations in female Wistar rats, indicating a rapid modulation of oxygen transport mechanisms. The dose- and time-dependent elevations observed-especially the peak in total hemoglobin at 500 mg/kg and oxyhemoglobin at 750 mg/kg within 30 minutes-suggest transient hematological stimulation followed by dose-sensitive shifts. By applying spectrophotometric analysis, this research provides novel quantitative insight into the systemic effects of a widely used herbicide.

These findings highlight the importance of incorporating hemoglobin-based biomarkers into pesticide toxicity assessments and underscore the need for further investigation into compensatory responses, oxidative stress, and long-term consequences. The study establishes a foundation for future research in veterinary toxicology and environmental health, particularly regarding erythrocyte dynamics and oxygen transport regulation.

References

[1] Basu D, Kulkarni R. Overview of blood components and their preparation. Indian journal of anaesthesia. 2014 Sep 1;58(5):529-37.

[2] Chinwuba UR, Chi-amaka NJ, Nonso EE, Precious E, Jeremiah O, Collins K, Uche NC, Ehimen AW, Chiwendu AO. Biochemical And Hematological Changes In Male Wistar Rats Following Subacute Exposure To Pesticide.

[3] Cohn CS, Shaz BH. Blood and its components. JAMA. 2023 Nov 21;330(19):1903-4.

[4] Daraghmeh DN, Karaman R. The redox process in red blood cells: Balancing oxidants and antioxidants. Antioxidants. 2024 Dec 31;14(1):36.

[5] de Kort M, Weber K, Wimmer B, Wilutzky K, Neuenhahn P, Allingham P, Leoni AL. Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: acceptable value ranges, definition of severity degrees, and vehicle effects. Toxicology Research and Application. 2020 Jul 1;4:2397847320931484.

[6] Drabkin DL. Spectrophotometric studies: XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. Journal of biological chemistry. 1946 Aug 1;164(2):703-23.

[7] Fernandes TC, Pizano MA, Marin-Morales MA. Characterization, modes of action and effects of trifluralin: a review. Herbicides-current research and case studies in use. 2013 Jun 12.

[8] Iolascon A, Bianchi P, Andolfo I, Russo R, Barcellini W, Fermo E, Toldi G, Ghirardello S, Rees D, Van Wijk R, Kattamis A. Recommendations for diagnosis and treatment of methemoglobinemia. American journal of hematology. 2021 Dec;96(12):1666-78.

[9] Patel S, Patel S, Kotadiya A, Patel S, Shrimali B, Joshi N, Patel T, Trivedi H, Patel J, Joharapurkar A, Jain M. Age-related changes in hematological and biochemical profiles of Wistar rats. Laboratory Animal Research. 2024 Feb 26;40(1):7.

[10] Rifai N. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics-E-Book: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics-E-Book. Elsevier Health Sciences; 2017 Jan 16.

[11] Rifkind JM, Mohanty JG, Nagababu E. The pathophysiology of extracellular hemoglobin associated with enhanced oxidative reactions. Frontiers in physiology. 2015 Jan 14;5:500.

[12] Rifkind JM, Nagababu E, Ramasamy S, Ravi LB. Hemoglobin redox reactions and oxidative stress. Redox report. 2003 Oct 1;8(5):234-7.

[13] European Food Safety Authority (EFSA). Conclusion on pesticide peer review regarding the risk assessment of the active substance trifluralin. EFSA Journal. 2009 Aug;7(8):327r.

[14] Zhang Q, He Z, Wang J. Acute Toxicity, Oxidative Stress, Toxicity Mechanism, and Degradation Dynamics of Trifluralin in Eisenia foetide (Annelida: Lumbricidae). Journal of Entomological Science. 2023 Jan 1;58(1):27-46.

Avicenna Veterinary Research
Vol. 1, No. 4, Winter 2025: 7-10
Bu-Ali Sina University

ORIGINAL ARTICLE

Clinical and Epidemiological Survey of Urinary Stones in Cats

Farnoosh Kaviani*, Erfan Jafari Moghadam
Department of Clinical Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article info

Article history:
Received 2025-08-30
Received in revised form
2025-08-28
Accepted 2025-08-01

Keywords:
Feline urolithiasis
Urinalysis
Risk factors
Qazvin

Abstract

Urinary tract stones in cats can have severe and dangerous complications, so evaluation the statistics of affected animals in different geographical areas can be helpful. The most common stone types are calcium oxalate (CaOx) and struvite that prevalence has changed over time. Breed, age, sex, lifestyle and underlying conditions influence the formation of urinary stones. In this study, 537 cats were evaluated over a six-month period at a veterinary clinic in Qazvin. Approximately 7% of cats have urinary tract stones, that 90% in the bladder and 10% in the kidney. Older male cats are more likely to develop calcium oxalate (CaOx) uroliths, while struvite stones occur in younger female cats. Persian and Domestic Shorthair breeds were most affected and were at greater risk. Urinalysis revealed hematuria (72%), crystalluria (89%), and urine pH >7 (40%). These results highlight value of urinalysis, combined with clinical assessment and imaging, for effective diagnosis and management in feline.

1. Introduction

Urinary tract stones (urolithiasis) are a major disease in cats that can affect both upper and lower urinary tracts and, in severe cases, can lead to serious clinical complications and even death (Kopecny *et al.*, 2021). s This condition is associated with clinical signs such as hematuria, dysuria, pollakiuria, urethral obstruction, and in some cases, acute renal failure. According to the Merck Veterinary Manual, urolithiasis is one of the most common causes of urinary tract diseases in cats and contributes significantly to patient visits to veterinary clinics (Gomes *et al.*, 2018). Complete urethral obstruction can result in uremia and renal failure within 36 to 48 hours, and if untreated, may progress to coma or death within about 72 hours. The term urinary stone refers to the presence of calculi in any part of the urinary tract, although most cases are reported in the bladder and urethra (Gomes *et al.*, 2018). Uroliths are classified based on mineral composition; therefore, qualitative and quantitative analyses are essential for selecting the appropriate therapeutic and preventive approach. Among the mineral compositions, calcium oxalate (CaOx) and struvite have historically been the most common types of urolith types in cats, although their relative frequencies have changed over time (Gomes *et al.*, 2018).

In recent years, a decrease CaOx stone and an increase in struvite stones have been reported, which requires more detailed investigations. Epidemiological knowledge of the prevalence and composition of urinary stones is an essential basis for treatment planning and preventive measures. Previous studies (including the analysis of 4495 canine and feline uroliths in the Benelux region and studies conducted between 1981 and 2008 have have emphasized the importance of collecting epidemiological data to better understand trends in urolith composition and prevalence (Picavet

*Corresponding author: F. Kaviani (Assistant Professor)
E-mail address: f.kaviani@basu.ac.ir
http://dx.doi.org/10.22084/avr.2025.31539.1018

et al., 2007). For instance, an epidemiological study in Mexico that examined data from 81 feline urolithe, it was found that oxalate (54.3%) and struvite (32.1%) to be the most common, followed by urate, cystine, and silica in smaller proportions (Mendoza-López *et al.*, 2019). These results emphasize the dominance of two main compositions (CaOx and struvite) in the formation of feline urinary stones (Kopecny *et al.*, 2021, Hesse et al., 2012). In addition, retrospective studies have shown that less common uroliths, such as urate stones, are often associated with metabolic disorders and liver disease. For example, urate stones in cats usually occur in association with underlying diseases and that careful laboratory and diagnostic workup is necessary to find the underlying cause (Dear et al., 2011). Clinical presentation in patients with urinary calculi can be highly variable. Some cats may remain asymptomatic, while others develop hematuria, dysuria, or even acute urethal obstruction. The type of stone, location, and associated conditions such as chronic kidney disease (CKD) can be critical in determining treatment and prognosis (Taylor *et al.*, 2025).

Urinary tract diseases are one of the main reasons for visiting veterinary clinics and can affect animals of all ages, breeds and sexes. urolithiasis is the second most important cause of clinical signs consistent with urinary tract disease in felids. It should not be viewed as a single disease entity but rather as a multifactorial disorder influenced by nutrition, metabolic abnormalities, genetic predisposition, and infections. Other risk factors include breed, sex, age, obesity, sedentary lifestyle, geographical location, and climate (Gomes *et al.*, 2018). Understanding the risk factors and pathophysiology of urolithiasis is critical for selecting appropriate therapeutic and preventive measures. This knowledge not only facilitates diagnosis and treatment but also helps identify at-risk populations and minimize exposure to risk factors. Epidemiological data on feline urolithiasis are therefore essential as the foundation for both treatment and prevention (Hesse *et al.*, 2012).

2. Materials and Methods

This study was conducted on cats referred to a veterinary clinic in Qazvin, Iran, between september 22, 2024, and March 20, 2025. Out of 537 referred cases, all cats presenting with urinary complaints (such as hematuria, burning urination, obstruction, or frequent urination), were screened via ultrasonography for urolithiasis. They were divided according to breed, age, and sex. the urine of these animals was obtained by cytocentesis and evaluated with urine dipstick. The samples were stored in sterile containers and examined within a maximum of 60 minutes. Uroliths detected by ultrasonography were categorized as bladder stones or renal stones (including pelvic and parenchymal locations). For each affected cat, data on sex, age, breed, stone location, and urinalysis results were recorded. Data were analyzed using SPSS software, and statistical significance was set at $P<0.05$ (t-test).

3. Result

During the six-month study period, 37 out of 537 cats (6.9%) were diagnosed with urolithiasis. Among these, 33 (89%) had bladder stones and 4 (11%) had renal stones (including pelvis or parenchyma). Gender analysis showed that the prevalence of the disease was higher in male cats than in females; 22 (59%) were males and 15 (41%) were females. In terms of age, the highest frequency was observed in the 4–6-year age group (15 cases; 41%), followed by the 7–10-year age group (10 cases; 27%), the 1–3-year age group (8 cases; 22%), and finally, over 10 years old (4 cases; 11%). Domestic shorthair (DSH) cats were most affected (20 cases; 54%), followed by Persian cats (10 cases; 27%), and other breeds (7 cases; 19%). In the urinalysis, Hematuria was the most frequent abnormality (25 cases; 68%), followed by proteinuria (10 cases; 27%). Crystalluria revealed struvite (12 cases; 32%), calcium oxalate (8 cases; 22%), and mixed/other types (17 cases; 46%).

These results suggest that bladder stones were the most common form of urolithiasis in the studied population, with middle age, male sex, and DSH breed representing major risk factors.

4. Discussion

In this study, the prevalence of urinary stones among cats visiting the clinic during the second half of 1403 was evaluated. Out of 543 referred cats, approximately 7% were diagnosed with urinary tract stones, of which about 90% were located in the bladder and 10% in the kidneys. Regarding sex distribution, three out of every five affected cats were male and two were female. The highest prevalence was observed in Persian and Domestic Shorthair cats.

Calcium oxalate formation was associated with increasing age, and struvite stones were more common in younger cats. Therefore, the chance of successful stone removal was higher in younger cats than other. For example, a retrospective study in the Republic of Ireland and Northern Ireland found that 54.3% of urinary stones were calcium oxalate, 32.1% were struvite, 7.4% were purine (urate and xanthine), and 6.2% were other types and Nearly 90% of the reported stones were from domestic short-haired cats Also males mainly affected by calcium oxalate stones and females more frequently presenting with struvite stones (Ortega et al., 2023). Similar studies in Mexico (Mendoza-López *et*

al., 2019), Thailand (Hunprasit *et al.*, 2019), Poland (Lew-Kojrys *et al.*, 2017), the United Kingdom (Geddes et al., 2023), and Kenya (Kimani *et al.*, 2021) Females are more likely to be affected by struvite stones (Dvorska *et al.*, 2015). Genetic predisposition and lifestyle, such as dry diets or indoor housing, were also identified as factors (Hsu *et al.*, 2022).

Urinalysis findings indicated crystalluria in 89% of cases and hematuria was also observed in 72%. Urine pH was above 7 in 40% of cases and urine specific gravity was lower than the physiological state of the animal (Lund *et al.*, 2013). These findings demonstrate the importance of thorough urinalysis for early detection and prediction of stone type, Researchs have also shown that acidity affects the type of stone (alkaline urine causes struvite stones and acidic urine causes calcium oxalate stones), so urinalysis is also helpful in treatment (Okafor *et al.*, 2019).

According to *The Merck Veterinary Manual* (12th ed., 2023), Epidemiological data suggest that the prevalence of urinary stones in cats is relatively low, but their clinical significance is high, including urinary obstruction and acute renal failure. Studies have indicated that complete urethral obstruction in cats can lead to uremia within 36–48 hours and may result in coma or death within 72 hours.

Finally, comprehensive clinical assessment urine and use imaging (ultrasonography and radiography), is essential not only for the diagnosis and treatment of urinary stones but also for preventing and guiding dietary (Ayoub et al., 2024). Recognizing risk factors such as age, sex, breed, diet, and environmental conditions is crucial for identifying susceptible populations and minimizing exposure to stone-promoting factors (Mendoza-López *et al.*, 2019).

References

[1] Ayoub SM, Mostafa MB, Abdelgalil AI. Studies on feline lower urinary tract disease in Egypt Cat population. Journal of Applied Veterinary Sciences. 2024 Jan 1;9(1):61-72.

[2] Dear JD, Shiraki R, Ruby AL, Westropp JL. Feline urate urolithiasis: a retrospective study of 159 cases. Journal of feline medicine and surgery. 2011 Oct;13(10):725-32.

[3] Dvorska J, Saganuwan SA. A review on urolithiasis in dogs and cats. Bulgarian Journal of Veterinary Medicine. 2015 Mar 1;18(1):1-8.

[4] Geddes RF, Davison LJ, Elliott J, Syme HM, O'Neill DG. Risk factors for upper urinary tract uroliths and ureteral obstruction in cats under referral veterinary care in the United Kingdom. Journal of Veterinary Internal Medicine. 2023 Mar;37(2):567-77.

[5] Gomes VD, Ariza PC, Borges NC, Schulz Jr FJ, Fioravanti MC. Risk factors associated with feline urolithiasis. Veterinary research communications. 2018 Mar;42(1):87-94.

[6] Hesse A, Orzekowsky H, Frenk M, Neiger R. Epidemiological data of urinary stones in cats between 1981 and 2008. Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere. 2012 Apr 1;40(2):95-101.

[7] Hsu HH, Ueno S, Miyakawa H, Ogawa M, Miyagawa Y, Takemura N. Upper urolithiasis in cats with chronic kidney disease: prevalence and investigation of serum and urinary calcium concentrations. Journal of Feline Medicine and Surgery. 2022 Jun;24(6):70-5.

[8] Hunprasit V, Pusoonthornthum P, Koehler L, Lulich JP. Epidemiologic evaluation of feline urolithiasis in Thailand from 2010 to 2017. The Thai Journal of Veterinary Medicine. 2019;49(1):101-5.

[9] Kimani, Wilson K., J. Nguhiu-Mwangi, John D. Mande, and Susan W. Mbugua. "Prevalence of urinary tract conditions and factors associated with urolithiasis in domestic cats in Nairobi, Kenya." (2021): 196-201.

[10] Kopecny L, Palm CA, Segev G, Larsen JA, Westropp JL. Urolithiasis in cats: Evaluation of trends in urolith composition and risk factors (2005-2018). J Vet Intern Med. 2021 May;35(3):1397-1405.

[11] Lew-Kojrys S, Mikulska-Skupien E, Snarska A, Krystkiewicz W. Evaluation of clinical signs and causes of lower urinary tract disease in Polish cats. Veterinární medicína. 2017 Jul 1;62(7).

[12] Lund HS, Krontveit RI, Halvorsen I, Eggertsdóttir AV. Evaluation of urinalyses from untreated adult cats with lower urinary tract disease and healthy control cats: predictive abilities and clinical relevance. Journal of feline medicine and surgery. 2013 Dec;15(12):1086-97.

[13] Mendoza-López CI, Del-Angel-Caraza J, Aké-Chiñas MA, Quijano-Hernández IA, Barbosa-Mireles MA. Epidemiology of feline urolithiasis in Mexico (2006–2017). Journal of Feline Medicine and Surgery Open Reports. 2019 Nov;5(2):2055116919885699.

[14] Merck Veterinary Manual. 12th ed. Kenilworth (NJ): Merck & Co., Inc.; 2023.

[15] Okafor CC, Pearl DL, Blois SL, Lefebvre SL, Yang M, Lund EM, Dewey CE. Factors associated with

hematuric struvite crystalluria in cats. Journal of feline medicine and surgery. 2019 Oct;21(10):922-30.

[16] Ortega CJ, Stavroulaki EM, Lawlor A, Lulich J, Cuq B. Retrospective analysis of 131 feline uroliths from the Republic of Ireland and Northern Ireland (2010-2020). Irish Veterinary Journal. 2023 Feb 6;76(1):2.

[17] Picavet P, Detilleux J, Verschuren S, Sparkes A, Lulich J, Osborne C, Istasse L, Diez M. Analysis of 4495 canine and feline uroliths in the Benelux. A retrospective study: 1994–2004. Journal of animal physiology and animal nutrition. 2007 Jun;91(5-6):247-51.

[18] Taylor S, Boysen S, Buffington T, Chalhoub S, Defauw P, Delgado MM, Gunn-Moore D, Korman R. 2025 iCatCare consensus guidelines on the diagnosis and management of lower urinary tract diseases in cats. Journal of feline medicine and surgery. 2025 Feb;27(20):1098612X241309176.

Avicenna Veterinary Research
Vol. 1, No. 4, Winter 2025: 11-19
Bu-Ali Sina University

REVIEW ARTICLE

A Mini Review on *Neospora Caninum:* Updates and Epidemiology in Iran

Jamal Gharekhani[a,*], **Mehdi Ebrahimi**[b]

[a] Iranian Veterinary Organization, Hamedan, Iran.
[b] Iranian Veterinary Organization, Tabriz, Iran.

Article info

Article history:
Received 2025-11-21
Received in revised form
2025-11-30
Accepted 2025-12-03

Keywords:
Neospora Caninum
Neosporosis
Epidemiology
Biology
Pathogenesis

Abstract

Neospora caninum is a globally distributed parasite affecting animals, especially dogs and cattle. Its pathogenesis depends on the balance between the parasite's ability to invade and multiply within cells and the host's capacity to limit infection. Encephalomyelitis and polymyositis are the most significant pathological findings. The tachyzoites multiply inside cells, causing necrosis and cell death. Clinical signs such as abortion and neuromuscular paralysis are predominant in cattle and dogs, respectively. The most common histopathological features include necrotic foci in the brain. The overall prevalence of *N. caninum* in dogs and cattle worldwide is 17.1% and 20%, respectively. In Iran, infection rates vary from 3.8% to 76.2% in cattle, zero to 54.6% in dogs, 0.9% to 9.9% in sheep, 6.2% to 10.8% in goats, 19.2% to 55.9% in buffaloes, 20% to 2.42% in horses, 52% in donkeys, 3.2% to 27% in camels, 14% to 19% in cats, zero to 20.4% in rodents, 17.3% in chickens, 9.8% to 30.4% in pigeons, 2.8% to 3.7% in sparrows, and 9.9% in crows. Managing the disease to reduce infection can improve animal productivity and help prevent economic losses. To control the disease on a farm or regionally, it is advisable to start with an initial assessment of the disease's epidemiology and risk factors.

1. Introduction

Neospora caninum (*N. caninum*) is an obligate apicomplexan parasite (Dubey *et al.,* 2007). A wide range of animals are hosts for this parasite, but the main definitive and intermediate hosts are dogs and cattle, respectively (Dubey and Schares, 2011). Transplacental transmission of *N. caninum* is the primary route of infection spread (up to 95%) in animals; however, horizontal transmission of the infection via the ingestion of oocysts has an important role in causing the disease and stormy abortions (Gharekhani and Yakhchali, 2019, 2020; Gharekhani *et al.,* 2021a). Dogs are the main hosts that have a significant impact on the life cycle of this parasite (Gharekhani *et al.,* 2019; Gharekhani *et al.,* 2020a).

Abortion and neuromuscular disorders are the top clinical manifestations in cattle and dogs, respectively (Gharekhani, 2014; Heidari *et al.,* 2014; Gharekhani *et al.,* 2013a; Gharekhani and Heidari, 2014a). Congenital infections in sheep and goats cause lambs to be born with encephalitis (Gharekhani *et al.,* 2013b, 2016, 2018). *Neospora hughesi* is a species closely related to *N. caninum,* which causes rapid-onset nervous system impairment and paralysis in horses, commonly referred to as Equine Protozoal Myeloencephalitis (EPM) (Gharekhani *et al.,* 2013c). Regarding human neosporosis, studies have shown that there are varying levels of anti-*N. caninum* antibody titers in patients with AIDS and neurological symptoms. This dis-

*Corresponding author: J. Gharekhani (PhD)
E-mail address: Gharekhani_76@yahoo.com
http://dx.doi.org/10.22084/AVR.2025.31983.1024

ease has not yet been confirmed in humans and requires further investigations (Gharekhani *et al.,* 2021b).

Neosporosis significantly affects the economy, mainly due to abortion (Gharekhani, 2014). The yearly estimated costs vary from a median of US$1.119 million within New Zealand's beef sector to around US$546.3 million in US dairy farms. In just these two nations, the total annual costs surpass US$1 billion (Richel *et al.,* 2013). Different laboratory methods, such as immuno-serology, pathology, bioassay, cell culture and various molecular biology, are used for detecting the infection (Gharekhani *et al.,* 2014, 2021a, 2022, 2023). A precise diagnosis of abortions linked to *Neospora*-infection is attained by evaluating the timing of the abortion, conducting histopathological examinations, employing different laboratory techniques, and eliminating other potential causes of abortion (Gharekhani *et al.,* 2012; Gharekhani and Tavoosidana, 2013; Gharekhani and Heidari, 2014b).

The control of *N. caninum* infection/neosporosis in farms is divided into two main categories. The programs aim to reduce infection in final hosts to limit the horizontal transmission of the parasite, as well as methods to decrease infection in intermediate hosts, prevent vertical transmission, and reduce the economic losses. Vaccination is also a control measure for both groups of hosts (Gharekhani and Yakhchali, 2022).

2. Epidemiology in Iran and other Countries

Neosporosis is a highly prevalent disease worldwide (Dubey *et al.,* 2007; Dubey and Schares, 2011; Gharekhani *et al.,* 2020a). There have been varying accounts of *N. caninum* infection from different hosts (Dubey *et al.,* 2007; Dubey and Schares, 2011). In a comprehensive evaluation (Anvari *et al.,* 2020), the pooled prevalence of *N. caninum* in dogs was 17.1%. Moreover, the infection rates were found to be 15.8% for males and 15.1% for females. Regarding different locations, the infection rate was 26.5% in Africa, 21.3% in Australia, 19% in Asia, 17.7% in Europe, and 15% in America. In a similar study, the pooled prevalence in the cattle population has been reported to be 20% (America 20%, Asia 18%, Europe 15%, Africa 13% and Australia 8%). The infection rate was higher in dairy cattle than in beef cattle and also in cows with a history of abortion 1.6 times higher than in others (Ribeiro *et al.,* 2019).

For the first time in Iran, *N. caninum* infection was reported in dairy cattle from Mashhad (Sadrebazzaz *et al.,* 2004). Reports of *Neospora* infection in various hosts, especially cattle and dogs, have been reported in Iran (Gharekhani *et al.,* 2020a). In a meta-analysis study, the seroprevalence of *N. caninum* in cattle was reported to be 23.6%, and a statistically significant association was detected between the infection rate and history of abortion. There was no statistically significant difference among infection, age, and breed (Ansari-Lari, 2020). In a recent report on infection from Iran, this rate was estimated to range from 3.8% to 76.2% in cattle, zero to 54.6% in dogs, 0.9% to 9.9% in sheep, 6.2% to 10.8% in goats, 19.2% to 55.9% in buffaloes, 20% to 2.42% in horses, 52% in donkeys, 3.2% to 27% in camels, 14% to 19% in cats, zero to 20.4% in rodents, 17.3% in chickens, 9.8% to 30.4% in pigeons, 2.8% to 3.7% in sparrows, and 9.9% in crows (Gharekhani *et al.,* 2020a). All studies conducted in Iran are summarized in Tables 1-5.

3. Clinical and Pathological Findings

Neuromuscular disorders are dominant in dogs. Motor nerve paralysis and muscle inflammation cause progressive muscle contraction and fibrosis, as well as joint stiffness (Gharekhani *et al.,* 2013a, 2019, 2020b). In addition, diarrhea, multifocal pulmonary consolidation (necrotic and purulent bronchopneumonia), fibrinohemorrhagic enteritis, myocarditis, and nonpurulent encephalitis are common (Dubey and Schares, 2011; Silva and Machado, 2016). In pregnant dogs that are chronically infected, bradyzoites switch back to tachyzoites and cross the placenta, causing infection of the fetus. finally, animals die due to CNS and muscular weakness (Silva and Machado, 2016).

Abortion is the most significant symptom in pregnant cows. The majority of abortions caused by *N. caninum* happen during the 5–6-month period of gestation. In the endemic abortions, about 3 to 5% of the herd aborts annually without any sudden increase, whereas in epidemic outbreaks, more than 12.5% of pregnant animals abort within 2 months (Dubey *et al.,* 2017). Exophthalmia, asymmetrical eyes, CNS disorders such as hydrocephalus, and spinal cord stenosis are rare symptoms (Bowman, 2015). The placental inflammation, focal non-suppurative encephalitis, non-suppurative myocarditis, myositis, and extensive infiltration of non-suppurative inflammatory cells into other tissues are common (Dubey *et al.,* 2017). Cysts containing bradyzoites form mainly in brain tissue. Multifocal necrosis and non-suppurative encephalomyelitis are the main lesions in aborted fetuses. The parasite invades the placental blood vessels, causing placental inflammation and vasculitis in the fetus; extensive placental necrosis is also observed after chorioallantoic degeneration (Sasani *et al.,* 2013).

4. Molecular Biology and Pathogenesis

The surface proteins of *N. caninum* play a crucial role in its survival, as they initiate the interactions between the pathogen and the surface molecules of host cells,

as well as the host's immune response. The surface of this parasite is coated with a family of glycosylphosphatidylinositol (GPI)-linked proteins (SRSs). Attachment of the parasite to host cells and interaction with the host immune response are mediated by SRS proteins to regulate the virulence of the parasite.

Table 1
Frequency of *Neospora caninum* infection in cattle in different regions of Iran.

Region	Sample type	Diagnostic method	No. of sample	Positive%	References
	blood	ELISA	200	19	(Razmi *et al.,* 2013)
	Brain of aborted fetus	PCR	200	11.5	(Razmi *et al.,* 2013)
	Blood	ELISA	116	24.3	(Mikhchi *et al.,* 2013)
Neishabour	blood	ELISA	100	26	(Nourollahifard et al., 2017)
Ahvaz	blood	ELISA	557	21	(Hajikolaei et al., 2008)
Golestan province	blood	ELISA	800	13.4	(Sattari et al., 2011)
Babol	blood	ELISA	237	32	(Youssefi et al., 2009)
Garmsar	blood	ELISA	104	38.5	(Ranjbarbahadori et al., 2010)
Arak	Brain of aborted fetus	PCR	38	26.3	(Khani *et al.*, 2018)
Tehran	blood	ELISA	768	38.8	(Salehi et al., 2010)
Kerman	blood	ELISA	285	12.6	(Nourollahifard et al., 2008)
Tabriz	blood	ELISA	236	17.8	(Gharedaghi, 2012a)
	blood	ELISA	76	18.4	(Nematollahi et al., 2013)
Shiraz	blood	ELISA	253	30.4	(Ansari Lari et al., 2017)
	blood	ELISA	180	32.1	(Tavanaee and Namavari, 2017)
Isfahan	blood	ELISA	611	32.1	(Morovati and Noaman, 2016)
	blood	ELISA	1500	26.3	(Hosseininejad et al., 2017)
	blood	ELISA	216	19	(Noaman and Nabinejad, 2020)
Lorestan	blood	ELISA	347	9.8	(Nayebzadeh *et al.,* 2015)
Shahrekord	Brain of aborted fetus	PCR	100	11	(Rafati and Jafarian, 2014)
	milk	PCR	100	24	(Alipour *et al.,* 2018)
Hamedan	blood	ELISA	400	20	(Gharekhani *et al.,* 2012)
	blood	ELISA	1406	17.4	(Gharekhani *et al.,* 2013a)
Sanandaj	blood	ELISA	336	17.6	(Adhami *et al.,* 2019)
Hamedan and Sanandaj	blood	ELISA	768	14.2	(Gharekhani and Heidari, 2014a)
Kurdistan	blood	ELISA	368	7.8	(Heidari *et al.,* 2014)
Sistan	blood	ELISA	184	3.8	(Noori *et al.,* 2019)

ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

Table 2
Frequency of *Neospora caninum* infection in dogs in different regions of Iran.

Animals type	Region	Sample type	Diagnostic method	No. of sample	Positive%	References
Stray dogs	Tehran	blood	ELISA	42	2.2	(Pouramini et al., 2017)
	Urmia	blood	IFAT	135	26.6	(Yakhchali et al., 2010)
	Tabriz	blood	IFAT	100	31	(Gharedaghi, 2012b)
	Hamedan	blood	IFAT	200	52.8	(Gharekhani et al., 2013a)
		blood	ELISA	180	5	(Gharekhani *et al.,* 2019)
Pet dogs	Tehran	Stool	IFAT	50	20	(Malmasi et al., 2007)
	Mashhad	Stool	PCR	85	0	(Razmi, 2009)
	Hamedan	blood	ELISA	184	4.9	(Gharekhani *et al.,* 2020b)
Farm dogs	Tehran	blood	IFAT	50	28	(Haddadzadeh et al., 2007)
	Mashhad	Stool	PCR	89	2.2	(Razmi, 2009)
	Lorestan	Stool	PCR	428	2.1	(Dalimi et al., 2014)

ELISA: enzyme-linked immunosorbent assay; IFAT: indirect immunofluorescence antibody test; PCR: polymerase chain reaction.

Table 3
Frequency of *Neospora caninum* infection in sheep and goats in different regions of Iran.

Animals type	Region	Sample type	Diagnostic method	No. of sample	Positive%	References
Sheep	Lorestan	blood	ELISA	586	1.5	(Ezatpour et al., 2013)
	Tabriz	brain tissue	PCR	70	8.5	(Asadpour et al., 2013)
	Tehran	brain tissue	PCR	330	3.9	(Arbabi et al., 2016)
	Hamedan	blood	ELISA	358	2.2	(Gharekhani *et al.,* 2013b)
	Mashhad	brain tissue	PCR	71	9.9	(Razmi and Naseri, 2017)
	Khuzestan	blood	ELISA	550	6.8	(Gharekhani *et al.,* 2018)
Goat	Hamedan	blood	ELISA	450	6.2	(Gharekhani *et al.*, 2016)
	Khuzestan	blood	ELISA	158	10.8	(Gharekhani *et al.,* 2018)

ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

Table 4
Frequency of *Neospora caninum* infection in cats, camels, buffaloes, equids, and rodents in different regions of Iran.

Animals type	Region	Sample type	Diagnostic method	No. of sample	Positive%	References
Cat	Ahvaz	blood	DAT	100	14	(Hamidinejat *et al.,* 2011b)
Rodents	Ahvaz	blood	DAT	150	6	(Mosallanejad *et al.,* 2018b)
	Meshkin Shahr	blood	IFAT	157	20.4	(Nazari *et al.*, 2020)
Camel	Mashhad	blood	IFAT	120	5.8	(Sadrebazzaz et al., 2006)
	Esfahan	blood	IFAT	310	3.2	(Hosseininejad et al., 2009)
	Yazd	blood	DAT	254	3.9	(Hamidinejat et al., 2013)
Buffaloe	Ahvaz	blood	ELISA	188	55.9	(Pourmahdi-Borujeni *et al.*, 2015)
	Urmia	blood	ELISA	83	19.2	(Rezvan et *al.*, 2019)
Horse	Tabriz	blood	DAT	100	28	(Gharedaghi, 2011)
	Shiraz	blood	DAT	200	40	(Moraveji et al., 2011)
	Mashhad	blood	DAT	150	30	(Hosseini *et al.*, 2011)
	Hamedan	blood	DAT	120	40.8	(Gharekhani et al., 2013c)
	Ahvaz	blood	DAT	235	20	(Tavalla *et al.,* 2015)
Donkey	Hamedan	blood	DAT	100	52	(Gharekhani et al., 2013c)

DAT: direct agglutination test; IFAT: indirect immunofluorescence antibody test; ELISA: enzyme-linked immunosorbent assay.

Table 5
Frequency of *Neospora caninum* infection in birds in different regions of Iran.

Birds type	Region	Sample type	Diagnostic method	No. of sample	Positive%	References
Chicken[a]	Shiraz	blood	DAT	150	17.3	(Sayari et al., 2016)
Pigeon[b]	Ahvaz	brain tissue	PCR	102	9.8	(Bahrami *et al.,* 2016)
Sparrow[c]	Ahvaz	brain tissue	PCR	210	2.8	(Bahrami *et al.,* 2015)
	Tehran	brain tissue	PCR	217	3.7	(Abdoli *et al.,* 2015)
Crow[d]	Tehran	brain tissue	PCR	55	9.9	(Abdoli *et al.,* 2018)

a=Gallus domesticus; b=Columba livia; c=Passer domesticus; d=Corvus cornix
DAT: direct agglutination test; PCR: polymerase chain reaction.

Proteins linked to the surface, micronemes, rhoptries, and dense granules play crucial roles during the intracellular lytic cycle of *N. caninum*. Due to their role in host-parasite dissemination, these elements could constitute potential drug targets and vaccine candidates (Soltani *et al.,* 2013). There are biological differences in some strains. Nc-Liverpool has a high pathogenicity and causes fetal death in pregnant cows. However, Nc-Nowra is used for attenuated vaccines due to its low virulence. Nc-1 causes very severe clinical signs such as fetal death in pregnant cows, as well as granulomatous polymyositis and polyradiculoneuritis in dogs (Calarco *et al.,* 2018).

The ability of pathogenic protozoa to persist and spread relies on their capacity to escape or undermine the immune responses of their host, both innate and adaptive. Also, hormonal alterations during gestation may trigger reactivation of the parasite (Lopes *et*

al., 2012). The transmission rate has a direct connection with gestational age, perhaps related to placental vascularization because the placenta seems to be more permeable in the last trimester (Dubey *et al.,* 2017; Gharekhani and Yakhchali, 2020). The primary placental damage causes the release of maternal prostaglandins that, in turn, cause luteolysis and abortion. Additionally, the development of fetal lesions could result from the proliferation of *N. caninum* in the fetus or due to insufficient oxygen/nutrition (Dubey and Schares, 2011). The balance between the capacity of the tachyzoite to penetrate and multiply inside the cells has a positive impact on the parasite's pathogenesis. The cell-invasion process has two different steps: the adhesion to the host-cell surface and penetration into the cell. Invasion is an active process that demands metabolic energy exclusively from the parasite (Silva *et al.,* 2016). The inhibitor of aspartyl protease in *Neospora* known as pepstatin, plays an important role in assembling and trafficking the MIC and ROP proteins into host cells, and has a significant impact on parasite invasion. (Khan *et al.,* 2020). NC-P43 is one of the most important and largest surface proteins that play a key role in parasite adhesion and invasion into host cells (Dubey *et al.,* 2017). Interferon-gamma (IFN-γ), IL-12, and Th1 lymphocyte responses play a prominent role in the control of acute *Neospora*-infection. Increased IFN-γ, by decreasing BCL-2 expression, causes apoptosis of infected cells. Activation of macrophages by IFN-γ leads to inhibition and killing of *Neospora* through the production of nitric oxide (Dubey *et al.,* 2017). *N. caninum* causes necrotic lesions and cell death by multiplying tachyzoites, and also causes neuromuscular symptoms in the host by destroying nerve cells. In the CNS lesions, the highest parasite invasion has been observed in astrocytes (Khan *et al.,* 2020).

5. Conclusion

Various researchers have frequently detected traces of this infection in different animal species across various regions of the world using multiple diagnostic methods (Dubey *et al.,* 2007). Many risk factors contribute to the occurrence and spread of this infection on farms. Due to their abundance, livestock farms are usually not free of these factors (Gharekhani and Yackchali, 2019). *Neospora* has various mechanisms that promote pathogenicity and tissue lesions in the host, thereby supporting the parasite's survival and transmission cycle (Dubey *et al.,* 2017). Infected animals are usually asymptomatic. Additionally, the disease has been recognized as one of the main causes of economic losses in the livestock industry (Reichel *et al.,* 2013). Understanding the epidemiology of the disease in a region and its transmission methods helps determine the most appropriate strategy for controlling and preventing the parasite (Riberio *et al.,* 2019). Certainly, using multiple methods simultaneously and addressing risk factors will be more effective in managing the disease on a livestock farm or within an area. Managing the disease to reduce infections will increase animal production and offset economic losses. To control the disease on a farm or within a region, it is recommended to begin with an initial assessment of the disease epidemiology and risk factors.

References

[1] Abdoli A, Arbabi M, Dalimi A, Pirestani M. Molecular detection of Neospora caninum in house sparrows (Passer domesticus) in Iran. Avian Pathology. 2015; 44 (4): 319-22.

[2] Abdoli A, Arbabi M, Pirestani M, Mirzaghavami M, Ghaffarifar F, Dalimi A, Sadraei J. Molecular assessment of Neospora caninum and Toxoplasma gondii in hooded crows (Corvus cornix) in Tehran, Iran. Comparative Immunology, Microbiology and Infectious Diseases. 2018;57:69-73.

[3] Adhami G, Hoghooghi R, Dalimi A. The seroepidemiological investigation into Neospora caninum in cattle in Sanandaj, Kordestan province. Journal of Veterinary Microbiology. 2014;10(2):83-92.

[4] Alipour M, Shakerian A, Rahimi E. Prevalence of Toxoplasma gondii and Neospora caninum in different types of raw milk and traditional dairy product samples. Journal of Food Safety. 2018;e12575.

[5] Ansari-Lari M. Bovine neosporosis in Iran: a systematic review and meta-analysis. Preventive Veterinary Medicine. 2020;176:104913.

[6] Ansari-Lari M, Rowshan-Ghasrodashti A, Jesmani H, Masoudian M, Badkoobeh M. Association of Neospora caninum with reproductive performance in dairy cows: a prospective study from Iran. Veterinary Research Forum. 2017;8(2):109-14.

[7] Anvari D, Saberi R, Sharif M, Sarvi S, Hosseini S, Moosazadeh M, Hosseininejad Z, Nayeri T, Daryani A. Seroprevalence of Neospora caninum infection in dog population Worldwide: a systematic review and meta-analysis. Acta Parasitologica. 2020;65:273-290.

[8] Arbabi M, Abdoli A, Dalimi A, Pirestani M. Identification of latent neosporosis in sheep in Tehran, Iran by polymerase chain reaction using primers specific for the Nc-5 gene. Onderstepoort Journal of Veterinary Research. 2016;83(1):1-7.

[9] Asadpour R, Jafari-Joozani R, Salehi N. Detection of Neospora caninum in ovine abortion in Iran. Journal Parasitic Diseases. 2013;37(1):105-9.

[10] Bahrami S, Boroomand Z, Alborzi A, Namavari M, Mousavi B. A molecular and serological study of Neospora caninum infection in pigeons from southwest Iran. Veterinary Arhive. 2016;86(6):815-23.

[11] Bahrami S, Hamidinejat H, Mayahi M, Baloutaki M. A survey of Neospora caninum infection in sparrows (Passer domesticus) in Khuzestan province, Iran. Archive Razi Institute. 2015;70(4):279-81.

[12] Bowman DD. Georgis parasitology for veterinarians. 2015; 10th edition, Elsevier publication, Netherland.

[13] Calarco L, Barratt J, Ellis J. Genome wide identification of mutational hotspots in the apicomplexan parasite Neospora caninum and the implications for Virulence. Genome Biology Evolution. 2018;10(9):2417-31.

[14] Dalimi A, Sabevarinejad G, Ghafarifar F, Forouzandeh-Moghadam M. Molecular detection of Neospora caninum from naturally infected dogs in Lorestan province, West of Iran. Archive Razi Institute. 2014;69(2):185-90.

[15] Dubey JP, Hemphill A, Schares G, Calero-Bernal R. Neosporosis in animals. 2017; CRC Press is an imprint of Taylor Francis Group, USA.

[16] Dubey JP, Schares G. Neosporosis in animals-The last five years. Veterinary Parasitology. 2011;180:90-108.

[17] Dubey JP, Schares G, Ortegamora LM. Epidemiology and control of Neosporosis and N. caninum. Clinical of Microbiology Review. 2007;20:323-69.

[18] Ezatpour B, Alirezaei M, Hassanvand A, Zibaei M, Azadpour M, Ebrahimzadeh F. The first report of Neospora caninum prevalence in aborted and healthy sheep from West of Iran. Comparative Clinical Pathology. 2013;24(1):19-22.

[19] Gharedaghi Y. Seroepidemiology of Neospora sp., in horses in East-Azerbaijan province of Iran. Research Journal of Biological Science. 2011;6(5):224-6.

[20] Gharedaghi Y. Seroepidemiology of Neospora caninum in cattle in East-Azerbaijan province, North-West Iran. Journal of Animal and Veterinary Advances. 2012a; 11(5):645-8.

[21] Gharedaghi Y. Seroprevalence of Neospora caninum in stray dogs of Tabriz Iran. Journal of Animal and Veterinary Advances. 2012b;11(6):723-6.

[22] Gharekhani J. Seroprevalence of Neospora caninum and Toxoplasma gondii infections in aborted cattle in Hamedan, Iran. Journal of Advanced in Veterinary and Animal Research. 2014;1(2):32-5.

[23] Gharekhani J, Esmaeilnejad B, Rezaei H, Yakhchali M, Heidari H, Azhari M. Prevalence of anti-Neospora caninum antibodies in Iranian goats. Annals of Parasitology. 2016;62(2):111-4.

[24] Gharekhani J, Haddadzadeh H, Bahonar A. Prevalence of IgG antibody to Neospora caninum in dairy cattle of Hamedan province, west of Iran. Veterinary Research Forum. 2014;5(2):149-52.

[25] Gharekhani J, Heidari H. Serology-based comprehensive study of Neospora infection in domestic animals in Hamedan province, Iran. Journal of Advanced in Veterinary and Animal Research. 2014a;1(3):119-24.

[26] Gharekhani J, Heidari H. Detection of anti-Neospora caninum antibodies in Iranian native cattle. Journal of Advance Veterinary and Animal Research. 2014b;1(4):228-31.

[27] Gharekhani J, Heidari H, Akbarein H. Seroepidemiology of Neospora caninum in Iranian native and crossbreed cattle: A cross-sectional study. Journal of Veterinary Research. 2012;67(4):325-9.

[28] Gharekhani J, Rafaat MR, Heidari R, Hajipour N, Trotta M, Villanueva-Saz S. Assessment of Neospora caninum infection in bulls using serological and molecular techniques. Veterinary Parasitology: Regional Studies and Reports. 2023;100940. https://doi.org/10.1016/j.vprsr.2023.100940.

[29] Gharekhani J, Tavoosidana GR. Serological survey of Neospora caninum (Sarcocystidae) infection in beef cattle from Western Iran. Scientia Parasitologica. 2013;14(2):95-8.

[30] Gharekhani J, Tavoosidana GR, Akbarein H. Serological study of Neospora caninum infection in dogs and cattle from west of Iran. Comparative Clinical Pathology. 2013a;23(4):1203-7.

[31] Gharekhani J, Tavoosidana GR, Naderisefar GR. Seroprevalence of Neospora infection in horses and donkeys in Hamedan Province, Western Iran. Veterinary World. 2013c;6(9):620-2.

[32] Gharekhani J, Tavoosidana GR, Zandieh M. Seroprevalence of Neospora in sheep from Western Iran. Veterinary World. 2013b;6(10):709-10.

[33] Gharekhani J, Yakhchali M. Neospora caninum infection in dairy farms with history of abortion in west of Iran. Veterinary and Animal Science. 2019;8:100071.

[34] Gharekhani J, Yakhchali M. Vertical transmission of Neospora caninum in Iranian dairy cattle. Annals of Parasitology. 2020;66(4):495-500.

[35] Gharekhani J, Yakhchali M. Control measures to reduce Neospora caninum abortions in dairy farms: a pilot study in Iran. Veterinaria Italiana. 2022;58(2):237-44.

[36] Gharekhani J, Yakhchali M, Abbasi-Doulatshahi E, and Barati E. Seroprevalence of Neospora caninum and Toxoplasma gondii infections in stray dogs of Hamedan suburb, West of Iran. Avicenna Journal of Clinical Microbiology and Infection. 2019;6(2):57-60.

[37] Gharekhani J, Yakhchali M, Afshari A, and Adabi M. Herd-level contamination of Neospora caninum, Toxoplasma gondii and Brucella in milk of Iranian dairy farms. Food Microbiology. 2021a;100:103873.

[38] Gharekhani J, Yakhchali M, and Berahmat R. Neospora caninum infection in Iran (20042020): a review. Journal of Parasitic Diseases. 2020a;44:671-86.

[39] Gharekhani J, Yakhchali M, Esmaeilnejad B, Mardani K, Majidi G, Sohrabi A, Berahmat R, Alaei M. Seroprevalence and Risk Factors of Neospora caninum and Toxoplasma gondii in Small Ruminants in Southwest of Iran. Archives of Razi Institute Journal. 2018;73(4):305-10.

[40] Gharekhani J, Yakhchali M, Heidari R. Molecular detection and phylogenetic analysis of Neospora caninum in various hosts from Iran. Comparative Immunology, Microbiology and Infectious Diseases. 2022;80:101737:1-9.

[41] Gharekhani J, Yakhchali M, Keramat F, Berahmat R. A literature review of Neospora caninum infection in humans. Avicenna Journal of Clinical Microbiology and Infection. 2021b;8(3):113-6.

[42] Gharekhani J, Yakhchali M, and Khaltabadi-Farahani R. Prevalence and risk factors associated to Neospora caninum (Apicomplexa: Toxoplasmatidae) in pet dogs from Hamedan, West of Iran, 2016. Avicenna Journal of Clinical Microbiology and Infection. 2020b;7(1):22-6.

[43] Haddadzadeh H, Sadrebazzaz A, Malmasi A, Talei Ardakani H, Khazraii Nia P, Sadreshirazi N. Seroprevalence of Neospora caninum infection in dogs from rural and urban environments in Tehran, Iran. Parasitology Research. 2007;101(6):1563-5.

[44] Hajikolaei MH, Hamidinejat H, Ghorbanpoor M, Goraninejad S. Serological study of Neospora caninum infection in cattle from Ahvaz area, Iran. International Journal Veterinary Research. 2008;2(1):63-127.

[45] Hamidinejat H, Ghorbanpour M, Rasooli A, Nouri M, Hekmatimoghaddam S, Namavari MM, Pourmehdi-Borojeni M, Sazmand A. Occurrence of anti-Toxoplasma gondii and Neospora caninum antibodies in camels (Camelus dromedarius) in the center of Iran. Turkey Journal of Veterinary and Animal Science. 2013;37:277-81.

[46] Hamidinejat H, Mosalanejad M, Razi Jalalil H, Avizeh R, Ghorbanpour M. Prevalence of Neospora caninum in domestic cats from Ahvaz, Iran. Parasite. 2011b;1(10):8-9.

[47] Heidari H, Mohammadzadeh A, Gharekhani J. Seroprevalence of Neospora caninum in slaughtered native cattle in Kurdistan province, Iran. Veterinary Research Forum. 2014;5(1):69-72.

[48] Hosseini MH, Moraveji M, Tahamtan Y, Rahimian A, Mohammadi G, Namavari M. Seroprevalence of Neospora spp. in horses in North-East of Iran. Iranian Journal of Parasitology. 2011;6(2):64-8.

[49] Hosseininejad M, Mahzounieh M, Shams Esfandabadi N. Neospora caninum Suspects as one of the most important causes of abortion in large dairy farms in Isfahan, Iran. Iranian Journal of Parasitology. 2017;12(3):408-12.

[50] Hosseininejad M, Pirali-Kheirabadi K, Hosseini F. Seroprevalence of Neospora caninum infection in camels (Camelus dromedarius) in Isfahan province, Center of Iran. Iranian Journal of Parasitology. 2009;4(4):61-4.

[51] Khan A, Shaik J, Sikorski P, Dubey JP, Grigg M. Neosporosis: An Overview of Its Molecular Epidemiology and Pathogenesis. Engineering. 2020;6:109.

[52] Khani M, Arabkhazaeli F, Hosseini SD, Shayan P. Molecular detection of Neospora caninum in aborted fetuses of cattle farms in Arak. Journal of Veterinary Research. 2018;73(4):457-63.

[53] Lopes M, Zamboni D, Lujan H, Rodrigues M. Immunity to Protozoan Parasites. Journal of Parasitology Research. 2012; Article ID 250793, 3 pages, doi:10.1155/2012/250793.

[54] Malmasi A, Hosseininejad M, Haddadzadeh H, Badii H, Bahonar A. Serologic study of anti-Neospora caninum antibodies in household dogs and dogs living in dairy and beef cattle farms in Tehran, Iran. Parasitology Research. 2007;100,1143-1145.

[55] Mikhchi A, Jafarabadi GA, Torshizi ME. Seroprevalence of Neospora caninum in Holstein dairy cattle in Northeast of Iran. Research Opinion of Animal and Veterinary Science. 2013;3(12):453-6.

[56] Moraveji M, Hosseini M, Amrabadi O, Rahimian A, Namazi F, Namavari M. Seroprevalence of Neospora spp. in horses in South of Iran. Tropical Biomedicine. 2011;28(3):514-7.

[57] Morovati H, Noaman V. Seroepidemiology of Neospora caninum in dairy cattle farms with a history of abortion in Isfahan province, Iran. Journal of Veterinary Science and Animal Husbandry. 2016;4(3):304.

[58] Mosallanejad B, Razi Jalali MH, Hamidinejat H, Peighambari E. A serological survey on Neospora caninum infection in wild rats (Rattus rattus) in Ahvaz district, Iran. Journal of Zoonosis Diseases. 2018b;3(1):1-9.

[59] Nayebzadeh H, Nourollahi Fard S, Khall M, Zakan N. Seroprevalence of Neospora caninum infection in dairy cattle in West of Iran. Istanbul Veterinary Journal. 2015;41(2):162-6.

[60] Nazari N, Shojaee S, Salimi S, Mohebali M , Ahmadifard N , Hamzavi Y, Zarei Z, Farahmand-Rad R, Bozorgomid A, Heydarian P. Serological survey of Neospora caninum and Toxoplasma gondii co-infection in rodents in Northwestern Iran. Iranian Journal of Parasitology. 2020;15(2):253-8.

[61] Nematollahi A, Moghaddam G, Jaafari R, Ashrafi Helan J, Norouzi M. Study on outbreak of Neospora caninum-associated abortion in dairy cows in Tabriz (Northwest Iran) by serological, molecular and histopathologic methods. Asian Pacific Journal of Tropical Medicine. 2013;6(12):942-6.

[62] Noaman V, Nabinejad A. Seroprevalence and risk factors assessment of the three main infectious agents associated with abortion in dairy cattle in Isfahan province, Iran. Tropical Animal Health and Production. 2020;52:2001-9.

[63] Noori M, Rasekh M, Ganjali M, Nourollahi-Fard SR. Seroprevalence of Neospora caninum infection and associated risk factors in cattle of Sistan areas, Southeastern Iran in 2016. Iranian Journal of Parasitology. 2019;14(2):340-6.

[64] Nourollahifard SR, Khalili M, Aminzadeh A. Prevalence of antibodies to Neospora caninum in cattle in Kerman province, South East Iran. Veterinary Arhive. 2008;78(3):253-9.

[65] Norollahifard SR, Khalili M, Fazli O, Sharifi H, Radfar MH. Seroprevalence of Neospora caninum in cattle of Neishabour, Northeast Iran. Slovenian Veterinary Research. 2017;54(1):5-9.

[66] Pouramini A, Jamshidi S, Shayan P, Ebrahimzadeh E, Namavari M, Shirian S. Molecular and serological detection of Neospora caninum in multiple tissues and CSF in asymptomatic infected stray dogs. Iranian Journal of Veterinary Medicine. 2017;11(2):105-112.

[67] Pourmahdi-Borujeni M, Hamidinejat H, Ghorbanpour M, Asefi H. Comparison of serological methods for the diagnosis of Neospora caninum infection in water buffalo (Bubalus bubalis). Journal Veterinary Microbiology. 2015;11(1):19-26.

[68] Rafati N, Jaafarian M. The determination of prevalence of in aborted fetuses in dairy cattle of Shahrekord area, Chaharmahal-va Bakhtiari province, by Nested-PCR. Journal of Veterinary Laboratory Research. 2014;6(1):45-50.

[69] Ranjbar bahadori S, Motevaselian A, Bokaie S, Yousefi M. Serological study of Neospora caninum in aborted dairy cattle in Garmsar, Iran. Comparative Biopathology. 2010;2:249-54.

[70] Razmi GR. Fecal and molecular survey of Neospora caninum in farm and household dogs in Mashhad area, Khorasan province, Iran. Korean Journal of Parasitology. 2009;47(4):417-20.

[71] Razmi G, Barati M. Prevalence of Neospora caninum and Toxoplasma gondii antibodies in bulk milk of dairy cattle, Mashhad, Iran. Archive Razi Institute. 2017;72(3):265-9.

[72] Razmi GR, Naseri Z. Molecular detection of Neospora caninum infection in ovine aborted foetuses in the Mashhad area, Iran. Annals of Parasitology. 2017;63(1):45-7.

[73] Razmi GR, Zarae H, Norbakhsh MF, Naseri Z. Estimating the rate of transplacental transmission of Neospora caninum to aborted fetuses in seropositive dams in Mashhad area, Iran. Iranian Journal of Veterinary Medicine. 2013;7(4):253-56.

[74] Reichel MP, Ayanegui-Alcrreca MA, Gondim L, Ellis J. What is the global economic impact of Neospora caninum in cattlethe billion-dollar question? International Journal of Parasitology. 2013;43:133-42.

[75] Rezvan H, Khaki A, Namavari M, Abedizadeh R. An investigation of the concurrency of anti-Neospora antibody and parasitemia in water buffalo (Bubalus bubalis) in Northwest of Iran. Veterinary Research Forum. 2019;10(1):79-84.

[76] Ribeiro CM, Soares I, Mendes R, Santis P, Katagiri S, Zavilenski R, Porto H, Afreixo V. Meta-analysis of the prevalence and risk factors associated with bovine neosporosis. Tropical Animal Health Production. 2019;51(7):1783-800.

[77] Sadrebazzaz A, Haddadzadeh H, Esmailnia K, Habibi G, Vojgani M, Hashemifesharaki R. Serological prevalence of Neospora caninum in healthy and aborted dairy cattle in Mashhad, Iran. Veterinary Parasitology. 2004;124(3):201-4.

[78] Sadrebazzaz A, Haddadzadeh H, Shayan P. Seroprevalence of Neospora caninum and Toxoplasma gondii in camels (Camelus dromedarius) in Mashhad, Iran. Parasitology Research. 2006;98(6):600-1.

[79] Salehi N, Haddadzadeh H, Shayan P, Vodjgani M, Bolourchi M. Serological study of Neospora caninum in pregnant dairy cattle in Tehran, Iran. International Journal Veterinary Research. 2010;4(2):113-6.

[80] Sasani F, Javanbakht J, Seifori P, Fathi S, Aghamohammad M. Neospora caninum as causative agent of ovine encephalitis in Iran. Pathology Discovery. 2013;1(1):1-5.

[81] Sattari A, Moshiri F, Musavi S. The seroprevalence of Neospora caninum antibodies in dairy cattle herds in Golestan province, Iran. Journal Veterinary Microbiology. 2011;7(1):60-4.

[82] Sayari M, Namavarim M, Mojaver S. Seroprevalence of Neospora caninum infection in free ranging Chickens (Gallus domesticus). Journal Parasitic Diseases. 2016;40(3):845-7.

[83] Silva RC, Machado GP. Canine neosporosis: perspectives on pathogenesis and management. Veterinary Medicine: Research and Reports. 2016;7:59-70.

[84] Soltani M, A Sadrebazzaz, M Nassiri, Tahmoorespoor M. Cloning, Nucleotide Sequencing and Bioinformatics Study of NcSRS2 Gene, an Immunogen from Iranian Isolate of Neospora caninum. Iranian Journal of Parasitology. 2013;8(1):114-28.

[85] Tavalla M, Sabaghan M, Abdizadeh R, Khademvatan S, Rafiei A, Razavi Piranshahi A. Seroprevalence of Toxoplasma gondii and Neospora spp. infections in Arab horses, Southwest of Iran. Jundishapur Journal of Microbiology. 2015;8(3):e14939.

[86] Tavanaee HR, Namavari M. Evaluation of attenuated variety of Neospora caninum for diagnosis of infection in cattle by agglutination test. Veterinary Journal (Pajouhesh Sazandegi). 2017;115:153-7.

[87] Yakhchali M, Javadi S, Morshedi A. Prevalence of antibodies to Neospora caninum in stray dogs of Urmia, Iran. Parasitology Research, 2010;106:1455-8.

[88] Youssefi MR, Arabkhazaeli F, Tabar Molla H. Seroprevalence of Neospora caninum infection in rural and industrial cattle in Northern Iran. Iranian Journal of Parasitology. 2009;4(1):15-8.

Avicenna Veterinary Research
Vol. 1, No. 4, Winter 2025: 21-28
Bu-Ali Sina University

REVIEW ARTICLE

New Active and Intelligent Packaging Technologies to Increase the Shelf life of Meat Products

Behnaz Bazargani-Gilani, Mahshad Javid Moghadam*, Fatemeh Moradiani
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article info

Article history:
Received 2025-12-01
Received in revised form 2025-12-16
Accepted 2025-12-17

Keywords:
Active Packaging
Smart Packaging
Meat
New Technologies
Food Safety

Abstract

Meat products are highly susceptible to microbial spoilage, oxidation, and quality deterioration because their biological structure is rich in protein, fat, and moisture. Traditional packaging, which serves only a passive protective role, is often inadequate and contributes to significant food waste. In recent years, active and intelligent packaging technologies have emerged as novel approaches to extend shelf life and enhance food safety. Smart packaging technology aims to communicate data regarding the administration and historical storage of food goods to elevate product quality and align with consumer expectations. Meat is a highly perishable food that can cause serious illness if spoiled. Various indicators and sensors have been developed to alert about meat spoilage in the industry. Intelligent packaging systems (IPSs) give consumers real-time information and indicate product status. This review article, based on research up to 2025, examines active packaging systems including antimicrobial, antioxidant, and oxygen-scavenging agents and intelligent systems such as gas sensors, time–temperature indicators, and Radio Frequency Identification (RFID). The application of these technologies to meat products, including red meat, poultry, and processed items, with focus on case studies, shows shelf-life increases of 20–50%. This technology has great potential to become a standard tool in meat quality control and could create a promising future for the industry.

1. Introduction

Meat products are a major source of protein in the global diet, but rapid spoilage caused by the growth of microorganisms such as Pseudomonas spp., Enterobacteriaceae and Salmonella spp., lipid oxidation, and physical changes poses a major challenge. FAO reports that more than 20% of the world's meat production is lost annually due to spoilage (FAO, 2023). Traditional packaging (polyethylene films) acts only as a physical barrier and cannot interact with the internal environment of the package. In contrast, active packaging, which releases or absorbs specific substances, and smart packaging, which enables real-time quality monitoring, offer advanced solutions. These technologies have been developed since the 1990s and made significant progress in recent years through the integration of nanomaterials, natural extracts, and artificial intelligence. Consumers and manufacturers increasingly demand strategies to extend the shelf life of food products and to monitor quality in real time. Recent studies (Ivanov *et al.*, 2025) found that an increase of just 2°C in cold chain temperature can raise the rate of biogenic amine formation by 55%, highlighting the importance of monitoring storage conditions and using reactive packaging. Active packaging methods have

*Corresponding author: M. J. Moghadam (Assistant Professor)
E-mail address: m.javidmoghadam@basu.ac.ir
http://dx.doi.org/10.22084/avr.2025.32004.1025

been used to modify the conditions of packaged food to improve safety and sensory properties and extend its shelf life (Mohebi and Marquez, 2014). Active and smart packaging has emerged as a transformative technology in food preservation, acting as a critical interface to monitor and respond to environmental interactions between packaged products and the surrounding environment (Zhang *et al.*, 2025).

Meat consumption has risen significantly over the past 50 years; the global fresh and processed meat market reached 277.5 million tons in 2020 and is projected to grow to 292.92 million tons by 2027. However, spoilage of meat products can cause economic losses for producers and health risks for consumers. To address this, smart packaging using markers and sensors such as barcodes and radio-frequency tags has been developed to ensure quality control and safety (Sani *et al.*, 2024). Despite significant advances, challenges such as high costs and environmental concerns remain (Mazhari and Mahmoudi-Meymand, 2002). The most commonly used technologies include pH indicators, gas sensors, temperature–time indicators, and RFID (Hidayat *et al.*, 2025). Active and smart packaging, as emerging technologies, can preserve food quality and safety throughout shelf life. These systems employ sensors that respond to environmental changes and are especially useful for meat products (Pourhamzeh, 2002) Nanotechnology offers substantial opportunities for innovative biodegradable packaging, providing improved barrier properties, mechanical strength, and active functions (Mahdoyan-Mehr and Sedaghat, 2002). Smart packaging has strong potential to become standard in the meat industry, particularly through integrating gas sensors and freshness indicators that can extend shelf life by up to 50%. This article reviews the mechanisms, applications, challenges, and future prospects of these technologies for meat products.

2. The Importance of Increasing the Shelf Life of Meat Products

The shelf life of meat products depends on several factors, such as the type of meat (red, white, processed), storage conditions (temperature, humidity) and packaging method. For example, fresh red meat can last only 3–5 days at refrigerated temperature (4°C), while processed products such as sausages may last up to 2 weeks. Modern technologies can increase this time by up to 50%, which is crucial in the global supply chain, especially in developing countries. Furthermore, driven by global trends like consumer demand for natural and sustainable products, the integration of plant extracts and biodegradable materials into packaging has become increasingly important. Smart packaging enables monitoring of food quality, safety, integrity, cleanliness, freshness and purity prior to consumption (Koneti *et al.*, 2023).

3. Active Packaging: Concepts, Types and Mechanisms

Active packaging denotes systems that engage proactively with the contents of the packaging or the ambient environment to sustain the quality of food (Mazaheri and Mahmoudi Meymand, 2024). Recent research indicates that the implementation of such systems in meat products enhances shelf life by mitigating microbial proliferation and oxidative processes. Active packaging encompasses various active components, including desiccants, scavengers, antimicrobial release mechanisms, and antioxidants, which are employed in the vicinity of the food to augment the efficacy of the packaging system (Mohebi and Marquez, 2014). Active packaging has risen to prominence as a revolutionary technology in food preservation, serving as a pivotal interface to monitor and react to environmental interactions between packaged goods and their surrounding environment (Zhang *et al.*, 2025).

4. Types of Active Packaging

4.1. Antimicrobial Systems

These systems serve to suppress the proliferation of bacteria, fungi, and pathogenic microorganisms through the release of antimicrobial compounds. Frequently employed materials encompass nano silver, zinc oxide (ZnO), botanical extracts including essential oils derived from basil and thyme, as well as bacteriocins exemplified by nisin. (Zhang *et al.*, 2025). The operational mechanisms of these compounds encompass the infiltration of the bacterial cell membrane, the induction of oxidative stress, the inhibition of essential enzymatic functions, and the infliction of DNA damage (Hakeem *et al.*, 2020). For instance, agar films infused with silver nanoparticles have demonstrated a reduction in bacterial proliferation while concurrently preserving the aesthetic properties, such as color and texture, of refrigerated beef (Heng *et al.*, 2021). Furthermore, the synergistic application of chitosan film in conjunction with clove essential oil within meat products has effectively suppressed the growth of Pseudomonas spp. and has extended the shelf life from 6 days to 12 days (Mohebi and Marquez, 2014). Anticipated advancements by 2025 entail nanoencapsulation techniques for the controlled release of active agents; for example, polylactic acid films incorporating nano encapsulated thymol have been shown to prolong the shelf life of mutton by as much as 14 days by diminishing total bacterial counts to below 6 log CFU/g. The strategic integration of these antimicrobial agents into polymeric matrices serves to enhance meat safety and is underpinned by standardized evaluation protocols that ensure regulatory compliance and quality assurance (Zhang *et al.*, 2025).

Table 1
Comparison of shelf life of various meat products in traditional and modern packaging.

Type of meat product	Shelf life in traditional packaging (days)	shelf life in active/smart packaging (days)	Percentage of increase
Fresh red meat	3-5	7-10	%50-100
Fresh poultry	4-7	10-14	%50-100
Processed sausage	7-14	14-21	%50
Hamburger	2-4	5-8	%100

Table 2
Types of active packaging and examples.

Type	Common materials	Mechanism	Practical example
Antimicrobial	Nanosilver, ZnO, Nisin, Basil/Thyme essential oils	Penetration into membrane, oxidative stress, DNA degradation	Chitosan film + clove essential oil: Pork shelf life from 6 to 12 days (Mohebi & Marquez, 2014)
Antioxidant/ Oxygen absorber	Rosemary extract, Vitamin E, Iron powder	Preventing lipid peroxidation	Whey protein film + rosemary: MDA <0.5 mg/kg in salami after 90 days (Zhang et al., 2025)
Moisture/Ethylene control	Modified starch, Silica gel	Moisture absorption, ripening inhibition	Starch-lemon film: +8 days shelf life (Fraunhofer IVV, 2024)

4.2. Antioxidant and Oxygen Scavenger Systems

In the context of meat, lipid oxidation represents a critical determinant of quality deterioration, as it results in the synthesis of undesirable compounds, notably malondialdehyde (MDA). Active packaging mitigates this phenomenon by either liberating antioxidant substances or sequestering surplus oxygen (Lee *et al.*, 2018). Frequently utilized antioxidant agents in this form of packaging encompass vitamin E, rosemary extract, natural polyphenols, and metallic adsorbents such as iron powder (Jin *et al.*, 2025).

For instance, whey protein films incorporating rosemary extract on salami successfully maintained malondialdehyde and hexanal concentrations below 0.5 mg/kg over a 90-day storage period at 5°C, thereby demonstrating enhanced oxidative stability compared to traditional packaging methods (Zhang *et al.*, 2025). Recent innovations encompass the application of nanomaterials, such as titanium dioxide, within chitosan films to enhance photocatalytic activity, which consequently mitigated oxidation in poultry meat and prolonged shelf life by as much as 7 days. One prominent active packaging technology, Modified Atmosphere Packaging (MAP), serves as a contaminant barrier and selectively inhibits spoilage microorganisms, thereby extending food shelf life (Mohebi and Marquez, 2014).

4.3. Humidity and Ethylene Control Systems

Maintaining humidity levels within the packaging is essential to avoid mold formation and alterations in texture. Bio-based films like starch-lemon, which possess moisture-absorbing capabilities, have demonstrated the ability to extend the shelf life of meat products by approximately 8 days (Fronfer IV, 2024). A new generation of modified starch-based moisture absorbers has proven to be around 2.5 times more effective than silica gel absorbers, highlighting the effectiveness of biodegradable materials in eco-friendly packaging solutions (Mundak, 2024). In addition, active ethylene-absorbing films reduce the rate of spoilage and maintain the color and freshness of the product. (Khodaei *et al.* 2023).

5. Materials and Manufacturing Technologies

Active and intelligent packaging is fabricated from a diverse array of materials, frequently comprising natural or synthetic polymers that incorporate active agents (such as antimicrobials or antioxidants) or exhibit intelligent functionalities (such as sensors). Such materials are required to exhibit characteristics including biodegradability, food safety, and economic viability (Jiang et al., 2023). Recent literature indicates that foundational materials encompass biopolymers (including chitosan, gelatin, starch, and polylactic acid or PLA) and nanomaterials (such as nano silver, zinc oxide or ZnO, and titanium oxide or TiO2) sourced from natural origins or agricultural by-products to promote environmental sustainability (Ahari and Soufiani, 2021). Furthermore, synthetic polymers like polyvinyl alcohol (PVA) and polyhydroxyalkanoates (PHA) are employed to enhance mechanical and sensory characteristics (Biji *et al.*, 2015).

6. Types of Materials Utilized

Biopolymers and Bio-based Materials: These substances are obtained from renewable sources such as food by-products (like fruit peels, shrimp shells, or

plant residues) and encompass chitosan (derived from chitin in crustacean shells), gelatin (originating from animal proteins), starch (extracted from corn or potatoes), cellulose (from plant sources), and Poly Lactic Acid (produced from lactic acid fermentation). These materials are biodegradable and can integrate active agents such as anthocyanins (extracted from red cabbage or black rice as pH indicators) or plant essential oils (such as lemon oil or curcumin) to impart antimicrobial or antioxidant attributes (Mkhari et al., 2025). For instance, chitosan is utilized in active films to inhibit bacterial growth, such as that of E. coli, owing to its intrinsic antimicrobial properties (facilitated by amino groups) (Puebla-Duarte *et al.*, 2023). Bio-based materials like PHB (polyhydroxybutyrate) are also suitable for intelligent packaging, possessing the capability to respond to environmental cues (such as temperature or humidity) (Ahari and Soufiani, 2021).

Nanomaterials: Metallic nanoparticles such as nanosilver (noted for potent antibacterial properties in fruits and vegetables) or ZnO and TiO2 (utilized for ethylene and moisture regulation in perishable items) are incorporated to enhance barrier and sensing functionalities. For example, cellulose nanocrystals (CNCs) derived from plant biomass are employed to create oxygen barriers within films (Jiang *et al.*, 2023). Nanocomposites such as nanoclay (to augment barrier properties in beer packaging) or halloysite nanotubes (for ethylene absorption in tomatoes) are also prevalent (Biji *et al.*, 2015).

Smart and Hybrid Polymers: Stimuli-responsive polymers like PNIPAAm (N-isopropylacrylamide) for controlled release contingent upon temperature (with a critical temperature below 32°C) or conductive polymers such as PEDOT: PSS for gas sensing applications are utilized. Hybrid formulations, including chitosan-starch or PLA-PEO for moisture indicators (exhibiting color change at humidity levels exceeding 60%), have been innovated (Mkhari *et al.*, 2025).

7. Manufacturing Methods

Electrospinning: This technique is used to produce nanofibers, enabling the inclusion of active components like curcumin in PVA fibers for prolonged release. Recently, it has been applied to create smart films that contain antimicrobial nanostructures, such as those made from PLA/PEO combined with phycocyanin to serve as pH indicators (Ahari and Soufiani, 2021; Mkhari *et al.*, 2025).

Extrusion and Molding: This industrial approach supports large-scale production of films, such as the extrusion of LDPE with curcumin or PLA mixed with -tocopherol for oxygen absorption. It enhances barrier properties by integrating nanocomposites like starch-clay (Biji *et al.*, 2015; Jiang *et al.*, 2023).

Solvent Casting: A simple, laboratory-scale method for producing thin films involves combining gelatin with anthocyanins and drying the mixture to create indicators for meat freshness. This approach is also effective for incorporating natural extracts such as essential oils (Puebla-Duarte *et al.*, 2023).

3D Printing: This modern technology allows for the creation of intricate structures, including chitosan-anthocyanin films for pork quality indicators and hydrogel-oleogel bigels containing anthocyanins to detect volatile amines. It offers excellent scalability and customization of properties (Mkhari *et al.*, 2025; Ahari and Soufiani, 2021).

Nanotechnology Integration: This encompasses methods like nano-coating (for example, applying nano silver to gelatin films) or encapsulation (such as using magnetic silica nanoparticles for turmeric oil) to enable controlled release. Techniques such as thin film deposition or solvent-based chemistry are utilized in sensor development (Puebla-Duarte *et al.*, 2023). These innovative technologies, which focus on sustainable materials and methods, hold great promise for reducing food waste. Nonetheless, challenges like production costs and the scalability of these processes in industry still need to be addressed (Biji *et al.*, 2015).

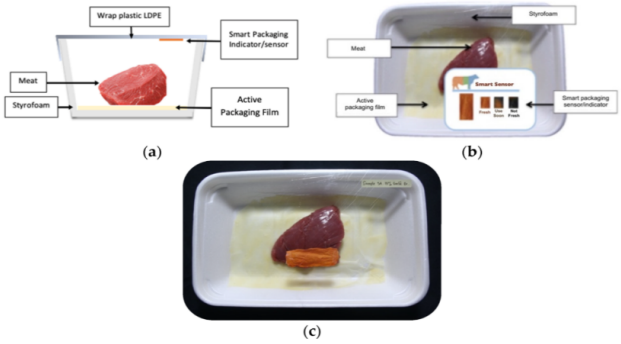

Fig. 1. "Design of a smart and active packaging system (a), prototype of smart packaging (b), and its application on fresh beef (c)." (Dirpan et al., 2022).

8. Smart Packaging: Real Quality Monitoring

Smart packaging offers immediate information concerning the status of food without needing to open the package, utilizing sensors and indicators. These technologies enhance quality, safety, and streamline the supply chain. The importance of monitoring or assessing food quality from production to consumption has grown due to increasing consumer satisfaction, minimizing food waste, and preventing foodborne illnesses (Mohebi and Marquez, 2014). In the present day, food packaging not only protects food from contamination and aids in distribution but can also monitor food from the manufacturing stage until it reaches consumers.

Smart packaging can be viewed as a system capable of executing intelligent tasks such as sensing, detecting, tracking, recording, and communicating, facilitating decisions to extend shelf life, improve quality, enhance safety, provide information, and alert about possible issues. Smart packaging systems comprise data carriers, indicators, and sensors. Indicators deliver visual cues that reflect the internal condition of food items. Digital sensors monitor alterations in the product through the use of transducers (koneti *et al.*, 2023).

9. Types of Smart Packaging

9.1. Time-Temperature Indicators (TTI)

TTIs reflect cumulative temperature variations through a permanent color change. This process is driven by lipid hydrolysis or enzymatic activity. In meat products, TTI based on lipase activity forecasts microbial development in chilled meat packaging, thereby extending the product's shelf life by mitigating temperature abuse. Research demonstrates a strong correlation (R=0.98) with microbial growth in meat. Time-temperature indicators (TTIs) have become vital instruments for thorough quality monitoring within meat supply chains, furnishing essential real-time information that influences various facets of food management, including quality assurance, consumer confidence, and compliance with regulations (Zhang *et al.*, 2025).

Furthermore, temperature monitoring indicators (TTIs) are classified into three groups based on their performance: critical temperature indicators (which identify exposure to temperatures that exceed or fall below a specified threshold), integrated temperature/time indicators or partial history indicators (which detect temperature mismanagement affecting quality), and integrated time-temperature indicators or full history indicators (which track the complete temperature history and identify abuse). TTIs operate based on various principles, including chemical types (like polymerization-based, photochromic, and oxidation reactions), physical mechanisms (such as diffusion, nanoparticles, and electronics), enzymatic types (e.g., hydrolysis using enzymes such as lipase), biological methods (like yeast and lactic acid bacteria), changes in photonic networks, and combinations of polymers and thermal dyes (Abekoon *et al.*, 2024). As an illustration, enzymatic TTIs induce pH shifts via the hydrolysis of lipid substrates, resulting in a color transition (for example, from green to orange/red), and this process is linked to microbial proliferation (Zhang *et al.*, 2025).

The functioning of TTIs involves irreversible changes influenced by time and temperature through mechanisms such as chemical, mechanical, electrochemical, enzymatic, or microbiological activities that result in observable effects, including color shifts, color movement, or physical deformation. For example, polymerization-based TTIs utilize the solid-state polymerization of monomers featuring acetylene groups; photochromic TTIs depend on the thermal fading of photochromic compounds for color alteration; and oxidation reaction-based TTIs are grounded in redox reactions with oxygen (Abekoon *et al.*, 2024). In meat-related applications, nanoparticle TTIs, utilizing silver (AgNPs) and gold (AuNPs) nanodispersions within 3D-printed plant resin containers, identify cumulative temperature fluctuations through colorimetric alterations; for instance, AgNPs above 22°C produce a yellow-green color shift due to an increase in nanoparticle concentration, whereas AuNPs below 4°C result in a red-purple color change through aggregation (cryoagglomeration) (Alcañiz *et al.*, 2025). These mechanisms are effective for monitoring the cold chain in perishable items such as meat and can extend shelf life by curbing microbial growth. In meat and seafood products, temperature time indicators (TTIs) like the OnVu^{TM} system are utilized to estimate color changes in cold meat and to monitor the shelf life of ready-to-eat cold-smoked items, which are linked to microbial growth (Zhang *et al.*, 2025). Recent developments feature enzymatic TTIs that evaluate the freshness of fish by measuring pH alterations resulting from lipid breakdown, thereby minimizing safety hazards (Zhang *et al.*, 2025). The benefits of TTIs encompass enhanced management of the cold chain, reduction of waste by indicating spoilage risks, and increased consumer satisfaction by offering storage details. These external indicators facilitate prompt decision-making and can be integrated with intelligent packaging systems for tracking and HACCP protocols (Abekoon *et al.*, 2024). However, there are drawbacks such as their exclusive focus on temperature (neglecting factors like humidity or oxygen), the irreversibility of many TTIs, and the high costs associated with small producers (Zhang *et al.*, 2025).

9.2. Gas and pH Sensors

These sensors identify volatile substances, including amines (TVB-N), CO2, and H2S. The detection methods involve either electrochemical reactions or color changes. Anthocyanin-agar films can detect putrescine in pork with a sensitivity range of 1-5 ppm and a response time of 2-5 minutes, allowing for an extension of shelf life up to 72 hours. Recent advancements feature titanium dioxide-polyaniline sensors capable of detecting ammonia in pork, showing a response of 0.82 at a concentration of 100 ppm. Odor is a crucial factor in determining the freshness of meat. Each type of meat has distinct characteristics of volatile compounds, resulting in a unique smell for every product. Research has indicated that biogenic amines like tyra-

mine, tryptamine, putrescine, and cadaverine have significant correlations with traditional quality indicators such as total bacterial count, pH, and TVBN in meat products (Mohebi and Marquez, 2014).

9.3. RFID and IoT Systems

RFID technology enables real-time monitoring and is combined with sensors to forecast freshness. In the case of pork, utilizing passive RFID along with machine learning achieves a freshness prediction accuracy of 96.9%. As an essential information technology in food packaging, radio frequency identification (RFID) technology encodes extensive product details on microchips, which include the product name, ingredients, functional attributes, origin, shelf life, weight, price, and usage instructions (Zhang *et al.*, 2025).

9.4. Integration of Active and Intelligent Packaging

Hybrid systems like chitosan films that incorporate color indicators and antimicrobial substances can simultaneously monitor and preserve food. This combination enhances the shelf life of chicken meat by an additional 6 days. The findings indicated that smart packaging provides various benefits compared to conventional packaging, including antimicrobial properties, antioxidant effects, and prolonged shelf life, particularly in industrial processing settings (Khodai *et al.*, 2023).

10. Application in Meat Products

10.1. Case Studies

Red meat shelf life was extended by 6 days using chitosan films with eugenol for chicken, which reduced microbial counts. Gelatin coatings with nisin inhibited Listeria in processed beef, extending shelf life up to 10 days. Nanoparticle H2S sensors accurately detected spoilage in poultry and seafood. Meat products' (poultry, red meat, and seafood) short shelf life is due to spoilage and oxidation from microbial activity and metabolic/enzymatic processes (Sani *et al.*, 2024).

10.2. Effects on Shelf Life

These innovations enhance shelf life by 20-50% by decreasing waste and boosting safety. For instance, with pork, active systems manage TBARS levels and prolong shelf life from 5 to 12 days. As a result, these systems have been utilized to enhance the shelf life and texture of meat and meat products. It is essential to monitor the amount of smart ingredients included in the packaging, as they significantly influence the quality, nutritional characteristics, and overall cost of food items (Khodai *et al.*, 2023).

10.3. Challenges and Future Outlook

The outlook for advanced and intelligent packaging technologies in meat products is certainly encouraging, yet it faces several challenges that necessitate creative solutions and strategic funding. A key obstacle is the elevated cost associated with manufacturing and deploying these systems, which might hinder widespread acceptance in developing nations. Furthermore, concerns regarding the migration of active substances (like nanoparticles or chemical agents) into food necessitate thorough safety evaluations and stringent regulations from bodies such as the FDA and EFSA to guarantee that these technologies remain safe for health. Another significant challenge is environmental sustainability, as many existing materials are derived from non-biodegradable plastics, contributing to ecological pollution. Transitioning from laboratory development to full-scale industrial production also presents obstacles, including issues with scalability, the consistency of performance, and reliability, all of which demand considerable investments in state-of-the-art equipment and refined processes (Sani *et al.*, 2024).

The future of this sector is brimming with new advancements. By incorporating artificial intelligence (AI) and machine learning into intelligent systems, more precise predictions regarding spoilage can be made; for instance, AI algorithms can evaluate sensor data and provide early warnings, potentially extending shelf life by as much as 50%. The emergence of innovative biodegradable materials, like starch-based biopolymers or chitosan enhanced with natural nanomaterials, will promote eco-friendly and sustainable packaging while assisting in minimizing environmental impacts.

Table 3
Types of smart packaging.

Type	Mechanism	Application in meat
Time-temperature indicator (TTI)	Enzymatic/chemical color change	Microbial growth prediction (R^2=0.98) (Zhang *et al.*, 2025)
Gas/pH sensor	Color/electrical change S against amines, CO2, H2S	Detection of putrescine in pork (sensitivity 1-5 ppm)
RFID + IoT	Digital tracking + ML prediction	96.9% accuracy in freshness prediction (Sani *et al.*, 2024)

Next-generation technologies will include stimuli-responsive systems that react to environmental alterations such as temperature or pH, boosting efficiency. Moreover, marrying the Internet of Things (IoT) with RFID technology can completely digitize the supply chain, facilitating traceability from farm to table, which not only enhances safety but also aids in decreasing global waste. The rising adoption of these technologies—projected to grow annually at over 10% until 2030—suggests significant commercialization potential, particularly in developing regions like Asia and the Middle East. Future studies should emphasize international partnerships, comprehensive field testing, and the establishment of global standards, so these innovations can become standard instruments within the meat industry and support worldwide objectives like food security and sustainability (Hidayat *et al.*, 2025; Zhang *et al.*, 2025).

11. Conclusion

Active and intelligent packaging solutions, representing significant advancements in the food sector, have resulted in a remarkable change in how meat products are preserved and their shelf life is extended. By employing sophisticated methods like the controlled release of antimicrobial and antioxidant substances, absorption of oxygen and moisture, and real-time monitoring of quality metrics such as pH, volatile gases, and temperature fluctuations, these systems effectively address conventional issues like microbial spoilage, lipid oxidation, and the deterioration of sensory qualities. Research highlighted in this article indicates that the use of these technologies across various meat products—from fresh red meat and poultry to processed items like sausages and hamburgers—has led to shelf life enhancements of 20 to 100 percent. This not only significantly curtails food waste (as reported by the FAO, over 20 percent of meat production ends up wasted each year) but also enhances food safety and reduces health hazards linked to pathogens such as Salmonella and Listeria. The combination of natural materials, including plant extracts like rosemary and thymol, with nanotechnology has not only bolstered the effectiveness of these systems but also advanced environmental sustainability, since many of these materials are biodegradable and serve as viable alternatives to traditional plastics.

Additionally, intelligent packaging incorporating tools like time-temperature indicators (TTI), gas sensors, and RFID systems facilitates real-time observation of the supply chain and empowers consumers to make better-informed choices. The analyzed case studies indicate that these technologies not only increase the shelf life of products (for instance, extending it from 5 to 12 days in pork) but also assist in lowering production costs while enhancing consumer satisfaction by offering clear information about product freshness and safety. Ultimately, these results highlight that active and smart packaging has transformed from a merely experimental idea or futuristic concept into a strategic and practical approach that is primed for broad implementation within the food sector. These innovations can enhance the competitiveness of meat products in the global marketplace, reduce waste, and aid in achieving sustainable development goals, such as minimizing food waste by 2030. Nonetheless, the overall effectiveness of these systems hinges on addressing current challenges and prioritizing applied research to ensure these technologies become an essential component of the food supply chain (Hidayat *et al.*, 2024).

References

[1] Ahari H, Soufiani SP. Smart and active food packaging: Insights in novel food packaging. Frontiers in Microbiology. 2021 Jul 9;12: 657233.

[2] Abekoon T, Buthpitiya BL, Sajindra H, Samarakoon ER, Jayakody JA, Kantamaneni K, Rathnayake U. A comprehensive review to evaluate the synergy of intelligent food packaging with modern food technology and artificial intelligence field. Discover Sustainability. 2024 Jul 22;5(1):160.

[3] Lanza G, Perez-Taborda JA, Avila A. Improving Temperature Adaptation for Food Safety: Colorimetric Nanoparticle-Based TimeTemperature Indicators (TTIs) to Detect Cumulative Temperature Disturbances. Foods. 2025 Feb 22;14(5):742.

[4] Biji KB, Ravishankar CN, Mohan CO, Srinivasa Gopal TK. Smart packaging systems for food applications: a review. Journal of food science and technology. 2015 Oct;52(10):6125-35.

[5] Hakeem MJ, Feng J, Nilghaz A, Ma L, Seah HC, Konkel ME, Lu X. Active packaging of immobilized zinc oxide nanoparticles controls Campylobacter jejuni in raw chicken meat. Applied and environmental microbiology. 2020 Oct 28;86(22):e01195-20.

[6] Hidayat SH, Novrain AY, Irwan I. A decade of intelligent packaging for meat with insight from smart packaging literature: Trends, innovations, and future prospects-a systematic review and network analysis. Canrea Journal: Food Technology, Nutritions, and Culinary Journal. 2025 Oct 9:201-35.

[7] Hong SI, Cho Y, Rhim JW. Effect of agar/AgNP composite film packaging on refrigerated beef loin quality. Membranes. 2021 Sep 29;11(10):750.

[8] Ivanov A, Buruiana DL, Trus C, Ghisman V, Vasile Antoniac I. Nanomaterials for Sensory SystemsA Review. Biosensors. 2025 Nov 11;15(11):754.

[9] Jain P, Meshram BP, Singh S, Gaikwad KK. Non-iron oxygen scavengers in food packaging: mechanisms, applications, and the shift towards green alternatives. Sustainable Food Technology. 2025;3(6):1668-80.

[10] Jiang Y, Zhang Y, Deng Y. Latest advances in active materials for food packaging and their application. Foods. 2023 Nov 7;12(22):4055.

[11] Khodaei SM, GholamiAhangaran M, Karimi Sani I, Esfandiari Z, Eghbaljoo H. Application of intelligent packaging for meat products: A systematic review. Veterinary Medicine and Science. 2023 Jan;9(1):481-93.

[12] Koneti PB, Raziuddin M, Devangare A. Advances in Intelligent Food Packaging of Meat. Journal of Meat Science. 2023 Jun.

[13] Lee JS, Chang Y, Lee ES, Song HG, Chang PS, Han J. Ascorbic acidbased oxygen scavenger in active food packaging system for raw meatloaf. Journal of food science. 2018 Mar;83(3):682-8.

[14] Lohita B, Srijaya M. Novel technologies for shelf-life extension of food products as a competitive advantage: A review. Food production, diversity, and safety under climate change. 2024 Apr 2:285-306.

[15] Mazaheri M, Mahmoudi Meymand M. A review of smart and active packaging in the food industry. Nesha Alam. 2024. 13(2), 47-54. (In Persia)

[16] Mkhari T, Adeyemi JO, Fawole OA. Recent advances in the fabrication of intelligent packaging for food preservation: a review. Processes. 2025 Feb 14;13(2):539.

[17] Mohebi E, Marquez L. Intelligent packaging in meat industry: An overview of existing solutions. Journal of food science and technology. 2015 Jul;52(7):3947-64.

[18] Pourhamzeh S. Novel Trends in the Packaging of Meat and Meat Products. Packaging science and art. 2021 Aug 23;12(46):1-8.

[19] Puebla-Duarte AL, Santos-Sauceda I, Rodríguez-Félix F, Iturralde-García RD, Fernández-Quiroz D, Pérez-Cabral ID, Del-Toro-Sánchez CL. Active and intelligent packaging: A review of the possible application of cyclodextrins in food storage and safety indicators. Polymers. 2023 Nov 3;15(21):4317.

[20] Dirpan A, Djalal M, Kamaruddin I. Application of an intelligent sensor and active packaging system based on the bacterial cellulose of Acetobacter xylinum to meat products. Sensors. 2022 Jan 11;22(2):544.

[21] Sani MA, Zhang W, Abedini A, Khezerlou A, Shariatifar N, Assadpour E, Zhang F, Jafari SM. Intelligent packaging systems for the quality and safety monitoring of meat products: From lab scale to industrialization. Food Control. 2024 Jun 1;160: 110359.

[22] Zhang J, Zhang J, Zhang L, Qin Z, Wang T. Review of Recent Advances in Intelligent and Antibacterial Packaging for Meat Quality and Safety. Foods. 2025 Mar 26;14(7):1157.

[23] Zouharová A, Bartáková K, Bursová Š, Necidová L, Haruštiaková D, Klimešová M, Vorlová L. Meat and fish packaging and its impact on the shelf life–a review. Acta Veterinaria Brno. 2023 Feb 2;92(1):95-108.

Avicenna Veterinary Research
Vol. 1, No. 4, Winter 2025: 29-33
Bu-Ali Sina University

ORIGINAL ARTICLE

Study of the Histological Structure of Hardin's Glands in Khalkhali Goats

Azin Beiranvand[a,*], Ali Kalantari-Hesari[a], Mohammad Babaei[b]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article info

Article history:
Received 2025-11-29
Received in revised form
2025-12-07
Accepted 2025-12-09

Keywords:
Harderian Glands
Goat
Histology
Azerbaijan

Abstract

The Khalkhali goat is a black dairy breed found in the Azerbaijan region. The Harderian gland is only found in some species. These glands secrete an oily substance rich in porphyrin that facilitates the movement of the eyelids over the eye and creates an immune response. So far, no study has been conducted on the histological structure of the Hardin glands in the eyes of Khalkhali goats. In this study, 4-goats slaughtered at the Zanjan slaughterhouse were used. After, the heads of the goats were separated from the trunk and the eyelids along with the Hardin glands were separated from the skull bone. The samples were placed in 10% formalin. The sample was fixated and entered the tissue passage and after preparing paraffin blocks, 5-μm thick sections were prepared and stained using the hematoxylin-eosin method. Histological examination showed that this gland in the Khalkhali goat is covered from all sides by a capsule of tissue. Blades of the capsule enter the parenchyma of the gland and divide it into lobules. The lobules of this gland include serous, mucous, and seromucous secretory units, and the secretions of these units are collected by intralobular ducts. It can be concluded that the Harderian gland in the Khalkhali goat breed was similar to the Harderian glands of the alpaca in terms of the type of secretions of the acini units and in terms of the interlobular and extralobular ducts, it was very similar to the existing reports on cattle, other goat breeds, and sheep.

1. Introduction

The goat, scientifically known as (Capra aegagrus hircus), is a domesticated animal of the order of the ungulates, the family of the Bovids, the subfamily of the Capidae, and the genus of the Goats. The first signs of goat domestication were found in Iran, dating back to about 10,000 BC. The anatomy of goats is not much different from that of sheep, except for some things like tail and hair, which also vary between breeds. The pupil of the eye in goats is a horizontal rectangle with convex corners (Rana, 2023).

The Khalkhali goat is a black dairy breed. The distribution area of this breed is in the Moghan plain and around Khalkhal city. The hair coat of this breed is long and thick and the ears are wide and short. The amount of milk produced by this breed of goat in one period is 130 liters (Nasirain *et al.*, 2009).

Among the anatomical and morphological features of the eye in small ruminants, one can mention the orbital bone, which is approximately 37 to 38 mm wide and approximately 41.2 mm long. The angle between the two eyes is approximately 90 degrees. The volume of the orbital cavity is approximately 1.1 compared to the eyeball. The orbital depth is 45 to 50 mm and the angle between the two orbital cavities is 115 to 120

*Corresponding author: A. Beiranvand (M.Sc. Student)
E-mail address: azin770823@gmail.com
http://dx.doi.org/10.22084/avr.2025.32030.1027

degrees. The orbital rim is completely surrounded by bone and is formed by the junction of the cheekbones, frontal bone, and lacrimal bone. The orbital rim is fissured and rough in small ruminants. The ducts of the eye glands are connected to each other by bones. The duct is a protruding bone located in the anterior and posterior parts of the eye canal and is approximately 3 mm wide and 2 mm long.

The eyelids of goats are movable and much thinner than those of cattle. The upper eyelid is thicker than the lower eyelid. These folds of skin protect the eye from external damage. They also help keep the cornea moist and regulate the amount of light entering the eye. The upper, lateral, and third eyelids are the lateral organs of the eye whose normal structure is essential for proper eye function. The eyelids protect the eye from damage and control the amount of light entering the eye by opening and closing the palpebral fissure. The inner eyelid or third eyelid is referred to in veterinary terminology as Palpebra tertia, but it also has other names such as eyelid membrane (Schlegel *et al.*, 2001).

The superficial glands of the third eyelid surround the base of the stalk of the T-shaped cartilaginous plate. These glands are structurally similar to the lacrimal glands of Boh and also participate in the secretion of a thin layer of tear film. These glands are of the serous type in horses and cats, of the seromucous type in cows and dogs, and of the mucous type in pigs. Most acinar cells secrete lipids. The deep third eyelid gland is only present in cattle and pigs (Heatley, 2005).

The lacrimal system consists of the lacrimal gland, the lacrimal sac, and the duct system that runs from the eye to the nasal area. The lacrimal gland is a broad, oval, pink-colored gland that surrounds the orbital tissue and is covered by bone, and may be partially hidden by the fat surrounding the eyeball. The lacrimal gland measures 2.8 cm in the mediolateral direction, about 2 cm in the anterior-posterior direction, and 5 mm when the layers are compressed between the bone and the eyeball. Both lacrimal ducts start at a corresponding point and converge towards the medial margin, passing through the orbit and finally opening into the lacrimal sac. The superficial layer of the canal may change from squamous to cuboidal, and a few goblet cells may be seen. The lacrimal sac is located in the depression of the lacrimal bone and transports tears from the lacrimal canal to the nasolacrimal bone canal. The lacrimal duct passes through the lacrimal canal and then the lacrimal groove ends in the maxillary bone and the posterior part of the nostril. When the duct leaves the lacrimal canal and enters the lacrimal groove, its medial surface is covered by nasal mucosa (Coulombre *et al.*, 1962; Kassab and El-Zoghby. 2010; Olopade *et al.*, 2005; Gelatt *et al.*, 2008).

The Harderian gland (deep gland of the third eyelid) is present only in some species. It is absent in species such as carnivores, primates, and humans. However, it is well developed in many laboratory animals, amphibians, reptiles, and birds (Sisson *et al.*, 1975). These glands secrete an oily substance rich in porphyrin that facilitates the movement of the eyelids over the eye. They are a site for the immune response, a source of lipids for thermoregulation and pheromones, and a protective organ for the retina-pineal axis (Emily *et al.*, 2015). Considering the role of this gland in the function and health of the eyelids and eyes, and also considering the fact that no study has been conducted so far regarding the histological structure of the Hardin glands in the eyes of Khalkhali goats, the histological structure of this gland was examined in the present study.

2. Materials and Methods

In this study, 4 goats (2 male goats and 2 female goats) slaughtered at the Zanjan livestock slaughterhouse were used. After slaughter, the goats' heads were separated from the trunk, the heads were placed on a table, and then the eyeballs and eyelids, along with the Hardin glands, were separated from the skull bone. The samples were placed in 10% formalin. After the minimum time required for tissue fixation, the samples were dehydrated, clarified, and embedded in paraffin using a tissue passager, and finally paraffin blocks were prepared from the samples. Each sample was stained with hematoxylin-eosin after being sectioned at 5 μm thickness. The stained sections were examined under a light microscope at magnifications of $\times 4$, $\times 10$, $\times 40$, and $100\times$.A Dino late camera and Dino capture version 2 software were used to capture images of the slides (Morovvati and Kalantari-Hesari, 2019).

3. Histological Results

Regarding the histological examination of the Hardin glands in the Khalkhali goat, it should be noted that this gland is surrounded by a capsule of relatively hard connective tissue, along with connective fibers and adipose tissue. Blades of the capsule enter the gland parenchyma and divide it into lobules. The lobules of this gland contain serous, mucous, and seromucous secretory units (Fig. 1, parts A and B), and the secretions of these units are collected by intralobular ducts. These intralobular ducts are lined with simple cuboidal epithelium or stratified cuboidal epithelium with a few visible layers (Fig. 1, part C). The intralobular ducts end in the interlobular ducts. The interlobular ducts are larger, located between the lobules, and are lined by a stratified cubic epithelium with a higher number of layers (Fig. 1, Part D).

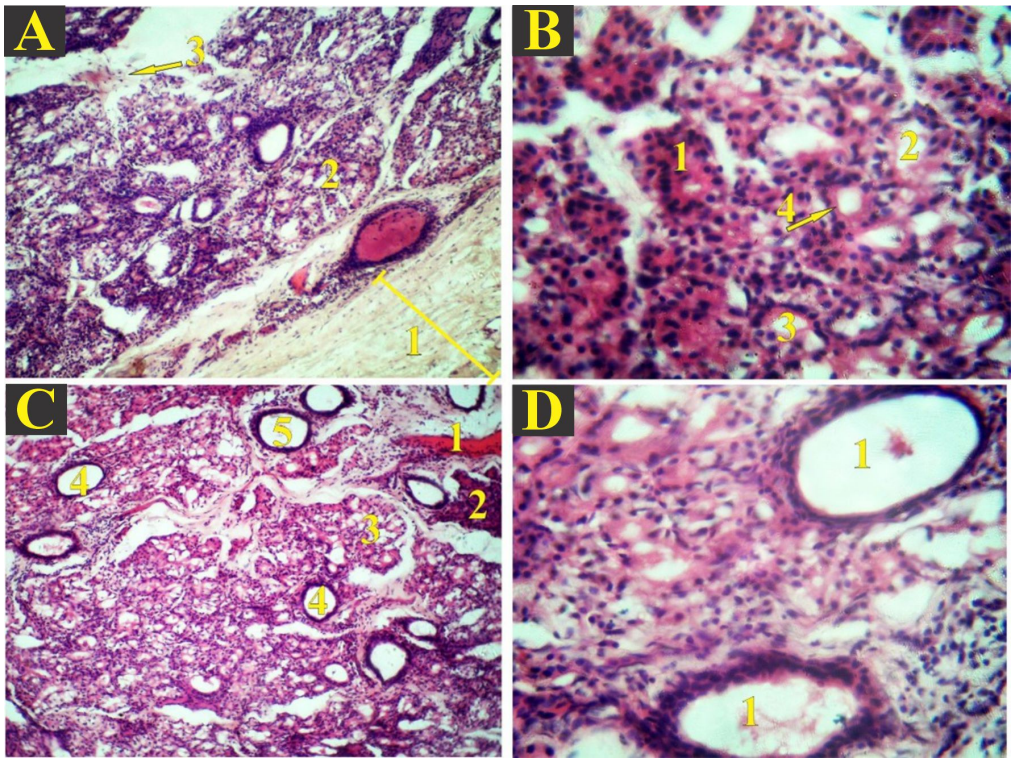

Fig. 1. Cross-section of the Harderian gland in the third eyelid of a Khalkhali goat with hematoxylin-eosin (H&E) staining and ×100 magnification. A) No. 1: gland capsule, No. 2: gland lobules, and No. 3: septa branching from the capsule. B) Secretory units of the third eyelid Harderian gland of a Mahabadi goat with H&E staining and ×400 magnification (No. 1: serous unit, No. 2: mucous unit, No. 3: seromucous unit, and No. 4: intralobular duct with simple cuboidal epithelium). C) No. 1: Septa branching from the capsule, No. 2: Serous secretory units, No. 3: Mucous secretory unit, No. 4: Intralobular collecting duct with stratified cuboidal epithelium and interlobular collecting duct with stratified cuboidal epithelium. D) No. 1: Interlobular collecting duct with stratified cuboidal epithelium.

Given that goats play an important role in the economy of some countries, knowledge of the anatomical condition and function of the various systems and structures of the animal's body can help diagnose diseases and abnormalities and also be useful in maintaining their health. The predominant glands in the eyes of mammals and domestic animals are the lacrimal glands, which may have different locations. The shape of these glands may also vary (Klećkowska-Nawrot *et al.*, 2015).

It has been stated that the secretory acinar units in the Harderian glands of alpaca are composed of long, irregular cone cells with a very narrow lumen in the middle, while the ducts or excretory ducts are lined by a layer of simple or stratified cuboidal epithelium (Klećkowska-Nawrot *et al.*, 2015). Similar reports have been reported in cattle, buffalo, goats and sheep in Iran (Gargiulo *et al.*, 1999; Gargiulo *et al.*, 2000; Kühnel 1968a, b; Shadkhast and Bigham. 2010; Sinha and Calhoun. 1966). Secretory units in the Hardin glands of Khalkhali goats were also composed of long and irregular cone cells. Also, the intralobular ductal epithelium was composed of simple cuboidal to stratified cuboidal type with a low number of layers, which was consistent with previous reports.

It has been shown that the Harderian glands in deer and goats are of the serous type (Kühnel., 1968a; Klećkowska-Nawrot *et al.*, 2013). In histological examination of the Harderian glands of alpaca, these glands were diagnosed as seromucous type (Klećkowska-Nawrot *et al.*, 2015). While in the present study, serous, mucous and seromucous secretory units were observed.

Histological examination of the Harderian gland in goats revealed that this gland is of the lobar-alveolar type with a holocrine secretion that is surrounded by a thin connective tissue capsule. Blades from this capsule penetrate into the gland tissue and divide it into lobules (Rajathi *et al.*, 2019). The results of the present study were consistent with previous reports regarding the connective capsule surrounding the gland.

It has also been reported that secretory units in goats consist of acini with a single layer of columnar cells lined with intracellular eosinophilic vacuoles (Rajathi *et al.*, 2019). The results of the present study were also consistent with previous reports regarding

the shape of secretory cells. It has also been stated that the duct system of the Hardin glands in goats starts from the intralobular ducts and ends in the main excretory duct on the surface of the eyelid. The intralobular ducts are covered by simple cuboidal or cylindrical epithelial tissue, which is mostly in a single layer. The union of these ducts forms interlobular ducts that may be lined by simple columnar to stratified squamous epithelium (Rajathi *et al.*, 2019). In the present study, secretions from acinar units were observed through intralobular ducts lined by simple cuboidal epithelium or stratified cuboidal epithelium with a low number of layers. The intralobular ducts end in larger interlobular ducts, which are located between the lobules and are lined with stratified cubic epithelium with a higher number of layers. According to the results of the present study and the existing reports in this field, the histological structure of the Hardin glands in the Khalkhali goat breed was examined in terms of the shape of the secretory cells similar to most animals. In terms of the type of secretions, the acini units were similar to the Hardin glands of the alpaca, and in terms of the interlobular and extralobular ducts, it was very similar to the existing reports on cattle, other goat breeds, and sheep.

Acknowledgment
The authors of the article would like to thank all those who helped in the preparation of this article.

Conflict of Interest
The authors declare no conflict of interest in this research.

References

[1] Coulombre AJ, Coulombre JL, Mehta H. The skeleton of the eye: I. Conjunctival papillae and scleral ossicles. Developmental biology. 1962 Dec 1;5(3):382-401.

[2] Gargiulo AM, Coliolo P, Ceccarelli P, Pedini V. Ultrastructural study of sheep lacrimal glands. Veterinary research. 1999;30(4):345-51.

[3] Gargiulo AM, Dall'Aglio C, Coliolo P, Ceccarelli P, Pedini V. Complex carbohydrate histochemistry and ultracytochemistry of the sheep lacrimal gland. Anatomia, Histologia, Embryologia. 2000 Feb;29(1):19-24.

[4] Gelatt KN, Brooks DE, Kallberg ME. The canine glaucomas. Essentials of veterinary ophthalmology. 2008 Apr 22;2:155-87.

[5] Kassab A, El-Zoghby I. Anatomical and histological studies of the aqueous outflow system in the eye of goat (Capra hircus). Journal of Veterinary Anatomy. 2010 Oct 1;3(2):13-22.

[6] Klećkowska-Nawrot J, Marycz K, Czogała J, Kujawa K, Janeczek M, Chrószcz A, Brudnicki W. Morphology of the Lacrimal Gland and Superficial Gland of the Third Eyelid of Roe Deer (Capreolus Capreolus L.). Pakistan Veterinary Journal. 2013 Apr 1;33(2).

[7] Klećkowska-Nawrot J, Nowaczyk R, Goździewska-Harłajczuk K, Krasucki K, Janeczek M. Histological, histochemical and fine structure studies of the lacrimal gland and superficial gland of the third eyelid and their significance on the proper function of the eyeball in alpaca (Vicugna pacos). Folia morphologica. 2015;74(2):195-205.

[8] Kühnel W. Vergleichende histologische, histochemische und elektronenmikroskopische Untersuchungen an Tränendrüsen. Zeitschrift für Zellforschung und Mikroskopische Anatomie. 1968 Dec;89(4):550-72.

[9] Miedel EL, Hankenson FC. Biology and diseases of hamsters. InLaboratory animal medicine 2015 Jan 1 (pp. 209-245). Academic Press. 209-245.

[10] Morovvati, H., Kalantari-Hesari, A. (2019) Text book of histotechniques and lab management. University of Tehran Press. First Edition.

[11] Nasirian, A., Pashmi, M., Haji Seyed javadi, M.M., Nouri, M., Haji Seyed javadi, M.A., Eghbali, M., Abbasi, M. (2009). Atlas of livestock breeds (cattle, sheep, goats and horses) of Iran and the world. Srva Press.

[12] Olopade JO, Kwari HD, Agbashe IO, Onwuka SK. Morphometric study of the eyeball of three breeds of goats in Nigeria. Int J Morphol. 2005;23(4):377-80.

[13] Rajathi S, Ramesh G, Raja K, Kannan TA, Sriram P, Hemalatha S. Microscopic anatomy of harderian gland in goats. J. Entomol. Zool. Stud. 2019;7:1413-8.

[14] Rana T, editor. Principles of Goat Disease and Prevention. John Wiley & Sons; 2023 Aug 15.

[15] Schlegel T, Brehm H, Amselgruber WM. The cartilage of the third eyelid: a comparative macroscopical and histological study in domestic animals. Annals of Anatomy-Anatomischer Anzeiger. 2001 Mar 1;183(2):165-9.

[16] Shadkhast M, Bigham AS. A Histo-Anatomical study of dorsal lacrimal gland in Iranian river buffalo. Vet Scan| Online Veterinary Medical Journal. 2010 Dec 31;5(1).

[17] Sinha RD, Calhoun ML. A gross, histologic, and histochemical study of the lacrimal apparatus of sheep and goats. American Journal of Veterinary Research. 1966 Nov;27(121):1633-40.

[18] Sisson S, Grossman JD. The anatomy of the domestic animals WD Saunders Company. Philadelphia, USA. 1940:228-1745.