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A Multivariate Approach to The Relationships of Cardiac Biomarkers with Oxidative and Inflammatory Status in Rats

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ABSTRACT

Cardiac biomarkers are considered one of the fundamental elements in the evaluation of cardiac function, while oxidative stress and inflammatory processes can also be regarded as important factors in the assessment of cardiovascular health. This study examined the relationships between cardiac biomarkers, oxidative stress, and inflammation, aiming to uncover the multidimensional interactions among these parameters. Cardiac parameters, inflammatory cytokines, and oxidative stress markers in heart tissue were analyzed in twenty-four male rats. Pearson and canonical correlation analyses were employed to assess the complex relationships between these datasets. Creatine kinase (CK) and creatine kinase–myocardial band (CK-MB) were positively correlated with malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and negatively correlated with glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase ($p<0.05$). Furthermore, cTnI showed a positive correlation with TNF-α and a negative correlation with GSH-Px. The canonical correlation coefficients for the cardiac-oxidative stress and cardiac-inflammation datasets were significant ($r_c=0.852$, $p<0.05$; $r_c=0.821$, $p<0.05$). The proportion of oxidative stress and inflammation parameters explaining the variance in cardiac biomarkers was 52.20% and 49.40%, respectively. Canonical correlation analysis, which incorporates multiple relationships, revealed the intriguing multidimensionality of the interactions among the parameters. The results suggest that the association between oxidant-antioxidant and inflammatory status is notably more intricate with CK and CK-MB than with cTnI. These significant findings offer valuable insights that could contribute to advancing diagnostic and therapeutic strategies in the field of cardiology.

INTRODUCTION

Cardiovascular diseases are a major cause of mortality and a significant public health concern in modern society (Balakumar et al., 2016; Haybar et al., 2023). According to the World Health Organization (WHO), an estimated 17.3 million people died from cardiovascular diseases in 2008, accounting for approximately 30% of all global deaths. It is projected that by 2030, cardiovascular disease-related deaths will increase to approximately 23.6 million, mainly driven by heart disease and stroke (Norton et al., 2011). Additionally, 5.8 million heart patients in the USA have healthcare costs exceeding 39 billion dollars (Norton et al., 2011). After discovering cardiac biochemical markers, clinicians can obtain invaluable information from serum cardiac biomarkers, patient history, and electrocardiographic analysis. This information makes it easier for clinicians to determine the diagnosis, select treatment, and assess prognosis. However, current cardiac biomarkers, while highly sensitive and specific to myocardial damage, have the disadvantage of being unable to precisely identify the extent of the damage (Howie-Esquivel and White, 2008).

According to clinical studies, inflammatory disorders are seen as a risk factor for cardiovascular diseases. It has also been reported that various inflammatory cells cause vascular oxidative stress (Steven et al., 2019). It is reported that cardiovascular diseases are closely related to increased oxidative stress and inflammation, and antioxidant and anti-inflammatory therapies could be necessary to treat cardiovascular diseases (Daiber et al., 2021). Inflammatory cytokines modulate the phenotype and function of all myocardial cells and may cause impairments in cardiac function by suppressing contractile function in cardiomyocytes (Hanna and Frangogiannis, 2020). In addition to cardiac biomarkers, immune components play an essential role in diagnosing, treating, and prognosing cardiovascular diseases. For instance, plasma levels of IL-1α, IL-18, IL-33, IL-6, and IL-8 positively correlate with atherosclerosis, while plasma levels of other interleukins, such as IL-35, negatively correlate with acute myocardial infarction or cardiac angina. Additionally, the IL-1 superfamily plays a critical role in various cardiovascular diseases, including atherosclerosis, with IL-20 having a pro-atherogenic role, while others, such as IL-10 and IL-19, serve an anti-atherogenic function (Haybar et al., 2023). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's antioxidant defense capacity, as small amounts of ROS are typically produced inside cells to play a role in cell signaling, and the body's antioxidant defense system can easily handle these small amounts, but in certain conditions, such as illness, the production of ROS can overwhelm the body's antioxidant system, leading to damage and cell death (Van der Pol et al., 2019). Increased oxidative stress in heart diseases like heart failure is responsible for damaging heart tissue (Ahmed and Tang, 2012; Aimo et al., 2020). Some studies suggest that antioxidant treatments can be used for heart conditions (Ahmed and Tang, 2012).

Given the ongoing challenge of predicting cardiovascular diseases despite the wide range of cardiovascular biomarkers available, the study aims to analyse the relationship between cardiac parameters, inflammatory cytokines, and oxidative stress using the Pearson and canonical correlation method. The goal is to uncover the detailed relationships between commonly used clinical parameters cardiac biomarkers (CK, CK-MB, cTnI), inflammatory cytokines (TNF-α, IL-6, IL-1β), and oxidative stress markers (MDA, GSH, GSH-Px, and catalase) in the study. By doing so, we hope to contribute to improving diagnosis and treatment processes for cardiovascular diseases.

MATERIALS AND METHODS

Ethics and animals

The Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (HADYEK) approved the study (Decision No: 2023/05-10). Twenty-four male wistar rats (220-250 g) were provided with food and water ad libitum and exposed to a 12-hour light and 12-hour dark cycle. The animals were anaesthetised with xylazine (10 mg/kg) and ketamine (80 mg/kg) and then euthanised. Blood samples were collected from the heart chambers for biochemical analyses, and heart tissue samples were collected after euthanasia.

Biochemical analyses

The collected blood samples were centrifuged, and cardiac parameters were analyzed using an electrochemiluminescence immunoassay. Oxidative stress markers (MDA, GSH, GSH-Px, and catalase) were assessed via spectrophotometric methods, and inflammatory cytokines (TNF-α, IL-6, and IL-1β) in heart tissue were quantified using commercial ELISA kits.

Statistical analyses

The study rigorously controlled the variables by applying the D'Agostino-Pearson test to assess normality and Levene's test to evaluate the homogeneity of variances. The Pearson correlation coefficient was used to assess the relationships between cardiac, oxidative stress, and inflammation parameters. Prior to conducting canonical correlation analysis, the variables were evaluated for multicollinearity with the scores for the variance inflation factor (VIF). Canonical correlation analysis was then used to examine the associations between the independent set (X), consisting of cardiac biomarkers (CK, CK-MB, and cTnI), and the dependent set (Y), which included MDA, GSH, GSH-Px, catalase, and inflammation markers (TNF-α, IL-6, and IL-1β). This analysis explored the interrelationships between variables in the cardiac parameter set and those in the oxidative stress and inflammation parameter sets. The canonical correlation coefficients' F values were analyzed using Wilks' Lambda values, a significant test. The redundancy index was used to calculate the ratio of explained variance. The significance level was set at $p<0.05$.

RESULTS

Pearson correlation coefficient between cardiac biomarkers, oxidative stress biomarkers and inflammatory cytokine levels

A moderate positive and significant correlation was observed between CK and MDA ($p<0.01$). In contrast, a negative and significant correlation was found between GSH, GSH-Px, and catalase ($p<0.05$, $p<0.05$, and $p<0.01$, respectively). A moderate positive and significant correlation was also identified between CK and TNF-α, IL-6, and IL-1β ($p<0.01$). Similar relationships were found between CK-MB and oxidative stress and inflammation parameters, as shown in Table 1. Furthermore, a moderate negative and significant relationship was discovered between cTnl and GSH-Px ($p<0.05$), and a moderate positive and significant relationship was found with TNF-α ($p<0.01$). Notably, no statistically significant relationship was found between cTnl and other oxidative stress and inflammation parameters ($p>0.05$). The correlation coefficients for the analysed parameters are presented in Table 1.

Canonical correlation between cardiac biomarkers and oxidative stress

The canonical correlation coefficient between the cardiac and oxidative stress biomarkers was found to be 0.852, indicating a statistically significant relationship ($p<0.05$). The oxidative stress parameters accounted for 52.20% of the variance in the cardiac parameters. Notably, CK, CK-MB, and cTnI demonstrated the most substantial impact on the cardiac parameters, while catalase, MDA, GSH, and GSH-Px significantly influenced the oxidative stress parameters. Further details, including the canonical correlation results, can be found in Table 2.

Table 1. Pearson correlation coefficient between cardiac biomarkers, oxidative stress biomarkers and inflammatory cytokine levels.

	MDA	GSH	GSH-Px	Catalase	TNF-α	IL-6	IL-1β
CK	0.639**	-0.523*	-0.477*	-0.675**	0.677**	0.578**	0.574**
CK-MB	0.514*	-0.548*	-0.553*	-0.575**	0.700**	0.559*	0.563**
cTnl	0.377	-0.331	-0.518*	-0.375	0.590**	0.314	0.262

*: $p<0.05$; **: $p<0.01$

Table 2. Canonical correlation between cardiac and oxidative stress biomarker levels

Variables		Canonical Loadings
Cardiac Parameters	CK	0.979
	CKMB	-0.892
	cTnl	-0.636
Oxidative Stress Parameters	MDA	0.741
	GSH	0.648
	GSH-Px	0.635
	Catalase	0.789
Canonical Correlation Coefficient		0.852 ($p<0.05$)
R^2		0.726
Explained Variance Ratio (%)		52.20

Canonical correlation between cardiac biomarkers and inflammatory cytokines

The canonical correlation coefficient between cardiac biomarkers and inflammatory cytokines was 0.821 ($p<0.05$). The rate of inflammatory cytokines explaining cardiac parameters was 49.40%. CK, CK-MB, and cTnI exhibited the most significant associations with cardiac parameters, while TNF-α, IL-6, and IL-1β demonstrated the most significant impact on inflammatory cytokines, respectively. Detailed results are available in Table 3.

Table 3. Canonical correlation between cardiac biomarkers and inflammatory cytokine levels.

Variables		Canonical Loadings
Cardiac Parameters	CK	0.945
	CKMB	0.947
	cTnl	0.637
Inflammatory Cytokines	TNF-α	0.900
	IL-6	0.725
	IL-1β	0.719
Canonical Correlation Coefficient		0.821 ($p<0.05$)
R^2		0.675
Explained Variance Ratio (%)		49.40

DISCUSSION AND CONCLUSION

Cardiac parameters are fundamental biomarkers that have been widely utilized in clinical practice for many years (Collinson, 1998; Penttila et al., 2000). In patients with cardiac pathology, assessing these parameters during standard diagnostic procedures is essential for evaluating cardiac function and identifying underlying pathophysiological conditions (Nawaytou and Bernstein, 2014). Despite advancements in diagnostic techniques, differentiating the potential causes of chest pain remains challenging, with approximately one in ten patients experiencing acute myocardial infarction being mistakenly discharged from the emergency department (Collinson, 1998). Previous studies have emphasized that the evaluation of multiple cardiac parameters is essential for the early diagnosis and management of acute myocardial infarction, ultimately contributing to reduced mortality rates (Kim et al., 2022). Creatine kinase (CK), creatine kinase-MB (CK-MB), and cardiac troponin I (cTnI) are commonly measured biomarkers for assessing cardiac status in cardiovascular diseases (Mladenka et al., 2009; Mladenka et al., 2014). A study conducted in rats demonstrated a strong correlation between cardiac troponin T (cTnT), stroke volume index, ventricular weight, and myocardial calcium levels (Mladenka et al., 2009). Similarly, another study reported that serum cTnT concentrations exhibit a significant correlation with parameters reflecting the extent of myocardial damage, such as calcium overload and stroke volume (Mladenka et al., 2013). However, in a separate study on rats, cTnT did not show a strong correlation with myocardial calcium levels, ventricular weight, or electrocardiographic (ECG) parameters, including T-wave, R-wave, and J-junction amplitudes (Mladenka et al., 2014). Given these findings, further investigation into the potential relationships between cardiac biomarkers, oxidative stress parameters, and inflammatory status may provide valuable insights for improving the diagnosis and treatment of cardiac diseases in clinical settings.

According to a recent study, associations between cardiac parameters, oxidative stress, and inflammatory biomarkers have been demonstrated (Gökçek, 2024). In particular, recent research has shown that groups with elevated cardiac biomarkers, such as CK-MB, CK, and cTnI, also exhibit higher levels of inflammatory cytokines and oxidative stress markers, along with decreased antioxidant capacity (Çömez et al., 2020). In the study, Pearson correlation analyses further revealed significant relationships between CK and CK-MB and multiple oxidative stress and inflammatory markers, including MDA, GSH, GSH-Px, catalase, TNF-α, IL-6, and IL-1β. In contrast, cTnI demonstrated significant correlations only with GSH-Px and TNF-α.

In preclinical research, multivariate analyses are crucial for examining complex datasets, with canonical correlation analysis (CCA) being one of the most widely utilized statistical methods, as it constructs linear combinations that maximize the correlation between two datasets, thereby identifying the most relevant relationships (Figueroa et al., 2020; Vargas, 2022). For instance, a recent study employed similar multivariate analysis techniques to investigate the associations between sperm parameters, oxidative stress markers, and inflammatory biomarkers in detail (Kaya and Olğaç, 2024). Given its ability to elucidate intricate relationships, CCA may prefer as an appropriate method for exploring the connections among cardiac biomarkers, oxidative stress, and inflammatory markers.

Oxidative stress is a well-established contributor to cardiovascular pathophysiology, while antioxidant defense systems play a crucial role in maintaining cardiac health (Tsutsui et al., 2011). A strong association between oxidative stress and cardiac biomarkers has been reported (Çömez et al., 2020; Gökçek, 2024). For instance, a study demonstrated a significant relationship between oxidative stress markers and cardiac parameters (Çömez et al., 2020). In an experimental study on rats, cTnT exhibited a weak negative correlation with total blood glutathione, whereas no significant association was observed with plasma TBARS (Mladenka et al., 2014). Similarly, another study reported weak or no correlation between cTnT and certain oxidative stress parameters, suggesting that cTnT may not be a reliable indicator of cardiovascular function in this context (Mladenka et al., 2013). In the present study, a correlation was identified between oxidative stress markers and cardiac biomarkers, with catalase, MDA, GSH, and GSH-Px contributing most significantly to this association. Inflammatory cytokines are also known to play a pivotal role in modulating cardiac function under both physiological and pathological conditions. Their involvement in various cardiac pathologies, such as myocardial infarction and heart failure, depends on the nature of the disease, as well as the extent and duration of tissue damage (Bartekova et al., 2018). Cardiac inflammation has the potential to impair cardiac function and induce lasting damage (Lafuse et al., 2020), to the extent that systemic inflammation is considered a contributing factor in heart failure (Carrillo-Salinas et al., 2019). A study further demonstrated that inflammatory conditions were strongly associated with heart failure in patients with uremia (Zhang et al., 2021). There is growing evidence supporting the correlation between cardiac biomarkers and inflammatory cytokines (Bartekova et al., 2018; Çömez et al., 2020). In a study investigating myocardial injury, increased CK-MB levels, along with impaired ECG findings, confirmed heart damage, while a positive correlation between CK-MB and inflammatory cytokines was observed (Durdagi et al., 2021). Furthermore, a recent study in rats reported a significant positive correlation between cardiac biomarkers (CK, CK-MB, and cTnI) and the gene expressions and protein levels of inflammatory cytokines, including TNF-α, IL-6, NF-κB, COX-2, and Nrf-2, in cardiac tissue following ketamine-induced cardiotoxicity (Çömez et al., 2020). Notably, TNF-α, IL-6, and IL-1β were identified as the primary contributors to this correlation, further highlighting the intricate interplay between inflammatory status and cardiac function.

In conclusion, cardiac biomarkers serve as essential tools in the clinical assessment of cardiac function; however, their diagnostic utility may be enhanced when considered alongside additional physiological markers. This study utilized various correlation analyses to explore the relationships between cardiac biomarkers, oxidative stress, and inflammatory status. Multivariate methods such as canonical correlation provide an advantage to researchers in the evaluation of health biomarkers that vary under the influence of multiple factors. The findings demonstrated that CK played a significant role in influencing cardiac parameters, while catalase and TNF-α were the most prominent contributors to oxidative stress and inflammatory responses, respectively. The complex interplay between cardiac biomarkers, particularly CK and CK-MB and oxidative stress and inflammatory parameters underscores the potential for a more comprehensive approach to diagnosing and managing cardiac diseases. These findings, rooted in the complex biochemical and physiological interactions governing cardiovascular health, highlight the importance of further research. Future studies may build upon this foundation to develop targeted therapeutic strategies and precision medicine approaches aimed at improving cardiovascular outcomes.

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Ethical Declaration

The Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (HADYEK) approved the study (Decision No: 2023/05-10).

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: I.G., U.K., Design: U.K., Data Collection or Processing: I.G., U.K., Analysis or Interpretation: I.G., U.K., Literature I.G., Writing: I.G., U.K.

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Evaluation of Granulomatous and Vascular Lesions in Feline Infectious Peritonitis

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ABSTRACT

Feline Infectious Peritonitis (FIP) is a fatal systemic viral disease that affects all cat breeds and has two distinc forms: effusive and non-effusive. In the effusive form, there is a common type of vasculitis that causes extravasation of fibrin-rich fluid, resulting in the accumulation of yellow exudative fluid in the body cavities. In the present study, we aimed to evaluate the organ distribution and severity of the pathological alterations associated with granuloma and vasculitis. It also seeks to describe FIPV antigen localization in FIP lesions. For this purpose, necropsies were performed on eight cats suspected of having died from FIP at the Faculty of Veterinary Medicine, Kırıkkale University. Tissue samples were routinely processed for immunohistochemical analysis. FIPV antigen was detected using immunoperoxidase staining, and the immunopositivity of vasculitic and granulomatous lesions in various organs was evaluated semi-quantitatively for each tissue sample. In effusive FIP cases, peritoneal fluid accumulation was significantly more common, and the lesions were characterized by granulomas progressing through the serosa of the intestine, kidney and liver. Vasculitis lesions, usually affecting small and medium-sized vessels, were characterized by endothelial hypertrophy and swelling, edema and hyalinization of the muscular layer, and adventitial neutrophil leukocyte and macrophage infiltration. Granulomas were characterized by microscopic findings with dense infiltration of macrophages and lymphocytes around a few thrombotic and/or degenerative vessels in the center. In the examined cases, FIPV antigen immunopositivity varied according to the organ involvement in each case, but was frequently concentrated around the periphery rather than in the center of vasculitis and granulomatous lesions.

INTRODUCTION

Feline infectious peritonitis (FIP) is a fatal systemic viral infection caused by Feline Corona Virus (FCoV) that affects especially domestic and group-living cats and can be seen in all cats (Foley et al., 1997; Kipar and Meli, 2014). It is an enveloped, single-stranded RNA virus belonging to the Alphacoronavirus genus within the Coronaviridae family and has two different pathotypes, Feline Infectious Peritonitis Virus (FIPV) and Feline Enteric Corona Virus (FECV) (Gonzáles et al., 2003; Payne, 2017). While the highest disease rate is seen in cats between the ages of 3 months and 2 years, it has been reported that this rate increases especially in male, pure breed and non-neutered cats (Rohrbach et al., 2001; Worthing et al., 2012).

While FCoV replicates in enterocytes, and subsequently a subclinical infection period develops. The most widely accepted theory for the development of FIP infection is that the virus mutates and spreads throughout the body after gaining the ability to infect macrophages and monocytes (Herrewegh et al., 1998; Pedersen, 2014). With the systemic spread and replication of FIPV, the expression of some cytokines increases, which facilitates the interaction of activated monocytes in small and medium-sized vascular endothelial cells (Kipar et al., 2006; Regan et al., 2008; Takano et al., 2009). In activated monocytes, the expression of matrix metalloproteinase-9 enzyme increases, which leads to disruption of endothelial function and allows monocytes to exit the vessel and spread to surrounding tissues (Pedersen, 2009; Takano et al., 2007). In the effusive form of feline infectious peritonitis, there is a diffuse vasculitis picture, which results in the leakage of fibrin-rich fluid out of the vessels, resulting in the accumulation of yellow, fibrin-containing exudate in the abdominal and thoracic cavity (Andrew, 2000). While fluid does not accumulate in the cavities in the non-effusive form, meningitis and obstructive hydrocephalus, associated ataxia, loss of reflexes in the eyes and nystagmus may occur in the effusive form (Montali and Strandberg, 1972). In the present study, we aimed to evaluate the distribution and severity of granulomatous lesions and vasculitis and to define FIPV immunolocalizations in effusive FIP cases.

MATERIALS AND METHODS

Sampling Methodology

The material of the study consisted of clinically suspected FIP cats that were routinely brought to Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology for necropsy and histopathologic diagnosis (Table 1). After systemic necropsy of 8 cats, tissue samples were fixed in 10% buffered formaldehyde for 48 hours for pathologic examination.

Table 1. Table of breed, sex, age and disease form of FIP disease cases that routinely visited Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology

Case number	Species	Age	Sex	Form of the disease
1	Tabby	2,5 years	Famale	Effusive
2	Tabby	10 months	Male	Effusive
3	Tabby	7 months	Male	Effusive
4	Tabby	1 year	Male	Effusive
5	Tabby	10 months	Famale	Non-Effusive
6	Tabby	6 months	Famale	Effusive
7	Tabby	2 years	Male	Effusive
8	Yellow Tabby	8 years	Male	Effusive

Histopathologic Examinations

Liver, kidney, lymph node, spleen, intestine, lung and heart tissues were routinely processed on a tissue processing device after trimming and embedded in paraffin. From the paraffin blocks, 4 µm thick sections were taken on positively charged slides and kept in an oven at 37°C for 24 hours. The sections were deparaffinized for routine staining and then rehydrated and stained with hematoxylin and eosin. At the last step, the tissues were again processed through alcohol and xylene series and covered with entellan. In microscopic evaluation, lesions characterized by granulomas and vasculitis were assessed and scored semiquantitatively.

FIPV Specific Immunoperoxidase Test

4 µm thick tissue sections were taken and kept overnight in an oven at 37°C and then deparaffinized in xylene, graded alcohol series for five minutes each and washed in phosphate buffer solution (PBS) and then placed in citrate buffer (pH 6.0) solution. The slides were subjected to antigen retrieval in a microwave oven at 1200 W for 20 minutes. After removing from the microwave, the cooled slides were kept in PBS followed by 0.3% H2O2 for 15 minutes and then again in PBS solution. To prevent nonspecific staining, 1/100 diluted FIPV antibody (FIPV3-70 SantaCruz) was dropped on the tissue sections, which were protein blocked for 7 minutes, without washing and incubated in an oven at 37°C for 2 hours. At the end of the incubation period, tissue sections were washed with PBS to remove the antibody. After 30 minutes incubation in biotin-labeled secondary antibody, the tissues were washed again with PBS. Then they were incubated in streptavidin peroxidase solution for 30 minutes, washed again and stained with DAB chromogen. Finally, the hematoxylin stained tissues were washed under tap water, dehydrated and covered with entellan. For the evaluation of FIPV immunopositivity in tissues, photographs were taken at 20x objective magnification from 5 different areas with vasculitis and granulomatous lesions and scored semiquantitatively as described below. 0=No staining, 1=1-10% area staining, 2=10-30% area staining, 3=30-50% area staining, 4=50-100% area staining.

RESULTS

Necropsy Findings

Although cachectic appearance and jaundice were prominent in most of the cats on general external examination, it was noted that the skin lost its elasticity and they were dehydrated. Yellow-colored cloudy fluid (Figure 1A) was observed in the abdominal cavity in 6 cases and in the thoracic cavity in one of the effusive form FIP cases examined in the study. In cases with abdominal effusion, granulomas and pyogranulomas of varying sizes were observed on the serosal surfaces of the affected organs (liver, kidney, intestines) (Figure 1B, C). In some cases, fibrin-induced adhesions between organs were present. In general, mesenteric lymph nodes were enlarged. Mesenterium was hyperemic and covered with pyogranulomas. The liver was swollen and pale in appearance. Kidneys were icteric or pale in almost all cases (Figure 1C). In 1 case with exudate accumulation in the thorax, the lungs were pale and edematous with fibrin deposits and adhesions between the lobes and with the pericardium.

Histopathologic Findings

Although the distribution of lesions in the organs differed according to effusive or non effusive FIP (Figure 2), in the affected abdominal cavity organs, the serosa of the intestine, liver and kidney was thickened with dense fibrinous, neutrophil leukocyte and macrophage infiltration. In most of the cases, increased cellular infiltration of macrophages, lymphocytes and plasma cells was observed in the submucosa layer of the small intestine and around the vessels. In the liver, dense granulomas with necrotic changes were observed in most areas. Hepatocytes were swollen, remark cords were irregular and bile stasis and biliary pigments were observed in the sinusoids. In the kidneys, diffuse or multifocal mononuclear cell infiltrations and necrotic granulomas were observed. In the spleen, inflammatory changes limited to the capsule on initial examination were observed to transform into granulomas with macrophages in the center and lymphocytes around it, extending into the subcapsular region in severe cases. In the lungs, especially in cases with pleural effusion, edema and fibrinous effusion in the subpleural, bronchial and bronchiolar lumens and mononuclear cell infiltration of varying severity around these bronchioles were observed. In the heart, mononuclear cell infiltration in the pericardium, phlebitis characterized by media necrosis and thrombophlebitis were noted. The vessel walls were thickened and there was a dense fibrin deposition in the vessel lumens.

Immunohistochemical Findings

In the examined cases, immunopositivity scores in tissues stained with FIPV antibody varied depending on the organ (Figure 3), but were frequently observed in regions with granulomatous lesions and vasculitis. In the intestines, viral antigen positivity was detected in the submucosa, while the serosa showed stronger immunopositivity, particularly surrounding granulomas. High immunopositivity scores were also noted around granulomas in the mesentery. In the liver, positive staining was observed in areas of mononuclear cell infiltration in the capsular and subcapsular regions, as well as in inflammatory cells localized in perivascular areas. In the kidneys, immunopositivity was especially prominent in the regions extending from the renal capsule to the subcapsular area, particularly in association with granulomatous lesions. In contrast, positivity scores in the lungs and heart were lower than those observed in other organs. In the brain, immunopositive staining was concentrated in the leptomeninges. Notably, even in cases presenting with the wet form of the disease, immunopositivity was evident. In the retina, positive immunoreactions were observed in macrophage-rich inflammatory infiltrates near the optic nerve. In this study, immunopositive findings were evaluated based on the density of staining around vasculitic and granulomatous lesions, with the results summarized in Tables 2 and 3.

Table 2. Immunopositivity of tissues stained with FIPV3-70 antibody around vasculitis

Case no	Vasculitis								
	Mesentery	**Liver**	**Kidney**	**Lung**	**Heart**	**Leptomeninges**	**Eye**	**Spleen**	**Other**
1	3	1	2	1	2	1	2	1	LN2, UB3
2	3	1	2	2	0	0	0	3	LN2
3	2	3	1	2	0	0	0	2	
4	3	2	3	2	0	2	2	2	
5	1	NE	NE	NE	NE	2	0	1	
6	4	4	2	0	4	0	2	NE	
7	0	0	0	2	2	0	0	1	
8	0	0	0	0	0	0	0	NE	

LN: Lymph node, UB: Urinary bladder, NE: Not examined

Table 3. Granulomatous inflammation immunopositivity of tissues stained with FIPV3-70 antibody

Case no	Granulomatous Inflammation								
	Mesentery	**Liver**	**Kidney**	**Lung**	**Heart**	**Leptomeninks**	**Eye**	**Spleen**	**Other**
1	4	1	4	2	2	3	3	2	LN3, UB3
2	3	1	3	0	0	0	0	3	LN3
3	2	4	1	3	0	0	0	2	
4	3	2	4	2	0	2	2	2	
5	1	NE	NE	NE	NE	1	0	1	
6	4	4	3	0	2	0	2	NE	
7	0	0	0	3	2	0	0	1	
8	0	0	0	2	0	0	0	NE	

LN: Lymph node, UB: Urinary bladder, NE: Not examined

DISCUSSION AND CONCLUSION

The mechanisms of pathogenesis, fluid effusion and granuloma formation in feline infectious peritonitis are not fully understood (Pedersen, 2009). The poor prognosis of FIP cases reaching clinics and the fact that there is still no clear treatment, except for antiviral molecules in clinical trials, seems to be directly related to the complex pathogenesis of the disease. For this reason, much of the research on FIP has focused on the relationship between the different mutational pathotypes of the virus and host immunity. In the effusive form of FIP, which results in death, abdominal effusion is known to be more common than thoracic effusion (Pedersen and Boyle, 1980). In a study of 25 cases, it was revealed that the development of the FIP form was closely related to the immune response of the host, and effusive form was observed in 64 of the cases (Pedersen, 2014). In the present study, it was similarly shown that effusive form developed in 7 of 8 cases, and serofibrinous effusions were frequently abdominal. It is also a matter of debate as to which organ lesions the fluid leaking into the body cavities mostly originates from. In the effusive form, the virus adheres to the parietal and visceral layers of the peritoneum and damages the serosa epithelial cells in the initial stages of the infection, which are the physical barrier, and the lesions associated with necrotic phlebitis that develop in the advanced stages constitute the main cause of fluid passage into the body cavities (Kipar et al., 2005). Here, it is thought that fluid passage into the abdominal cavity may be due to increased vascular permeability in the liver capsule and parenchyma. In cases of non-infectious ascites, increased portal hypertension and intravascular pressure in the liver and transudate passage through Glisson's capsule into the abdominal cavity support this view (Angelo and Kurzrock, 2007). In FIP, increased permeability of the vessel wall directly results in leakage of fibrin and blood plasma from the serous capsule into the abdominal cavity. This occurs to a lesser extent in the inflamed serosa of the mesenterium and intestinal serosa, which is normal in the absence of functional blood circulation in these organs and tissues. Therefore, while planning drug protocols for the treatment of FIP, it may be beneficial to combine strategies to reduce vasculitis and FIPV-related vascular leakage, especially in the liver. On the other hand, there is still insufficient information on how vasculitis develops in FIP and how effective it is in granuloma formation and fibrinous fluid effusion.

Clinical disease in cats develops when the virus gains the ability to replicate in monocytes/macrophages (Francisco et al., 2016). The virus, which is transported to the target organs via macrophages leaving the bloodstream, localizes in the reticuloendothelial system and perivascular areas of many organs after the infected macrophages leave the bloodstream and the virus gains the ability to enter the tissues. In all this situation, the virus attracts antibodies, the complement system is activated, and more neutrophils and macrophages come to these areas. As a result, typical granulomatous/ pyogranulomatous changes occur (Nafe, 1984; Pedersen, 1995). In this study, the localization of FIPV in granulomatous lesions was mostly localized around granulomatous foci, with immunopositivity in monocyte/macrophage cytoplasm in the vessel lumen. Interestingly, in severe lesions with necrotic vasculitis and hyalinization of the vessel wall, low-intensity levels of FIPV were observed. This seems to support the role of mediators such as Th1 cytokines, cell adhesion molecules and VEGF, which may have a direct viral invasion effect at the onset of vascular lesions but are more activated in advanced vessel wall damage and fluid leakage. Further studies are needed on the aforementioned vascular damage and the pathogenesis of fluid exudation in FIP and the effect of mediators.

Typical FIP vasculitis is a phlebitis mediated mostly by activated virus-infected monocytes, a few neutrophils and T-cells (Kipar et al., 2005). Based on these features, FIP vasculitis can be distinguished from immune-mediated vasculitis (Kipar et al., 2005). However, in acute cases, necrosis of the vessels could be observed and it was even observed that it occurred in vessels where inflammatory reactions were previously detected, confirming that the disease may have a multiphasic nature (Boudreaux et al., 1989; Hayashi et al., 1977; Weiss et al., 1980). Thus, due to the morphologic features of acute vascular lesions, evidence has been provided that type III hypersensitivity reaction contributes to the pathogenesis of FIP in some cases (Boudreaux et al., 1989; Montali and Stranberg, 1972; Weiss et al., 1980). Perivascular granulomatous lesions due to FIP infection have been associated with immunopathogenic mechanisms such as type III and type IV hypersensitivity reactions (Jacobse-Geels et al., 1982; Paltrinieri et al., 1998; Pedersen and Boyle, 1980). Type III hypersensitivity is seen when antigens bind to antibodies and the resulting immune complexes are deposited on the vessel walls (Wills-Karp, 2008). Type IV hypersensitivity is a late hypersensitivity reaction caused by excessive stimulation of T-cells and macrophages, which also contributes to granuloma formation (Benacerraf and Levine, 1962). Vasculitis resulting from monocyte activation was supported by the identification of increased vascular permeability and induction of effusions following the release of vascular endothelial growth factor (VEGF) by FIPV-infected monocytes.

Whether the cat develops the disease in effusive or non-effusive form depends on an insufficiently activated cellular immunity or a strong humoral response. Although often described as distinct syndromes, effusive and non-effusive FIP are caused by an excessive inflammatory response following disease-specific vasculitis and pyogranulomatous lesions. The effusive form is more common than the non-effusive form, and cases of both are common (Pedersen and Boyle, 1980). In general, the balance between cellular and humoral immune response is a critical determinant in infected animals. In part, a more vigorous T cell immune response is thought to cause dry form FIP (Pedersen, 2009; 2014).

In this study, necropsies of naturally infected cats with FIPV were performed and the tissues were stained with histopathological and immunohistochemical immunostaining after the necessary procedures and the lesions caused by the virus in the organs were identified. When the results of the study were evaluated, vasculitis was characterized by endothelial hypertrophy and endothelial swelling in small vessels, while in medium-sized vessels it was characterized by media necrosis and adventitial inflammatory cell infiltration and fibrin deposition in the lumen.

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Ethical Declaration

Since the study material was routine specimens brought to Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, no ethics committee was required.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept, Design, Data Collection or Processing, Analysis or Interpretation, Literature Search, and Writing took placed by O.K. and T.S.Y.

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Prevalence and Risk Factors of Ectoparasite Infestation of Buffaloes from Coastal Regions of Bangladesh

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ABSTRACT

Ectoparasitic infestation can bring a threat to cost-effective buffalo production by causing mechanical damage, including, irritation, anaemia, allergic reaction, loss of condition, and even death. The current study was aimed to determine the ectoparasite status of buffaloes in the coastal regions of Bangladesh. For this study, 270 buffaloes from three selected districts (Noakhali, Bhola and Bagerhat) were randomly selected and examined visually. Buffaloes from different sexes and age ranges were selected based on their availability, and several risk factors, such as season and management systems, were also considered for the current study. Four (4) species of ectoparasites, namely, *Haemaphysalis bispinosa*, *Rhipicephalus microplus*, *Linognathus vituli*, and *Damalinia bovis*, where 47, comprising at 17.41% of overall prevalence was documented among the study areas. In contrast to the species-wise prevalence, *H. bispinosa*, *R. microplus*, *L. vituli*, and *D. bovis* were detected in 26, 11, 3, and 7 buffaloes, comprising a prevalence of 9.63%, 4.07%, 1.11%, and 2.59%, respectively. Moreover, the highest prevalence (18.52%) was seen in the adult compared to young buffaloes (12.96%), and females (18.92%) were more likely to be infected than males (15.57%). In addition to this, seasonal variations in the presence of ectoparasites were observed, with Winter having the highest infection levels (28.89%), followed by Summer (16.67%), and Rainy (6.67%). The ectoparasites, encountered in this study, pose significant threats to public health due to their vector importance, therefore, necessitating regular monitoring and effective control measures should be implemented in this study area.

INTRODUCTION

Buffaloes, along with cattle, are imperative members of the family Bovidae, which include various species, of which water buffaloes are the most widely distributed throughout the world, with a population of 200 million (Borghese A, 2005; Minervino et al., 2020). There are currently 1.457 million buffalo in Bangladesh, which are reared for both personal subsistence and large-scale bathan farming in salty coastal regions. Moreover, buffalo are mostly employed as draft animals, however, they are also partially raised for milk and meat production (Hamid et al., 2016). Entities that live on the skin or skin surface, such as lice, ticks, mites, and fleas, are known as ectoparasites and can be extremely important in spreading certain infections (Sahito et al., 2017). For instance, a number of bacterial, viral, rickettsial, and protozoal infections are contaminated by ticks and mites (Apanaskevich et al., 2018). Ectoparasitic infestation can bring a threat to cost-effective buffalo production by causing mechanical damage, including, irritation, anemia, allergic reaction, loss of condition, and even death (Iqbal et al., 2014).

Among the ectoparasites, mites can cause severe losses, including rejecting skin, reduced production, anemia, and even death, when encountered in a good number (Arora and Arora, 2012; Sayyad et al., 2016). Mange mites have been linked to significant financial losses because of their harm to skin, anemia, poor physical health, slowed growth rates, and milk supply (Aatish et al., 2007). Moreover, ticks, one of the ectoparasites, have been known to pose a serious risk because of the diseases they can spread, including babesiosis, theileriosis, and anaplasmosis, as well as the allergic condition, extreme irritation, and tick toxicosis they can create in the host body (Sajid et al., 2008). In addition to this, ticks are important biological vectors of several disease-causing microbes, such as bacteria, viruses, rickettsia, and protozoans (Jongejan and Uilenberg, 2004), causing morbidity of buffaloes (Rony et al., 2010; Sultana et al., 2015). A good number of buffaloes reared in large-scale bathan farming are also affected by irritation, abscesses due to tick-bite, and severe blood loss which cause negative stressful effects on the animals (Manan et al., 2007). Furthermore, reduced hide quality can negatively impact the tanning industry and destroy a nation's Gross Domestic Products (GDP), are mostly caused by louse infestation, which affects the buffaloes raised under innumerable management systems to varying degrees (Green et al., 2001; Aatish et al., 2007; Shamim et al., 2015).

Different agroclimatic conditions, animal husbandry techniques, and pasture management largely impact the prevalence and intensity of various ectoparasitic conditions in a region. According to the literature, these factors would serve as a guideline for developing tactical and strategic control of these ectoparasites. In Bangladesh, very limited research has been conducted on the prevalence and the associated risk factors related to ectoparasites in buffaloes, especially in the southern areas. As a result, the current study was conducted to investigate the ectoparasite status of buffaloes in the southern areas of Bangladesh.

MATERIALS AND METHODS

Study areas and periods

Ectoparasites (ticks and lice) were carefully collected through the visual inspection by registered veterinarians from three selected districts due to the availability of buffaloes. These districts (Noakhali, Bhola and Bagerhat) are located in the southern parts of Bangladesh (Figure 1). Additionally, the Department of Livestock Services (DLS) verbally granted authorization for sampling, and all necessary processes were completed under the Animal Welfare Act of 2019. The cross-sectional study continued for one year comprising July 2023 to June 2024, where three predominant seasons, namely Rainy (July to October), Winter (November to February), and Summer (March to June) were covered by this study period. In each season, a single round of sampling from every study area was conducted, during which ectoparasites were collected from all selected animals within a short, fixed period to minimize temporal variation.

Sample size

270 buffaloes from the aforementioned locations were selected randomly and examined visually for this investigation. Based on their availability, buffaloes of different sexes or age ranges were chosen for the current investigation, where several risk factors, like season, managemental systems, etc. were also included. During sampling, 87 individuals were male and the rest 183 were female. Additionally, buffaloes were divided into two age groups: young (less than a year old) and adults (more than a year older), where the majority of the buffaloes were adults.

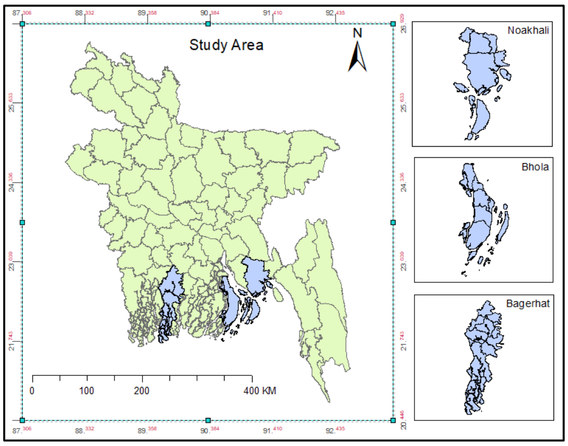

Figure 1. Location of study area

Sample size

270 buffaloes from the aforementioned locations were selected randomly and examined visually for this investigation. Based on their availability, buffaloes of different sexes or age ranges were chosen for the current investigation, where several risk factors, like season, managemental systems, etc. were also included. During sampling, 87 individuals were male and the rest 183 were female. Additionally, buffaloes were divided into two age groups: young (less than a year old) and adults (more than a year older), where the majority of the buffaloes were adults.

Collection of ectoparasites

Following proper confinement, the buffaloes were inspected in predetermined body parts (neck, ears, back, and tip of the tail), and any ectoparasites, especially, ticks and lice were removed subsequently from each animal. After being gathered, the ectoparasites were put into plastic tubes filled with 70% alcohol, and they were then morphologically recognized using particular traits (Soulsby E, 1982; Ruprah N, 1985; Wall and Shearer, 2008; Taylor et al., 2012; Mullen and Durden, 2018).

Processing of ectoparasites

The collected ectoparasites underwent a series of procedures, including dehydration, staining, and cleaning, before being slide mounted. First, the ectoparasites were made visible by allowing them to dissolve in 10% KOH for a whole night at room temperature. Following cleaning, the samples were put back into 50% ethanol and then into distilled water for 30 minutes each to get them ready for staining. The specimens were stained using Hematoxylin-Eosin (H & E) dye, and the slides were left in the stain for a whole night. By dipping the specimens in 3% Acid-Alcohol, the excess stain was eliminated as the specimens got darker. After that, the specimen was dehydrated into a series of ascending graded of ethanol for thirty minutes on each to complete this procedure. After dehydration, the samples were quickly cleaned with xylene and placed on a new slide having Canada balsam, and put a cover slip on it. Then, the prepared slides were left for one to two days to become dry, and morphological identification of the ectoparasites was performed under a microscope (4X and/or 10X).

Statistical analysis

Data on individual buffalo were entered into MS-excel spread sheet program to create a database. Then the data was transferred to SPSS version 20 for further analysis. Prevalence of ectoparasites in relation to sex, age, season, and rearing system were analyzed using Chi-square statistical test, where $p<0.05$ was considered to be lest limit of significance.

RESULTS

Morphological identification of ectoparasites

A total of 270 buffaloes from three different districts were included in this study where ectoparasites were collected and observed under microscope for morphological identification through a proper scientific way. Four species of ectoparasites, namely, *Haemaphysalis bispinosa*, *Rhipicephalus microplus*, *Linognathus vituli*, and *Damalinia bovis*, were identified.

Overall prevalence of ectoparasites

Through a proper examination of the randomly selected buffaloes from study areas, a total of 47, comprising at 17.41% of overall prevalence was documented which indicated relatively low prevalence of ectoparasites. With respect to area-wise infestation, the highest prevalence was encountered in Noakhali (25.56%), followed by Bagerhat (16.67%), and Bhola (10.00%). The overall prevalence along with study areas were documented in Table 1.

Table 1. Overall prevalence of ectoparasites

Areas	No. of buffaloes examined	No. of buffaloes infected	Prevalence %	P-value
Noakhali	90	23	25.56	0.069
Bhola	90	09	10.00	
Bagerhat	90	15	16.67	
Total	**270**	**47**	**17.41**	

Species-wise prevalence of ectoparasites

A total of four ectoparasites in buffaloes were encountered in this study which is shown in Table 2. Among the examined buffaloes, *H. bispinosa*, *R. microplus*, *L. vituli*, and *D. bovis* were detected in 26, 11, 3, and 7 buffaloes, comprising 9.63%, 4.07%, 1.11%, and 2.59% of prevalence, respectively.

Table 2. Species-wise prevalence of ectoparasites

Species	No. of buffaloes infected (N=270)	Prevalence %	P-value
Haemaphysalis bispinosa	26	9.63	<0.001*
Rhipicephalus microplus	11	4.07	
Linognathus vituli	3	1.11	
Damalinia bovis	7	2.59	

'*'- indicates significance at <0.05

Age-wise prevalence of ectoparasites

During sampling, buffaloes were categorized into two age groups: young (<1 year) and adult (>1 year), with a larger proportion of adults included, as the adult buffaloes were more commonly available and accessible in the study areas. The prevalence of ectoparasites was higher in adult buffaloes (18.52%) compared to young ones (12.96%), as shown in Table 3. However, the difference was not statistically significant ($p=0.412$), indicating no strong association between age and ectoparasite infestation in the studied population.

Table 3. Age-wise prevalence of ectoparasites

Variables	No. of buffaloes examined	No. of buffaloes infected	Prevalence %	P-value
Young	54	7	12.96	0.412
Adult	216	40	18.52	

Gender-wise prevalence of ectoparasites

In relation to gender during sampling, 54.81% of the study population was female, and the remaining 45.19% was male buffalo. Regarding prevalence by gender, a slight difference was documented, where females (18.92%) were more likely to be infected than males (15.57%), although there was no significant association ($p=0.544$). The gender-wise prevalence is given in Table 4.

Table 4. Gender-wise prevalence of ectoparasites

Variables	No. of buffaloes examined	No. of buffaloes infected	Prevalence %	P-value
Male	122	19	15.57	0.544
Female	148	28	18.92	

Seasonal prevalence of ectoparasites

As we mentioned before, the cross-sectional study was continued for one year comprising three predominant seasons, namely Rainy (July to October), Winter (November to February), and Summer (March to June). Seasonal variations in the presence of ectoparasites were observed (Table 5), with the Winter having the highest infection levels (28.89%), followed by Summer (16.67%), and Rainy (6.67%). Statistical analysis revealed that these differences were significant ($p=0.004$), indicating a strong seasonal influence on ectoparasite prevalence.

Table 5. Seasonal prevalence of ectoparasites in buffaloes

Variables	No. of buffaloes examined	No. of buffaloes infected	Prevalence %	P-value
Summer	90	15	16.67	0.004*
Rainy	90	6	6.67	
Winter	90	26	28.89	

'*'- indicates significance at <0.05

Prevalence regarding management system

This study found that the buffaloes raised in a free-range system had a higher prevalence of ectoparasites (40.00%) than buffaloes raised in semi-intensive (28.57%) systems. However, this difference was not statistically significant ($p=0.215$), which is documented in Table 6.

Table 6: Prevalence regarding management system

Variables	No. of buffaloes examined	No. of buffaloes infected	Prevalence %	P-value
Free-range system	152	31	20.39	0.215
Semi-intensive system	118	16	13.55	

Regions of infestation by ectoparasites on buffaloes

After reviewing the literature, the body of buffaloes was identified to have five (5) main regions: the head with ears, the neck and chest region, the back with tail region, the abdomen, and the legs. A total of 134 ectoparasites were collected in this investigation, where most of the ectoparasites were detected from two regions, i.e., the head with ears, and the area surrounding the back and tail region. Conversely, the lowest percentage of tick availability was found in the neck and chest region. Table 7 showing the attachment of ectoparasites to the host's body was statistically significant (p=0.002), indicating a preference for certain attachment sites on the host body.

Table 7. Attachment of ectoparasites on the host's body

Attachment of ectoparasites	No. of ectoparasites counted	Prevalence %	P-value
Head with ears	42	31.34	0.002*
Neck and chest region	13	9.70	
Back with tail region	35	26.12	
Abdomen	24	17.91	
Legs	20	14.93	

'*'- indicates significance at <0.05

DISCUSSION AND CONCLUSION

Through the morphological identification, four species of ectoparasites, namely, *H. bispinosa*, *R. microplus*, *L. vituli*, and *D. bovis*, were identified according to the keys and descriptions of various authors. Among them, *D. bovis* was characterized by a rounded head, three-segmented antennae, and dark transverse abdominal bands, while *L. vituli* possessed piercing mouthparts, five-segmented antennae, and enlarged second and third pairs of legs (Rony et al., 2010; Kumsa et al., 2012). On the other hands, both ticks were morphologically distinguished by their shape of basis capituli and festoons. *H. bispinosa* exhibited a rectangular basis capitulum with 11 festoons on their posterior margin, whereas *R. microplus* had a hexagonal basis capitulum, lacking festoons (Makwarela et al., 2024; Hornok et al., 2025).

Moreover, a total of 47 buffaloes, comprising at 17.41% of overall prevalence was documented which indicated relatively low prevalence of ectoparasites. The findings of this study are very similar to the reports of Hussain et al. (2006), Kakar and Kakarsulemankhel (2008), and Batista et al. (2018), who reported a prevalence ranging of 18-30% from different parts of the world. However, Sajid et al. (2008), and Islam et al. (2009), found higher prevalence of ectoparasite from Pakistan and Bangladesh, respectively. The results of the current study may differ from those of previous studies due to the different geographic locations, climate of the experimental areas, study techniques including the methods of sampling, etc. (Rony et al., 2010). In addition to this, the identified ectoparasites was reported previously by various authors with a moderate prevalence (Islam et al., 2009; Rony et al., 2010). Furthermore, we found very lower prevalence of lice in buffalo, which may be associated with the wallowing habit of them to prevent insects (Hussain et al., 2006; Tasawar et al., 2008).

The results of age-wise prevalence in this study were in agreement with Islam et al. (2009), Mamun et al. (2010), Desoky (2014), who found that prevalence of ectoparasitic infestation was higher in older group of buffaloes. Sarkar et al. (2010), postulates that the robust innate immunity and age resistance of young cattle contribute to their reduced susceptibility to tick infection, hence reducing the ectoparasitic burden. In case of gender, females were more likely to be infected than males, due to the close relation of the female with their calves during milking and the managemental systems where most of the sampled buffalo were confined within the farm. Additionally, production stressors like pregnancy and breastfeeding increase the vulnerability of female animals to infection (Mamun et al., 2010).

Seasons play a critical role in the transmission of ectoparasites due to the influence of environmental conditions on the survival of these ectoparasites. Sanjay et al. (2007), reported the seasonal prevalence of ectoparasites were significantly higher in winter, which is in accordance with our study. This behavior in the summer could be caused by the late-winter temperature increase, which would gradually raise the burden and the percentage of infestation in May and June (Roy et al., 2001). This discrepancy between the current and previous results can be explained by the differences in topography, soil type, humidity, and above all the world's alarming climate (Rony et al., 2010). Free-range buffalo are exposed to a variety of environments such as, open pasture land, and water bodies where ectoparasites like ticks, lice, and flies thrive. These areas often harbor higher densities of ectoparasites, increasing the likelihood of infestations (Rabbi et al., 2006 Rony et al., 2010). Because of an evolutionary strategy to lessen competition with other species and diminish host defensive systems, the ectoparasites have different attachment sites on the buffalo's body (De Castro J, 1997; Abd El-Aleem et al., 2014). Several variables, including the length of mouthpart, and morphology, affect the preferred attachment sites. Additionally, ectoparasites choose a location with a plentiful blood supply and little host resistance, allowing them to pierce the skin readily (Dantas-Torres F, 2010; Patel et al., 2013).

The epidemiological study of ectoparasites in buffaloes from selected parts of Bangladesh shows relatively low prevalence (17.41%). However, the presence of important ectoparasite species including *H. bispinosa*, *R. microplus*, and *L. vituli* is alarming due to their capability to transmit various diseases. Moreover, higher infestation in winter indicates the need for season-specific ectoparasite control in the study population. The findings also highlight the urgent need for routine surveillance and the implementation of effective ectoparasite control strategies to minimize their impact on animal productivity and reduce associated zoonotic risks.

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Ethical Declaration

The Department of Livestock Services (DLS) verbally granted authorization for sampling, and all necessary processes were completed under the Animal Welfare Act of 2019.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: S.A., Design: S.A., M.K., Data Collection or Processing: M.H., S.U., Analysis or Interpretation: M.I., Literature Search: S.A., M.K., Writing: S.A.

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CASE REPORT

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Pathology of Concurrent Infection of Metastrongylus apri with *Trichuris suis* in a Yorkshire Pig

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ABSTRACT

A two-month-old Yorkshire pig from Undisan Village, Bangli Regency, Bali, Indonesia was found dead, with a history of coughing, decreased appetite, and bloody diarrhea. Fecal examination revealed the presence of *Metastrongylus* spp. and *Trichuris suis* eggs. Gross pathology findings included verminous pneumonia, hemorrhagic gastritis, and hemorrhagic typhlitis. Worms were observed in the lungs, as well as on the mucosal surface of the large intestine and cecum. The worms inside the lungs was identified as *M. apri*, while those in the colon and cecum were *T. suis*. Histopathological examination revealed mild tracheitis with epithelial deciliation and goblet cell proliferation, emphysematous bronchiolitis, hemorrhagic gastritis, and hemorrhagic typhlitis. Based on these findings, it can be concluded that the pig was diagnosed with Metastrongylosis and Trichuriosis.

INTRODUCTION

The pigs are susceptible to infections by various parasites, including helminths. Parasitic infections such as helminthiasis can significantly impact the health and productivity of pigs, leading to economic losses in the pig farming industry (Panayatova-Pencheva et al., 2019; Kouam and Ngueguim, 2022). These effects include reduced growth rates, weight loss, and increased mortality. Additionally, the cost of anthelmintic treatments for worm control is considered high by many farmers (Nwafor et al., 2019).

Metastrongylosis is a lungworm infection caused by nematodes of the genus *Metastrongylus*, which affect both wild boars and domestic pigs through an intermediate host, earthworms. Pigs become infected when ingesting earthworms containing third-stage larvae of *Metastrongylus* spp. (Wallgren and Pettersson, 2022). Several species of *Metastrongylus* are present (Gassó et al., 2014). Among these species, *M. apri* is the most common infecting pigs (Helke et al., 2015). The lung worms can cause growth disorders in infected pigs. Clinically, affected pigs exhibit coughing due to bronchitis (Frontera et al., 2005; López and Martinson, 2017). Moreover, the significant importance of this lungworm is its zoonotic potential (Calvopina et al., 2016; Ghahvei et al., 2024). Human infections have been reported in Europe and America, with clinical signs of coughing with bloody sputum and progressive dyspnea (Calvopina et al., 2016).

A study by Oliveira et al. (2023), reported a 17.2% (10/58) prevalence of *M. apri* in necropsied wild boars in Brazil. In Uganda, *Metastrongylus* spp. were detected in 7.6% (95% confidence interval [CI]) of 932 pigs based on fecal sample analysis (Roesel et al., 2017). Similarly, a prevalence of 10.4% (95% CI: 8.1-13.2) was reported in Cameroon from a total sample of 597 pigs (Kouam and Ngueguim, 2022). The last epidemiological report on Metastrongylosis in Bali, Indonesia, was published by Dharma et al. (1988). Their study examined 104 pig lung specimens from the Denpasar Slaughterhouse and found *M. apri* infections in 21 pigs (21.19%), with larval migration lesions observed in two cases (1.92%). More recently, a study in Kupang, East Nusa Tenggara, Indonesia reported an 18% prevalence of *Metastrongylus* spp. based on coprological examination of 100 samples (Chrismanto et al., 2023).

Trichuriosis in pigs is an infection caused by the whipworm *Trichuris suis* (Bünger et al., 2022). *T. suis* is globally distributed and can infect pigs as well as several primate species, including humans. The parasite is transmitted via ingestion of soil contaminated with infective eggs. Once ingested, the larvae hatch in the ileum, cecum, and colon. High infection doses of *T. suis* can lead to clinical signs such as anorexia, anemia, and diarrhea, which contribute to weight loss. In some cases, blood or mucus may be present in the feces (Pittman et al., 2010).

Several studies have reported the prevalence of *T. suis* infection in pigs. In Chongqing, China, the prevalence was 10.13% among 2971 pigs (Lai et al., 2011). A similar rate of 20% was reported in Myanmar among 500 pigs (Bawm et al., 2024). In Bali, Indonesia, the prevalence was notably higher, reaching 71.7% among 117 pigs (Pinatih et al., 2024). This case report describes the pathological features of a pig co-infected with Metastrongylosis and Trichuriosis.

MATERIALS AND METHODS

Case history

A two-month-old Yorkshire pig from Undisan Village, Bangli Regency, Bali, Indonesia (8°26'20.3"S 115°23'43.4"E) experienced decreased appetite, lethargy, coughing, and bloody diarrhea starting on January 1, 2022, according information from the owner. The pig was housed in cages with cement floors. Previously, there was a history of pig deaths with similar symptoms. The pig died on January 8, 2022, due to lack of treatment. The carcass was subsequently necropsied at the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University, Indonesia.

Pathology examination

Prior to necropsy, a fully comprehensive external examination of the carcass was conducted. The necropsy procedure was then performed, with pathological evaluation of the organs conducted both *in situ* and *ex situ*. Gross pathological changes were documented, and affected organs were sampled by excising tissue sections measuring 1×1×1 cm. The preparation of histopathology slides followed the standard protocol of the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University. After sectioning, tissue samples were immediately fixed in 10% neutral buffered formalin for 24 hours. Following fixation, the samples were rinsed under running water and placed into labeled tissue cassettes. The cassettes were then loaded into an automated tissue processor for dehydration (using graded alcohol), clearing (with toluene), and paraffin infiltration. After processing, the tissues were embedded in paraffin to form blocks and then sectioned at a thickness of 5 µm. Then the tissues were processed and stained using Harris Hematoxylin and Eosin (H&E) staining, following the protocol described by Sewoyo et al., (2022). The histological slide was then examined under a microscope at 100× and 400× magnification.

Parasitology examination

Worm samples in the lungs were collected by incising the part that had undergone gross pathological changes, while worm samples in the cecum were collected directly from the mucosa. Worms were identified morphologically directly under a microscope at 40× magnification without preservation or staining. The species of worms *Metastrongylus* spp. were classified based on the morphological identification key of Gassó et al., (2014). *T. suis* samples were identified morphologically based on Otranto and Wall (2024). Fecal samples were also collected for the purpose of examining the morphology of worm eggs using native, sedimentation, and floating methods. Floatation examination uses a saturated salt floatation agent (Zajac et al., 2021).

RESULTS

The identification of worms was based on the morphological characteristics of adult worms, their eggs, and their predilection sites (Figure 1). *Metastrongylus* spp. were retrieved from the caudal lobes of both the left and right lungs, while *T. suis* was found on the mucosal surface of the cecum.

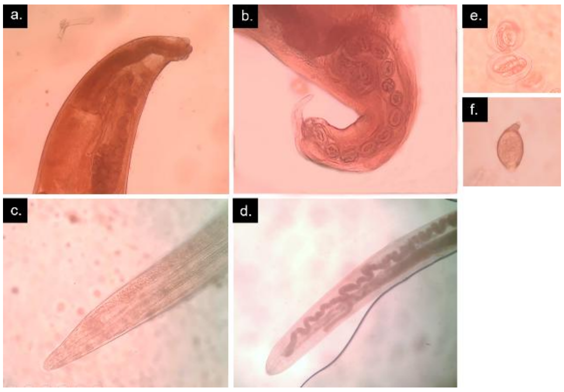

Figure 1. Morphological characteristics of *Metastrongylus apri* (a, b, e) and *Trichuris suis* (c, d, f). (a) Anterior end of a female *M. apri*. (b) Posterior end of a female *M, apri*. (c) Posterior end of a female *T. suis*. (d) Posterior end of a female *T. suis*. (e) *M. apri* eggs identified using the sedimentation method. (f) *T. suis* eggs detected using the flotation method with a saturated salt solution. (100× and 400× magnification)

The *Metastrongylus* spp. identified as *M. apri* based on morphological examination. The *M. apri* specimens collected were adult female worms (Figure 1a,b). Morphologically, the posterior end (Figure 1b) lacked prevulvar cuticular dilatation, with the vulva positioned slightly anterior to the anus and posterodorsal to the prevulvar swelling. The anterior end displayed characteristic trilobed labia (Figure 1a). *M. apri* eggs, identified through sedimentation examination, were ellipsoid in shape, with a thick, rough shell and were larvated upon excretion.

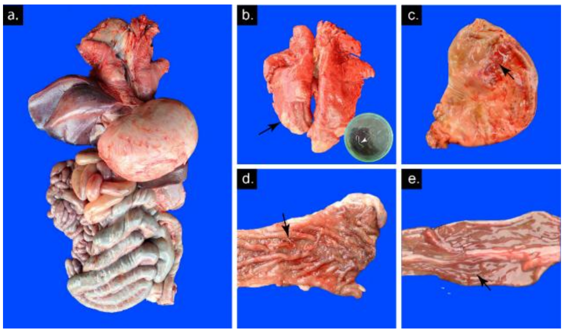

Figure 2. Gross pathology of pig internal organs. (a) Gross pathology of organs *ex situ*: The stomach and intestines appear distended, with hyperemia on the serosal surface of the small intestine and colon. (b) Lungs: The lungs were hyperemic with multifocal areas of inflammation (arrows), and worms are present (arrowheads). (c) Stomach: Hemorrhage in the gastric mucosa. (d) Cecum: Thickening of the mucosa, with hemorrhage and the presence of worms (arrows). (e) Small intestine: Hyperemic small intestinal mucosa

T. suis worms appeared as white, whip-like structures adhered to the cecal mucosa during gross examination (Figure 2d). The female worms exhibited a distinct morphology, with a slender anterior end and a thick, curved posterior end. *T. suis* eggs, detected using the flotation method, were lemon-shaped, with a thick, smooth shell and prominent transparent polar plugs at both ends. The egg contents were granular, unsegmented, and brownish in color.

Examination of the external body revealed no pathological changes. However, *ex situ* organ observation revealed abnormalities in several organs (Figure 2a). The stomach appeared distended, with the serosal surface of the small intestine and colon appearing reddish and showing prominent capillary blood vessels. The lungs were generally reddish, with a grayish lesion slightly raised at the distal ends of both lung lobes (Figure 2b). The gray lesion was suspected to be an area of inflammation. Upon incision, several worms were found in the lungs. Hemorrhage was observed on the surface of the gastric mucosa (Figure 2c). Upon opening the intestinal tract, small, hair-like worms were found in the colon and cecum (Figure 2d). The surface of the colon appeared thickened and reddish, with accompanying hemorrhage. No worms were found in the small intestine, but hyperemia was present (Figure 2e). The other organs, such as brain, esophagus, heart, pancreas, spleen, kidney, liver and bladder appeared unremarkable.

Histopathological examination of the organs revealed changes in the trachea, lungs, stomach, small and large intestine. In the upper respiratory tract, the trachea exhibited pseudostratified epithelial deciliation, goblet cell proliferation, and mild mononuclear inflammatory cell infiltration in the submucosa (Figure 3a). In the lungs, inflammatory cell infiltration was observed in the peribronchial region, accompanied by exudate in the bronchiolar lumen. Emphysema was also present (Figure 3b).

In the gastric mucosa, exudate and desquamation were noted in the columnar epithelium, while the lamina propria was infiltrated by mononuclear inflammatory cells and showed hemorrhage (Figure 3c). The small intestine appeared congested, with several blood vessels in the mucosa and submucosa were engorged with red blood cells. The lamina propria were infiltrated with mononuclear cells. The large intestine displayed mucosal erosion, along with eosinophilic and mononuclear inflammatory cell infiltration in the lamina propria. Cross-section of helminths were also present in the mucosa (Figure 3d).

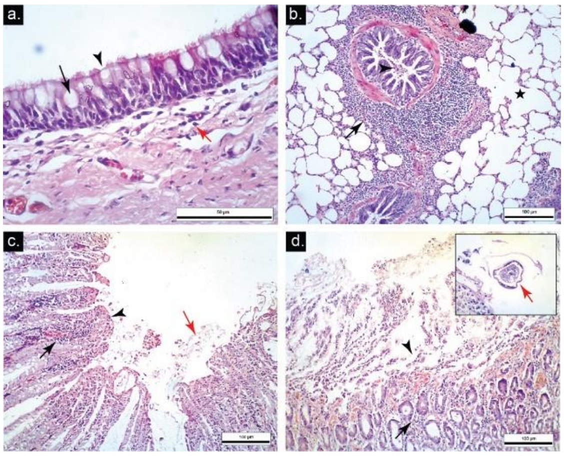

Figure 3. Histopathology of pig internal organs with Metastrongylosis and Trichuriosis. (a) Tracheitis: Deciliation (arrowhead), goblet cell proliferation (black arrow), and mild inflammatory cell infiltration in the submucosa (red arrow). (b) Emphysematous bronchiolitis: Exudate in the bronchiolar lumen (arrowhead), peribronchiolitis (black arrow), and emphysema (star). (c) Hemorrhagic gastritis: Desquamation of the mucosal epithelium (arrowhead), exudate in the lumen (red arrow), and mononuclear inflammatory cell infiltration with hemorrhage in the lamina propria (black arrow). (d) Hemorrhagic typhlitis: Erosion of the intestinal mucosa (arrowhead), eosinophilic and mononuclear inflammatory cell infiltration with hemorrhage in the lamina propria (black arrow). Cross-section of *T. suis* were observed in the mucosa (red arrow). (H&E staining, 100× and 400× magnification)

DISCUSSION AND CONCLUSION

Metastrongylus spp. is a nematode worm from the family Metastrongylidae that has a slender body shape of up to 6 cm, two lateral trilobed lips, and small dorsal bursa ray. The species are distinguished by the size and shape of the male spicules (Otranto and Wall, 2024). In addition to male morphology, worm species can be distinguished based on female morphology. Six species of *Metastrongylus* have been described, i.e., *M. apri* (also called *M. elongatus*), *M. salmi, M. pudendotectus, M. confusus, M. asymetricus*, and *M. madagascariensis* (Helke et al., 2015). The first three species are the most commonly reported worldwide and are usually found in mixed infections. Female *M. pudentodectus* and *M. asymetricus* have prevulvar cuticular dilatation, whereas *M. apri, M. confusus* and *M. salmi* do not. In distinguishing between the species *M. apri, M. confusus, and M. salmi,* the location of the vulva can be observed. In *M. apri*, the vulva is located slightly anterior to the anus and posterodorsal to the prevulvar swelling. In *M. confusus*, it is also slightly anterior to the anus but situated within the prevulvar swelling. In *M. salmi*, the vulva is positioned at the ventral base of the prevulvar swelling (Gassó et al., 2014). Based on this identification key, the female *M. apri* worm was morphologically identified in the present case.

M. apri is a nematode parasite that infects the respiratory tract of both wild boars and domestic pigs. Epidemiological studies indicate that *M. apri* infections are more prevalent in wild boars than domestic pigs. Poglayen et al. (2016), reported that the prevalence of *M. apri* infection in wild boars reached 80.7% (46/57). Young pigs are more susceptible to infection and harbor higher parasite burdens (García-González et al., 2013; Poglayen et al., 2016). The prevalence and intensity of *M. apri* infection are closely associated with highland regions and areas with high rainfall, as these environments favor the presence of earthworms, the intermediate host of the parasite (Gassó et al., 2014).

Macroscopically, pigs infected with *M. apri* exhibit lesions predominantly in the apical lobes of the lungs, characterized by irregularly shaped grayish areas. Histopathological examination revealed lymphocytes and neutrophils infiltration within the lumina of the bronchi and bronchioles, desquamation of the bronchial and bronchiolar epithelium, emphysema, thickening of the alveolar septa, and peribronchial lymphoid hyperplasia (Panayotova-Pencheva et al., 2019). Clinically, *M. apri* infection manifests respiratory symptoms such as coughing and dyspnea, which may be exacerbated by co-infection with other parasites, such as *Ascaris suum* (Frontera et al., 2005; 2007). Coinfection has also been reported to intensify the immune response (Frontera et al., 2007). Effective control of *Metastrongylus* infection can be achieved through anthelmintic administration, particularly ivermectin and abamectin, both of which demonstrate high efficacy in reducing parasite burdens (Lopes et al., 2014).

Trichuris worms have whip-like characteristics and are also called whipworms because their thick rear end narrows into a long, threadlike front end (Bünger et al., 2022). The front end of the worm is pointed and embedded in the mucosa. The male worm has a tightly coiled tail and a single spicule in a sheath that can protrude, whereas the female is curved. *T. suis* eggs have a characteristic lemon-like shape, measuring 50-68 × 21-31 µm, with a thick, smooth shell and prominent transparent polar plugs at both ends. The species that infects the large intestines (cecum and colon) of pigs is *T. suis* (Otranto and Wall, 2024). *T. suis* is a globally distributed parasite of pigs, with prevalence rates varying by geographic location, season, and farm management practices. *T. suis* infection is of significant concern in pig farming due to its impact on growth performance and economic losses (Pittman et al., 2010; Bünger et al., 2022). Clinically, *T. suis* infection causes diarrhea, anorexia, anemia, growth retardation, dehydration, and emaciation. Brewer et al. (2019) reported that *T. suis* infection leads to typhlitis and colitis, characterized by ulceration, edema, hemorrhage, and the presence of mucoid exudate on the mucosal surface, often accompanied by adult worms. John and Saikumar (2017) described gross pathological changes in infected pigs, including cecal thickening and catarrhal inflammation. In this case, cattarhal inflammation in the cecum was not found.

Histopathological findings by John and Saikumar (2017) include multifocal necrosis of the colonic epithelium and infiltration of eosinophils and mononuclear inflammatory cells, which is in line with this study. Another study by Limarta et al. (2024) reported that severe *Trichuris suis* infection (2329 worms), accompanied by *Eimeria* sp. infection, resulted in gross pathological changes, including mucosal openings and hemorrhages in the cecum and colon, hemorrhages in the stomach and small intestine, and an uneven liver coloration with white spots. Histopathological examination further revealed necrotizing hemorrhagic enteritis, necrotizing verminous colitis, necrotizing gastritis, and necrotizing hepatitis. The gross and histopathological changes observed in that study were similar to those in our case, although to a lesser extent. In conclusion, we observed pathological changes in the trachea, lungs, and gastrointestinal tract of Yorkshire pig resulting from infection with *M. apri* and *T. suis*, confirmed by parasitological examination of the parasite. These findings confirm that the infections of both parasites still occur in Bali, Indonesia. Due to the confirmed presence of the parasite in this region and its importance regarding public health, conducting epidemiological studies is recommended to determine the current prevalence, particularly *Metastrongylus* spp.

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Ethical Declaration

-

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: P.S., Design: P.S., R.P., Data Collection or Processing: R.P., Analysis or Interpretation: P.S., I.C., Literature Search: P.S., I.C., Writing: P.S., I.C.

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REVIEW ARTICLE

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Regional Anaesthesia Techniques for Feline Tooth Extractions

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ABSTRACT

Dental diseases are one of the most common diseases in pets, occurring in 80% of old and young cats. Viral diseases, plaque formation, periodontal diseases, tooth and gum problems caused by the disorder of the jaw structure, which are common in cats, make tooth extraction operations compulsory. Regional nerve blocks are used to reduce postoperative pain sensation in the tooth extraction area and to reduce the amount of general anesthetic concentrations used. In this review, regional nerve block techniques in cats will be discussed.

INTRODUCTION

The pain receptors in the dental hard and soft tissues are free nerve endings. A-δ fibers transmit sharp localized pain; A-β fibers conduct touch and pressure; and C fibers provide the sensations of burning, aching, and throbbing. These fibers are stimulated by the *Nervus* (*N.*) *maxillaris* and *Nervus mandibularis*, which form the sensory branches of the trigeminal nerves. Therefore, the anatomy of the area where regional anesthesia will be applied should be well known (Rochette, 2005).

Benefits of regional anesthesia methods: reduction in the concentration of inhaled anesthesia, which minimizing complications such as hyperventilation, bradycardia and hypotension. At the same time, preoperative nerve blockages provide analgesia and increase patient comfort and reduce pain sensation, eliminating the need for systemic analgesia (Beckman and Legendre, 2002). There are four nerve blocks that can be applied to animals undergoing tooth extraction.

Infraorbital nerve block

In the maxilla, the nervus maxillaris divides into two nerve branches which are *N. pterygopalatinus* and *N. infraorbitalis* when it reaches the *Fossa pterygopalatine*: *N. pterygopalatinus* innervate the nasal cavity, soft and hard palate (Rochette, 2005). *N. infraorbitalis* divides into the caudal maxillary alveolar branch before entering the infraorbital canal to innervate the teeth. The caudal maxillary alveolar branch innervates the maxillary first molars, gingiva and oral mucosa. After entering the canal, *N. infraorbitalis* is called the middle maxillary alveolar nerve and innervates the premolar teeth and the associated gingiva. The rostral superior alveolar nerve branches just before exiting the infraorbital canal, innervating the canine, inciciv tooth and associated gingiva. *N. infraorbitalis* nerve fibers at the cranial end innervate the upper lip and cutaneous structures (Woodward, 2008; Calvet et al., 2024).

Desensitised area width depends on how far the local anesthetic is injected in the infraorbital canal. A superficial blockade (if left only at the entrance of the infraorbital canal) desensitizes the rostral maxillary teeth, the foramen and surrounding soft tissues, the gingiva, the upper lip, the tip of the nose and the rostral aspect of the tongue. If the anesthetic substance is injected further into the caudal canal, it is sufficient to desensitize the entire maxillary teeth and surrounding soft tissue (Keating, 2016).

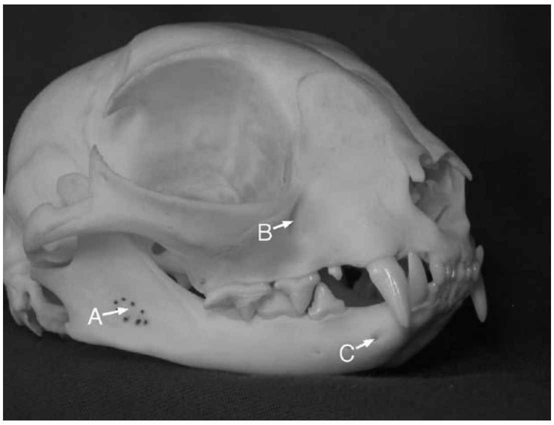

Figure 1. In the cat skull, the arrow next to A indicates the location of the foramen mandibulare. The arrow next to B indicates the location of the foramen infraorbitale. The arrow next to C indicates the location of the foramen mentale (Rochette, 2005)

To locate the foramen infraorbitale, the upper lip is lifted upward and an imaginary line is drawn downward from the medial canthus of the eye. It is located about 1 cm above the 3’rd premolar tooth in dogs and about half cm above the 2’nd premolar tooth in cats (Figure 1), (Keating, 2016.). In dogs, the location of the foramen can be felt by palpation, however in cats palpation of the foramen is quite difficult. Therefore, it is more likely to inject the local anesthetic into the skin or upper lip instead of the infraorbital canal. After locating the foramen, the needle to be used is advanced into the infraorbital canal at a 45 degree angle, (Figure 2). When administering local anesthetics, the entire needle is not inserted into the canal, because the orbit and infraorbital canal are in participation. Especially in brachiocephalic breeds, the canal is short and can cause neurological damage and bleeding (De Vries and Putter, 2015).

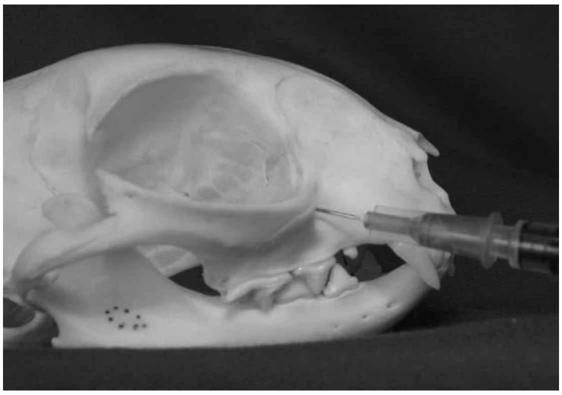

Figure 2. Positioning of the injector in the infraorbital canal (Rochette, 2005)

The needle is inserted into the infraorbital canal, aspiration is performed, it is checked whether it is in the vein. If not in the vein, the drug is injected slowly and steadily (Beckman, 2013). In cats, the infraorbital canal is very short (3-4 mm) and excessive needle advancement may cause ocular damage. This method can be used for canine incisors and premolars, but if we want to desensitise molars, it is more advantageous to use the maxillary nerve block technique. Excessive mouth opening should also be avoided in cats as it can compromise blood flow through the maxillary artery, in which there is no collateral circulation to the cerebrum and retina and can may lead to irreversable damage. Mouth gags with a maximum length of 20-30 mm should be used for cats (Grubb and Lobprise, 2020).

Maxillar nerve block

In the intervention applied to the maxillary nerve block, all premolar and molar teeth, hard and soft palates, tongue, tip of the nose, upper lip and bone and other soft tissues of the maxilla are affected. There are two types of approaches to access the maxillary nerve block and this varies depending on the animal species and size: percutaneous and intraoral approaches (Beckman, 2013).

Extraoral approach

With the patient's mouth open, an imaginary perpendicular line is drawn from the medial canthus of the eye to the arc. zygomaticus. The needle is advanced in the pterygopalatine fossa until it hits the bone tissue and the foramen maxilla is reached (Dugdale, 2010). After checking whether the tip of the injector is in the vein, the drug is injected (Figure 3), (Grubb and Lobprise, 2020). This method has been used successfully in large breed and medium-sized dogs, but in cats it would be more appropriate to use the oral approach because the foramen maxilla and bulbus oculi are narrow and distance between them is close (Keating, 2016).

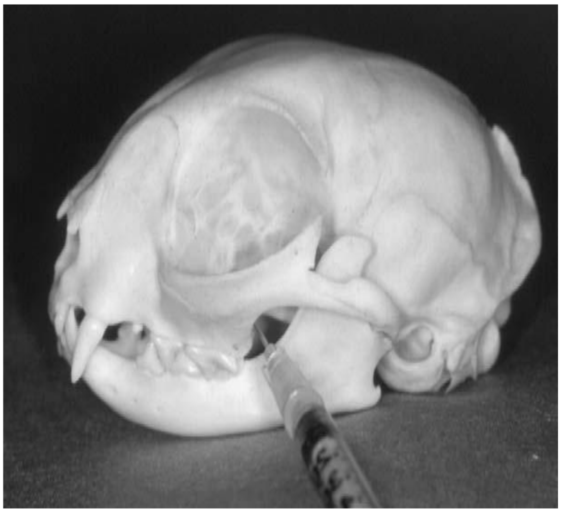

Figure 3. Extraoral approach technique to the foramen maxilla in the cat (Rochette, 2005)

Intraoral approach

The patient's mouth is opened and the lips are pulled back caudally. The needle is positioned perpendicular to the end of the maxillary 2’nd molar root and It is slowly advanced 2-4 mm. After checking whether the tip of the injector is in the vein, the drug is injected (Perry et al., 2015).

Mental nerve block

The nervus mandibularis enters the mandible through the foramen mandibulare and travels in the canalis mandibulare (Rochette, 2005). It is called *N. inferior alveolaris* to innervate premolar and molar teeth in the mandibular canal. *N. inferior alveolaris* takes the name of n. mentale after leaving the foramen mentale and affects the canine and incisive teeth, gingiva and lower lip (Dursun, 2008).

The area of local anaesthesia is related to how far the drug is given caudally in the foramen mentale. A superficial blockage affects only the soft tissue in the canine rostral midline, while a deep blockage affects the canine, incisor and soft tissue (Keating, 2016). But in the cats, it affects canine, incisive, premolars and sometimes molars along a with soft tissue. However, correct blocking in cats is not as easy as in dogs. Both the location of the foramen and the width of the foramen may prevent the correct spread of anesthesia (Beckman, 2013). The foramen mentale can be palpated ventrally at the level of the 2’nd premolar in the dog. Since the cat does not have a 2’nd premolar, it is located exactly in the middle, equidistant from the canine teeth and 3’nd premolars (Figure 1). During blockade, the lower lip is pulled downwards, the needle is positioned slightly outwardly at the designated point and gently advanced 1-3 mm (Rochette, 2005).

If the needle hits a wall to the right and left, but moves smoothly along the caudal pathway, the foramen has been reached. Aspiration is performed, the drug is injected slowly after checking whether it is in the vein. If there is no swelling of the lip or gingiva due to leakage, we have a second confirmation that we are in the foramen (Beckman, 2013).

Inferior alveolar nerve block

Inferior alveolar nerve blockage affects all canine, incisive, premolar and molar teeth, tongue, lower lip, bone tissue and soft tissues, in short, the entire mandible (Beckman, 2013). The foramen mandibulare is between the 3’rd molar tooth medial to the ramus mandibulare in the dog and the angular fold of the mandible. In the cat, it is between the 1st molar tooth and the angular fold of the mandible (Figure 1), (Snyder L et al., 2019). The foramen mandibulare can be difficult to palpate in medium breed dogs and cats. However, palpation of the foramen is not necessary and the approximate site for injection can be determined using known locations (Goudie-DeAngelis et al., 2016). There are two types of approach to the foramen mandibulare: extraoral approach and intraoral approach.

Extraoral approach

An imaginary line is drawn from the ventral border of the ramus mandibularis and the lateral canthus of the eye. The finger inserted into the mouth is placed at the base of the molar root. The point where the needle will enter is limited. The needle is placed perpendicular to the ramus mandibulare and the drug is injected through the skin under the guidance of the finger (Grubb and Lobprise, 2020).

Intraoral approach

The patient's mouth is opened, the needle is placed between the distal face of the molar and the mandibular salivary gland. It is advanced 2-3 mm at an angle of 45° towards the angular direction of the ramus mandibulare. When the bone tissue of the ramus mandibulare is felt, the plunger of the syringe is controlled and the drug is injected (De Vries and Putter, 2015).

Both methods have complications that can occur as a result of the procedure. Incorrect positioning of the syringe may cause trauma to the tongue, excessive drug volume may affect the n. lingualis (Grubb and Lobprise, 2020).

As a result, regional anesthesia can provide a more comfortable procedure for our patients and a more satisfying experience for our clients. Nerve blocks stop the pain stimulus before it occurs. The most commonly used blocks for oral and dental surgery are infraorbital, maxillary, mental and mandibular blocks. Regional anesthesia reduces the need for general anesthesia, provides relief of intraoperative and postoperative pain, and contributes to a smoother postoperative recovery. These benefits increase customer satisfaction by making the patient's postoperative recovery more comfortable and ensuring that the patient is discharged with less drug residue.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: B.K., Design: B.K., Data Collection or Processing: B.K., Z.D.Ü., Literature Search: B.K., Z.D.Ü., Writing: B.K., Z.D.Ü.

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REVIEW ARTICLE

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Heredity on the Cardiovascular System in Dogs: Mitral Valve Insufficiency and the King Charles Dog

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ABSTRACT

This review addresses the genetic basis of cardiovascular disease in dogs, with a particular focus on mitral valve insufficiency (MVI), which is common in the Cavalier King Charles Spaniel breed. MVI is a common degenerative heart disease, especially in small breed dogs, which progresses with age. Genetic predisposition plays a critical role in the pathogenesis of heart disease, and is also important for both early diagnosis and long-term treatment strategies. In the Cavalier King Charles Spaniel breed, polygenic inheritance and loci identification have been reported to be associated with early onset of the disease. In this study, the stages and clinical, echocardiographic and radiologic findings of MVI were detailed in line with the classification systems proposed by ISACH and ACVIM, and also modern diagnostic methods and clinical use of biomarkers were discussed. In addition, the contribution of proteomic research to the understanding of cardiac pathophysiology was emphasized and its relationship with platelet function and coagulation processes in heart failure was evaluated. In conclusion, it is suggested that further studies at the genetic and molecular level will provide innovative approaches in the fields of diagnosis, treatment and preventive medicine in veterinary cardiology practice.

INTRODUCTION

Cardiovascular diseases (CVDs) are serious health problems that affect animal welfare and their lifespan negatively. Understanding the genetic background of these diseases is essential for an effective treatment process. Recent studies have reported that genetic factors are the important risks for developing of heart diseases. This should be considered in the context of the One Health concept both for humans and animals.

Veterinary cardiologists have been studying genetic predisposition in CVDs since the 70s. The genetic prevalence of heart diseases is high in some specific dog species such as King Charles Spaniel, Dachshund, Cavalier. It has been known that understanding the genetic basis of CVDs can help in the treatment process. Particularly atrial fibrillation, mitral valve insufficiency (or mitral regurgitation, MVI) and subvalvular aortic stenosis have been interested more by researchers (Ontiveros and Stern, 2021; O'Brien et al., 2021; Arcuri et al., 2024).

Mitral valve insufficiency is accepted the major heart disease in dogs with the age that encountered in almost all breeds. It has been found more commonly in some breeds especially the Cavalier King Charles Spaniel and the Dachshund (O'Brien et al., 2021). Although some dogs have a rapid and early onset of the disease, many others live long periods without any clinical signs (Atkins et al., 2009; Meurs et al., 2018). In this review, in addition to a general overview of CVDs in dogs, studies on MVI and treatment which are frequently encountered especially in King Charles Spaniel dogs can be reviewed.

Genetic Etiology of CVDs in Dogs

CVDs involves a complex interplay of genetic, neurohormonal, inflammatory and biochemical changes in body. The heart valve diseases, MVI, chronic valve diseases and tricuspid valve insufficiency are the most important disease encountered in dogs. The age, sex, physiological condition and breed are important occurrences of these diseases (Ontiveros and Stern 2021; O'Brien et al., 2021; Arcuri et al., 2024). In addition, Cavalier King Charles Spaniels, Chihuahuas, Miniature Poodles, Miniature Pinchers and Whippets are considered as important dog breeds. Especially Cavalier King Charles

Spaniel dogs are more genetically predisposed to CVDs than other breeds. Importantly, MVI is one of the most crucial diseases for this breed.

Researchers have showed that polygenic inheritance is effective in the formation of MVI in Cavalier King Charles Spaniel dogs (O'Brien et al., 2021). The two locus on chromosomes 13 and 14 that are associated with the early onset of the MVI was reported. Researchers determined that especially in dogs over 8 years of age, this disease is more common (Borgarelli and Buchanan, 2012). However, in a postmortem study, it was shown that under MVI was found 35% in 5 years old of age Cavalier King Charles Spaniel dogs and 97% in dogs over 9 years of age (Whitney, 1974). Also, Beardow and Buchanan (1993), reported that mitral murmurs were determined in 9% of Cavalier King Charles Spaniel dogs under 1 year of age, 56% over 4 years of age and 100% over 10 years of age. Additionally, it was observed that MVI was higher in males than females as well as at an earlier age (Olsen et al., 2003).

Several classification systems have been categorized the severity of MVI in dogs. Earlier systems, especially the International Small Animal Cardiac Health Council (ISACH) scheme, dogs were classified into a four-class system (Classes I-IV) based on the clinical symptoms exhibited by dog. However, the American College of Veterinary Internal Medicine Cardiology Specialty consensus panel (ACVIM) has developed a more rigid system for some CVDs (Atkins et al., 2009; Keene et al., 2019; O'Brien et al., 2021).

Classification of CVDs and The Symptoms

ISACH has classified the CVDs in animals according to clinical, auscultation, radiologic and echocardiographic findings. The evaluations and modifications have been still made based on the same classification by the committee from teh date 1994 (ISACH 1994; Table 1).

Table 1. Classification of radiologic and echocardiographic findings of heart failure (modified from ISACH, 1994)

Class	Clinical Findings	Echocardiographic Findings	Radiological Findings
1. Asymptomatic heart failure	- No clinical symptoms. - Systolic murmur and arrhythmia - Weakening of systolic heart sounds	- No dilation in the left atrium and left ventricle.	- No pulmonary vein engorgement.
2. Moderate heart failure (asymptomatic)	- Exercise intolerance - Increased respiratory rate - Dyspnea - Cough	-Mild/moderate increase in internal diameters of the left atrium and left ventricle.	-Mild/moderate enlargement of the left atrium and left ventricle. - Thickening of the pulmonary veins is observed.
3. Severe heart failure	- Marked dyspnea - Marked ascites - Exercise intolerance - Hypoperfusion - Signs of cardiogenic shock	- Prominent left atrial dilation - Increased left ventricular volume - Eccentric hypertrophy observed - Hyperdynamic left ventricle detected - Increased end-systolic diameter	- Marked cardiomegaly - Dorsal deviation of the trachea - Increased pressure on the left main bronchus - Thickening in perihilar and caudodorsal lungs - Alveolar infiltration and changes in auscultation
4. End-stage heart failure	- Severe symptoms of congestive heart failure are observed.	- Enlargement and increased internal diameter of the left atrium and left ventricle - Significant decrease in myocardial function	- Severe cardiomegaly - Marked alveolar infiltration is detected.

As in humans in dogs and cats, heart failure can lead to impaired hemostasis. Also it has been reported that thrombus formation can occur due to platelet overwork (Smith et al., 2015). Circulatory insufficiencies in such organs (lung and brain) due to thromboembolism can cause the clinical pathological findings. The findings include peracute walking difficulties or paraplegia due to arterial thromboembolism, respiratory distress due to pulmonary embolism, syncope or seizures due to thrombus in the brain vessels. Therefore, using drugs which regulate the platelet activity in CVDs is accepted for both treatment and prophylaxis in humans as well as in animals (Wendy 2011; Smith et al., 2015). However, studies reported that while thromboembolism is a risk in hypertrophic cardiomyopathies in cats, there is a lower risk in heart failure in dogs (Smith et al., 2015).

Table 2. Classification of Heart Failure Findings according to the consensus panel of the American College of Veterinary Internal Medicine Cardiology Specialty (compiled from ACVIM)

Stage	Findings
Stage A	There is no audible heart murmur, but no obvious structural abnormality is found on examination, which would put the risk of developing heart failure above average.
Stage B	Dogs in Stage B show structural abnormalities. However, clinical signs of heart failure associated with mitral valve disease have never been observed.
Stage C	Mitral valve insufficiency is severe enough to cause current or past clinical signs of heart failure. Stage C includes all dogs with mitral valve insufficiency that have experienced an episode of clinical heart failure and are not resistant to standard heart failure treatment.
Stage D	Patients exhibit clinical signs of heart failure that are resistant to standard treatment used for Stage C heart failure.

Mitral Valve Insufficiency (Mitral Regurgitation, MVI) and Findings

Guidelines for the diagnosis and treatment of mitral valve disease in dogs were reported by the American College of Veterinary Internal Medicine Cardiology Specialty consensus panel (ACVIM) in 2009 (Atkins, 2009). However, new strategies for diagnostic, medical, surgical and dietary treatment recommendations have been more detailed and updated over the years (Table 2).

Mitral regurgitation is known as a leakage of the mitral valve during blood flow from the left ventricle to the left atrium due to deterioration of its structure (De Madron 2015; Abbott 2016). As the mitral valve is distrupted, the blood from the left ventricle cannot directed to the aorta, and thereby the blood passes into the left atrium. Accordingly, left atrium adaptation cannot be achieved due to the increasing blood volume and chordae tendineae rupture, which causes mitral regurgitation. Also, pulmonary edema and signs of left-sided congestive heart failure occur (Eriksson et al., 2010). Nevertheless, some other important findings of MVI include the increased left ventricular filling pressure, pulmonary hypertension and myocardial dysfunction (Keene et al., 2019). Compensatory mechanisms such as left ventricular hypertrophy, dilatation or increased neurohormonal system activity are initially seen as effective mechanisms to maintain hemodynamic pressure against mitral regurgitation. However, with the progression of the disease, collagen fiber accumulation and myocyte damage in the heart are existed. Also, as the disease progresses even death can be observed (Vereb et al., 2023).

The basic reason of MVI in dogs has been reported as degenerative mitral valve disease which especially encounters in small breeds (Chetboul et al., 2016). Additionally, the polygenic inheritance effective in the formation of MVI in Cavalier King Charles Spaniel dogs and two locus on chromosomes 13 and 14 are associated with the early onset of the disease are noticed (Madsen et al. 2011). Borgarelli and Buchanan (2012) found that age and sex especially in dogs over 8 years of age are effective in disease. In a postmortem study, it was determined that MVI can be found in 35% of Cavalier King Charles Spaniel dogs under 5 years of age, and 97% of dogs over 9 years of age (Whitney, 1974). Furthermore, Beardow and Buchanan (1993), presented the mitral murmurs detected in 9% of Cavalier King Charles Spaniel dogs under 1 year of age, 56% over 4 years of age, and 100% over 10 years of age. Also, some researchers showed the high incidence of MVI in males at an earlier age than females (Olsen et al., 2003). According to the ACVIM, the therapeutic approach for this disease is based on the latest consensus guidelines.

Diagnosis of Cardiovascular Diseases and Technologies Routine Analyses

Despite the new generation technologies, anamnesis and physical examination methods are the first important step for the diagnosis of a disease. The anamnesis should be evaluated together with the physical examination. The first steps towards diagnosis are checking the external appearance, auscultation, pulse measurement, respiratory examination, and listening to the heart. The most clinical findings in cardiovascular diseases are respiratory problems such as cough, difficulty breathing, tachycardia, weak pulse, weight loss and abdominal distension. Particularly in MVI, tachycardia, a systolic sound shorter than diastole, and the qualities of first and second sound are accepted other important auscultation findings.

Table 3. Echocardiography techniques (compiled from Fries et al., 2019; Tidholm et al., 2024)

Type of Echocardiography	Ability to Show Anatomical Structures	Sensitivity to Abnormal Blood Flow	Examination of Blood Flow	Measurement of Ventricular Functions
M-Mode Echocardiography	Limited	Not Sensitive	Abnormal valve motion Ventricular septal hypertrophy Dilation	Left ventricular function measurement
2-D Echocardiography	Very Good	Not Sensitive	Dilatation of great vessels	Ventricular ejection fraction
PW (Pulsed Wave) Doppler	None	Sensitive	Indirect	Blood flow velocity (aorta and valves)
Color Doppler	None	Sensitive	Direct	None
CW (Continuous Wave) Doppler	None	Sensitive	Blood flow velocity and pressure gradients	Blood flow velocity (aorta and valves)

Although the diagnosis of heart diseases cannot be diagnosed directly, the general condition of the heart, rhythm and cardiovascular disorders can be revealed by electrocardiography. Electrocardiography has been successful in the diagnosis of cardiac arrhythmias that has been used in animal and human health for many years (Table 3). However, along with electrocardiography, echocardiography is one of the best imaging methods in the diagnosis of heart diseases today. It has also been reported that important information about the contractile strength of the heart and diastolic heart failure can be provided by these methods (Fries et al., 2019; Klein et al., 2022; Tidholm et al., 2024). Echocardiography imaging formats are obtained in 2-D (two-dimensional) or M-Mode (Mobilization mode). Especially in in 2-D echocardiography, cardiac structures can be seen in the right and left parasternal long axis, short axis and apical acoustic windows (Figure 1, 2). Some researchers have reported that echocardiography is far from being a screening test because it requires expertise, and also is expensive (Fries et al., 2019; Tidholm et al., 2024).

Routine analyses of heart failure include complete blood count (erythrocyte, hemoglobin, hematocrit, leukocyte, platelet), serum-electrolytes (Na, K, Cl), creatinine, urea, aspartate aminotransferase (AST), CK-MB (Creatine Kinase-MB isoenzyme), lactate dehydrogenase (LDH) and alpha hydroxybutyrate-dehydrogenase (α-HBDH), which are isoenzymes of creatine phosphokinase (CPK), and LDH/α-HBDH ratio. Although there is no blood test to diagnose heart failure definitively, there is strong evidence that circulating hormones and enzymes called as biomarkers, can detect heart failure. Initially, it was determined that while cardiac output, glomerular filtration ratio, urine output, venous oxygen pressure and serum sodium levels decreases, urea/creatine ratio and blood lactate levels increase (Baisan et al., 2016). It was reported that plasma norepinephrine levels also increase in all conditions that cause heart failure. Additionally, plasma levels of aldosterone and angiotensin II are increased besides the acid-base imbalance (do Carmo et al., 2024).

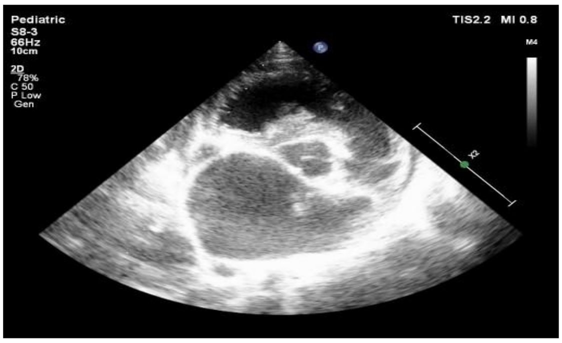

Figure 1. Echocardiographic mitral regurgitation in a Cavalier King Charles Spaniel dog with mitral valve insufficiency (right ventricle; Permissions of figures have been obtained from Bilgiçer and his veterinarians)

Figure 2. Echocardiographic mitral regurgitation in a Cavalier King Charles Spaniel dog with mitral valve insufficiency (mosaic image of the region of aortic narrowing on colour Doppler; Permissions of figures have been obtained from Bilgiçer and his veterinarians)

In recent years, neurohormonal biomarkers especially N-terminal-prohormone B-type natriuretic peptide (NT-proBNP) and cardiac Troponin I (cTnI) have gained an important role in small animal cardiology (Ljungvall et al., 2010; Oyama 2013). Brain Natriuretic Peptide (BNP) is secreted in the cavities and its production increases due to increasing pressure in heart failure. ProBNP is also a marker which reliefs in early diagnosis before symptoms appear in asymptomatic heart diseases. It is also important for the classification of respiratory and heart failure symptoms. It provides information about the prognosis of heart failure, and the evaluation of therapeutic responses (do Carmo et al., 2024). It has been reported that if the proBNP level is below 49 pmol/L in dogs, no heart failure is accepted with 80% accuracy (Boswood 2009; Oyama et al., 2008). According to genetic structure, it was observed that the genetic structure of NT-proBNP is different and can only be detected with special kits prepared for dogs (Oyama et al., 2008). It's also stated that the genetic structure of NT-proANP and NT-proBNP in cats is different from humans and can be detected with kits prepared for cats (Connolly et al., 2008). However, there is a room in examining biomarkers for heart diseases in dogs (Kim et al., 2018; Lee et al., 2018).

Proteomics Analysis: Protein analysis for the identification of a disease is incomparably more difficult than DNA analysis. While DNA consists of only four building blocks, natural proteins are made up of 20 different amino acids and the three-dimensional structure of the chain greatly influences the function of the protein. An organism has one genome but many proteomes. Protein is composed of the words Protein and Genome, which is the name given to the proteins expressed by the genome of an organism or tissue. Proteome analysis is an important technology that enable the determination of their structural properties and the disclosure of protein functions. Proteomics reveals the structures, localization, quantities, post-translational modifications and interactions of proteins with other molecules in a specific process. It's also defined as the technology of quantitative analysis of proteins in cells, tissues or body fluids under several conditions (Francois et al., 2025). There are five types of proteomics according to the purpose of the application; expression proteomics (which determines the proteins expressed from the cell or tissue), structural proteomics (determining the protein in three dimensions); functional proteomics (which examines the functions of proteins), chemoproteomics (which examines which small molecules interact with cells), and cell-map proteomics (determining protein-protein interaction and subcellular localization of proteins).

Platelet proteomics studies allow for the characterization and elaboration of basic biological behaviors in the organism that affect platelet hemostasis. Additionally, it authorises the determining the roles of platelets in disease and health conditions (Burkhart et al., 2014). In a study, significant changes in serum proteins were observed in dogs with Dilated Cardiomyopathy (DCM), and it was suggested that these proteins play an important role in DCM (Kocatürk et al., 2016). Regarding serum proteomics in dogs with heart failure, Locatelli et al. (2019), compared data from dogs with mitral valve disease (n=8) with a healthy control group (n=4); they found 8 significant proteins. In that study, compared to the control group, an increase in 1 protein and a decrease in 3 proteins were identified in class B2 patients; an increase in 8 proteins and a decrease in 8 proteins in class C patients; and an increase in 2 proteins and a decrease in 10 proteins in class D patients. Despite limited information on platelet proteomics studies in humans, any studies have been reported to examine heart failure and platelet proteomics together in dogs. In this context, it should be aimed to increase the ability of platelet proteomes to distinguish between healthy and diseased conditions, to address the pathophysiology of heart failure from different aspects, and to specificize the coagulation functions of platelet proteomes.

Despite the studies conducted with traditional methods in human and veterinary medicine, there is a need for molecular level studies to reveal the activation of the coagulation system in heart diseases. It is thought that the activation of platelets, which play a primary role in coagulation, shows many morphological and functional changes. These changes are not fully revealed by the evaluation methods that may be effective in the emergence of this need. According to the technological developments, protein identification on serum and cells enables the identification of disease-health problems and obtaining new details (Burkhart et al., 2014).

Treatment of Cardiovascular Diseases

Improving The Cardiac Workload: Cardiovascular diseases primarily require the use of medications that reduce cardiac workload, including diuretics such as furosamide or thiazide, angiotensin-converting enzyme inhibitors such as enalapril or benezapril, and some important mixed vasodilators such as pimobendan.

1. Diuretic therapy: Furosemide is an important pharmacologic agent commonly used to reduce cardiogenic pulmonary edema observed in congestive heart failure. It works by inhibiting the absorption of electrolytes, especially Na and Cl, in the renal excretory. According to the ACVIM, furosemide is recommended at doses of 1 - 4 mg/kg as initial applications depending on the severity of clinical findings in dogs with congestive heart failure (Atkins et al. 2009; Keene et al., 2019).

2. Positive Inotrope Therapy: Positive inotropes should be used to maintain contractility in the heart. For this purpose, it is recommended to use pimobendan (0.25-0.5 mg/kg/day, orally), which is both a vasodilator and an inotrope and therefore called inodilator. Pimobendan is an orally active drug that combines calcium sensitizing properties on myofilaments with c-AMP and phosphodiesterase III inhibition properties. Pimobendan is chemically a pyridazone-benzimidazole derivative. It has positive inotropic effect, and pre- and afterload reduction ability to improve energy utilization in the heart (Chetboul et al., 2007; Oullet et al., 2009). Pimobendan is used orally in dogs at a standard dose of 0.2-0.3 mg/kg at 12-hour intervals (Oullet et al., 2009, Atkinson et al., 2009), while Fuentes et al. (2002), recommend a dose of 0.3-0.6 mg/kg once daily.

Another drug used for positive inotropic therapy is Benazepril. In a study, treatment with Benazepril and Pimobenden on congestive heart diseases of dogs, it was reported that an increase in partial contraction strength and left ventricular systolic internal diameter at the end of 15days. It was also determined that no change was observed in the ratio of left atrium to aortic root. However, it was observed that the long-term treatment of Pimobendan increased the systolic function but worsened mitral valve disease and caused specific lesions in mitral valves (Chetboul et al., 2007). The recommended dose of benazepril is 0.12 to 0.25 mg per pound orally once or twice daily in dogs.

3. Angiotensin Converting Enzyme Inhibitors: Angiotensin-converting enzyme inhibitors (ACE inhibitors) are the most commonly used drugs in human and canine cardiology for left-sided congestive heart failure and MVI. There have been several studies reported the usage of ACE to improve quality of life, exercise opportunities and life expectancy in patients with heart failure (Amberger et al., 2004; Chetboul et al., 2007). Enalapril is an important ACE inhibitor approved by the Food and Drug Administration (FDA) as clinically safe and effective in dogs (The IMPROVE Study Investigators 1995; Nakayama et al., 2007). The drug acts by eliminating the enzymes that convert angiotensin I to angiotensin II. Angiotensin II has a peripheral vasoconstrictor effect which increases the thirst and aldosterone excretion. It adversely affects vital circulation by causing an increase in blood pressure and peripheral vascular resistance in acute heart failure (Bilal, 2011). In a study in Cavalier King Charles Spaniel dogs with severe mitral heart failure, it was reported that plasma aldosterone level decreased significantly after 3 weeks of ACE administration, and had improving effects on aldosterone (COVE Study Group, 1995). The recommended dose for dogs is 0.25 - 0.5 mg/kg orally at intervals of 12 hours or 24 hours (Kvart et al., 2002).

Correcting Arrhythmia (if occur): Researchers determined that in patients with supraventricular tachycardia (atrial fibrillation, etc.), digoxin (0.005-0.008 mg/kg, PO) can be used alone or in combination with a calcium channel blocker such as diltiazem (1 mg/kg, 3x1, PO) (Borgarelli and Haggstrom, 2010).

Other Important Considerations in Treatment: One of the important considerations in the treatment phase is arterial thromboembolism. It is reported that the incidence of such these disorders in dogs with the course of heart disease is not common. Winter et al. (2012) mentioned that there was no heart disease occurred in any of the 26 dogs with thromboembolism. On the contrary, Lake-Bakaar et al. (2012), determined the aortic thrombosis that related to the heart in 6 of 31 dogs. These contradictory studies reported that the period of hemostasis course during thromboembolism should be determined, and anti-platelet drugs (clopidogrel, aspirin, etc.) should be used as in humans (Smith et al., 2015). The changes in the coagulation chain in these diseases was accepted important in the development of diagnosis, treatment and prophylaxis strategies in animals (Lake-Bakaar et al., 2012; Winter et al., 2012).

DISCUSSION AND CONCLUSION

In veterinary cardiology, genetic predisposition is important for early diagnosis of cardiac diseases and treatment approaches. In this review, the effects of genetic factors on cardiovascular disorders, especially in important dog breeds such as the Cavalier King Charles Spaniel, were discussed, and the role of genetic predisposition in common diseases such as mitral valve insufficiency was emphasized.

Researchers reported that mitral valve insufficiency most common acquired in small breed dogs (Atkins et al., 2009). It has been indicated that it is particularly observed with findings of mitral valve degeneration and valve insufficiency. In additionally, it is known that polygenic inheritance is effective in the development of MVI in the Cavalier King Charles Spaniel breed. There is a relationship between the loci located on the related chromosomes (13th and 14th), and the possibility of this disease occurring at an early age. Studies have shown that mitral valve regurgitation can be seen at an earlier age, and is more severe in males than females. It also emphasizes the critical role of genetic factors of the disease, and the fact that the disease becomes more prominent with age.

Although radiographic, electrocardiographic, and echocardiographic criteria are currently available for the diagnosis and grading of MVI, they are not always practical due to limited access to techniques such as echocardiography, the cost of examination, and the need for specialist review. Therefore, researchers have begun to focus on electrocardiographic methods (Kim et al., 2020; Vezzosi et al., 2021; Seddigh Nia et al., 2022). Seddigh Nia et al. (2022), has been observed that rhythm-conduction problems and cardiac enlargements related to the mitral valve insufficiency can be diagnosed electrocardiographically quickly and easily, and thereby the disease can be followed. Vezzosi et al. (2021), used two radiographic measures for the valvular enlargement detection, and they reported that the methods can be criteria for stage of disease.

The use of advanced diagnostic methods in veterinary cardiology that supported by genetic testing and advanced imaging techniques such as echocardiography increases the possibility of early diagnosis. In addition, the dissemination of genetic analyses in veterinary practice may contribute to reducing the prevalence of the disease, and also to controlling genetic predisposition through selection programs. In conclusion, addressing the genetic aspects of cardiovascular diseases can make a big difference in diagnostic and therapeutic approaches. It is thought that future genetic and proteomic studies will expand the knowledge of veterinary medicine in this field, and contribute to the development of preventive strategies, especially for predisposed breeds.

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: E.B., N.S., Design: E.B., N.S., Data Collection or Processing: E.B., N.S., Literature Search: E.B., N.S., Writing: E.B., N.S.

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