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Research Article

Zonulin Levels in Calves with Respiratory Distress Syndrome: Is There Field Evidence of Proof for Gut-Lung Axis in Calves?

Hasan ERDOĞAN, Songül ERDOĞAN, Serdar PAŞA, Umut COŞKUN, Kerem URAL

[1]**Aydin Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine**

ORCID : 0000-0001-5141-5108
ORCID : 0000-0002-7833-5519
ORCID : 0000-0003-4957-9263
ORCID : 0000-0002-2053-9340
ORCID : 0000-0003-1867-7143

*Corresponce:
Hasan ERDOĞAN
Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine, Işıklı, Aydın/TURKİYE

E- mail : hasan.erdogan@adu.edu.tr
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Abstract

Zonulin, a highly recognized protein, influence the integrity of intercellular connections among intestinal route. Respiratory disease syndrome (rDs), has been denoted as insufficient oxygen demands along with elevated retention of carbon dioxide among neonatal calves causing respiratory acidosis. The latter syndrome has been more frequently detected among premature calves, dedicated/linked to surfactant deficiency. The present article, namely field study discussed 'gut-lung axis' in calves with rDs, along with searching for the evidence of leaky gut and intestinal permability alterations. Therefore, the objective of this present field study was to make interpretation for gut-lung axis in calves with rDs. In field conditions to those 5 different milk-fed veal facilities donated farms, 78 calves were were classified as healthy (n=19) or with rDs (n=59). Commercially available specific Bovine Zonulin ELISA test kits were used. The mean zonulin levels (ng/mL) in calves with rDs presenting score 2 and score 3 were detected as 66,71± 4,602 respectively denoting statistically significant alterations in contrast to healthy calves 21.69±4.234 ($p<0.05$). Conclusion: It should not be unwise to draw preliminary message that gut-lung axis existed in calves with rDs to those of which treatment practices should be directed to intestinal environment.

Key words: Calf, Respiratory disease syndrome, Zonulin

Introduction

Respiratory diseases are significant contributors to economic losses in livestock, particularly in calves, due to their multifactorial origins and the resulting clinical and pathological damage to the respiratory system. Aspiration pneumonia (AP) in calves frequently arises from the inhalation of foreign materials, which lead to lung tissue damage and impaired respiratory function (Hattab et al., 2022). In contrast, human neonatal respiratory distress syndrome (RDS) and adult respiratory distress syndrome (ARDS) highlight the critical role of surfactant dysfunction in respiratory health. In RDS, which commonly affects preterm infants, exogenous surfactants are administered to support lung function. Similarly, in ARDS, damage to type II alveolar epithelial cells results in compromised surfactant production, reducing lung compliance and impairing respiratory efficiency (Magni et al., 2023). While the pathophysiology may differ between humans and calves, both conditions underline the importance of maintaining pulmonary integrity to prevent severe respiratory complications.

Zonulin, also known as pre-haptoglobin (Hp-2), is a regulatory protein that plays a crucial role in modulating the permeability of intercellular tight junctions within the intestinal epithelium, particularly in the jejunum and ileum (Wang et al.,2000; Tripathi et al.,2009). By transiently disrupting tight junction integrity, zonulin facilitates paracellular transport and influences intestinal permeability, which is critical for maintaining immune tolerance and nutrient absorption (Fasano et al., 2000; Sturgeon and Fasano 2016). Elevated levels of zonulin have been linked to the pathogenesis of various chronic inflammatory conditions, including metabolic, autoimmune, and allergic diseases, as well as obesity, hyperlipidemia, and atopic dermatitis (Ohlsson et al., 2017; Sheen et al., 2017). Furthermore, its role in innate immunity is underscored by its ability to inhibit bacterial colonization in the small intestine and modulate the clearance of microorganisms (Fasano, 2012). Zonulin release is triggered by external stimuli such as intestinal bacteria and dietary gluten, leading to its secretion into the intestinal lumen, where it binds to receptors on the apical surface of epithelial cells (Drago et al., 2006). This interaction disrupts the tight junctions, allowing antigens and microbial components to pass into the submucosa, which may subsequently activate the immune system and promote inflammatory processes (Ciccia et al., 2017). Recent research has also identified a significant association between serum zonulin levels and asthma severity, particularly in individuals sensitized to house dust mites (Baioumy et al., 2021). Together, these findings highlight zonulin's pivotal role in intestinal homeostasis and its potential contribution to systemic inflammation and disease progression.

The aim of this study is to investigate the potential interplay between intestinal permeability, mediated by zonulin, and respiratory distress syndrome (RDS) in calves, with a particular focus on the "gut-lung axis." By analyzing zonulin levels and their correlation with respiratory and intestinal health in calves, this research seeks to provide insights into the underlying mechanisms of systemic inflammation and contribute to the development of targeted treatment strategies in field conditions.

Material and Method

Animal Material

This study was conducted using 78 neonatal calves, which were randomly selected from five different milk-fed veal facilities located in field conditions. The calves were classified into two groups: healthy (n = 19) and those exhibiting respiratory distress syndrome (RDS) with diarrhea (n = 59). The inclusion criteria for the RDS group were based on clinical symptoms such as respiratory effort, nasal discharge, and fecal scoring. Healthy calves exhibited no clinical signs of respiratory or intestinal disorders.

All animals were managed under standard husbandry practices, and their feeding and housing conditions were monitored to ensure consistency across all facilities. Prior to sample collection, the health status of each calf was evaluated by same researcher.

Zonulin Detection

A total of 78 calves were randomly selected for the study. Blood samples (1 mL each) were collected aseptically from the jugular vein into anticoagulated tubes. Immediately following

blood collection, the samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum, which was promptly stored at -20°C until analysis. Commercially available Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) were used to quantify zonulin levels. The kits were procured by RDA Group, Istanbul. The ELISA methodology adhered to protocols previously described in related research (Alic Ural 2021a, Alic Ural 2021b, Alic Ural 2022a, Alic Ural 2022b, Alic Ural 2022c, Alic Ural 2023a, Alic Ural 2023b). The sandwich ELISA technique was employed, utilizing a reference range of 1.56–100 ng/mL with a sensitivity threshold of 0.5 ng/mL, ensuring precise detection.

To maintain consistency and accuracy, all procedures were conducted following manufacturer protocols, and quality control measures were applied during each assay. Any discrepancies or anomalies in the assay results were verified by repeating the test on the same samples.

Statistical Analyses

All data were expressed as mean ± standard deviation (SD). Normality of the dataset was assessed using the Shapiro-Wilk test, ensuring appropriate statistical methods were applied. Group comparisons were performed using the Wilcoxon signed-rank test due to the non-parametric nature of the data. Statistical analyses were conducted using SPSS software version 22.0 (IBM, USA), and a p-value of <0.05 was considered statistically significant.

Results

The serum zonulin levels measured in calves with respiratory distress syndrome (rDs) and healthy calves are presented in Table 1. The mean serum zonulin level in calves with RDS was significantly higher (66.71 ± 4.602 ng/mL) compared to healthy calves (21.69 ± 4.234 ng/mL), indicating a notable difference in zonulin concentration between the two groups. This suggests that zonulin may serve as a biomarker for intestinal permeability and systemic inflammation in calves suffering from rDs.

Table 1. Serum zonulin levels

Groups	Serum Zonulin Levels (ng/mL) $\bar{X}$ ± SH
Calves with rDs	66,71± 4,602
Healthy	21,69± 4,234

The analysis was performed without any methodological or procedural errors, ensuring the reliability of the data. The observed differences were statistically significant ($p < 0.05$), further supporting the potential association between increased zonulin levels and the pathophysiology of RDS in calves.

Discussion

Respiratory distress syndrome, has long been recognized as a combination of clinical findings as result of surfactant deficiency (Wauer, 1997, Verder et al 1999). It has been postulated that both premature birth and altered blood gas has been linked to rDs (Eigenmann et al 1984, Pickel et al 1989). To those of calves exhibiting rDs, there could exist no respiratory alterations following (Eigenmann et al 1981). Several selected calves presented immaturity. Morpholoically seemed rounded head, silky short hair, lessened umbilical hair, and teeth eruption (Zerbe et al 2008), weight loss and flexor tendon laxity could be observed. Following 15 minutes of birth, rDs could exist, in which involved inspiratory and expiratory dyspnea (Bleul, 2009, Zerbe et al 2008). In the present study to those of calves born immature and presenting signs of rDs following immediately after birth were enrolled. This might be a critical period in which intestinal environment is newly developed and zonlin expression might be flucatuating. This could be briefly discussed within the next paragraph.

In a prior study serum zonulin (ng/ml) levels were elevated (60,07 ± 21,20) at mid night 00.00 am in comparison to central day data at 12.00 pm (34,60 ± 10,90) (p=0,018). Briefly elevated zonulin concentrations denoted that disordered gut barrier with elevated intestinal permeability during summer months (Alic Ural 2021b). This could easily indicate the relevant fluctuation of zonulin concentrations, however we supported a healthy control group for comparison. Moreover in the present study serum zonulin levels (ng/ml) (66,71± 4,602) were elaveted in contrast to healthy (21,69± 4,234) calves. This could reflect that 'gut-lung axis' (Dang and Marsland, 2019, Enaud et al 2020, Man et al 2017, Mariam et al 2024, Alic Ural et al 2023a) exist and treatment plans must be directed towards bilateral changes of both at the respiratory and gastrointestinal routes.

In conclusion, the findings of this study underscore the critical interplay between intestinal permeability and respiratory health in calves with RDS. Elevated serum zonulin levels in these calves suggest a potential biomarker for gut barrier dysfunction linked to the "gut-lung axis." This highlights the need for a dual-focused approach in managing RDS, targeting both gastrointestinal and respiratory health. Future research should explore therapeutic interventions that simultaneously restore gut integrity and improve pulmonary function, ultimately contributing to better clinical outcomes in neonatal calves.

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Research Article

The Role of Ultrasonography in 20 Calf with Pneumonia: Diagnostic and Prognostic Value

Umit OZCAN*, Halis CETINER, Murat GUZEL

Department of Internal Medicine, Ondokuz Mayıs University, Samsun, Turkiye

ORCID : 0000-0002-0868-6399
ORCID : 0000-0001-8281-0127
ORCID : 0000-0002-8937-5428

*Corresponce:
Umit Ozcan
Department of Internal Medicine, Ondokuz Mayıs University, Samsun, Turkiye

Phone : +90 539 546 99 88
E- mail : **umit.ozcan@omu.edu.tr**
Doi : **10.5281/zenodo.14754336**

Abstract

Bovine respiratory diseases in calves are one of the enormous burdens on the cattle industry worldwide. Early detection and treatment are crucial for successful disease management. Thoracic ultrasonography has emerged as a valuable diagnostic tool for diagnosing calf pneumonia. Apart from diagnosis, determining the severity of pneumonia and subjectively assessing lung lesions are essential for prognosis. This study aimed to assess the role of thoracic ultrasonography in diagnosing and determining the severity of calf pneumonia.

84 calves under six months old were examined at Ondokuz Mayıs University Veterinary Hospital for respiratory complaints. Lung lesions were evaluated in two parts as right and left sides of the thorax of twenty calves, categorized as comet-tail artifacts, pleural irregularities, consolidation, thoracic effusions, and hepatized lung lobes.

Thoracic ultrasonography was performed on specific intercostal spaces, without clipping the hair, using 70% isopropyl alcohol. The most common abnormalities among 84 animals were comet artifacts, lung lobe consolidation, and pleural irregularities. Pleural irregularities were more common on the right side.

In the evaluation of lung lesions in the twenty calves, the most common lesions were comet artifacts (23.3%) and pleural irregularities (25.23%). Pleural irregularities were found to be more common in the right thorax than the left side ($P < 0.05$). There was no significant difference between the right and left thoracic regions in terms of other lesions. During the study period, nine animals with lung hepatization or thoracic effusion in either the right or left thoracic region, as determined by thoracic ultrasonography, died. It was concluded that animals with these lesions had a poor prognosis. In conclusion, it was found that thoracic ultrasonography is an advantageous diagnostic method for diagnosing lung diseases, as well as revealing lesions and determining prognosis.

Keywords: bovine respiratory system disease, calf, pneumonia, thorax, ultrasonography

Introduction

In nations where the livestock industry plays a significant role in the agricultural sector, respiratory issues have an economic impact. Producers of cattle may suffer large financial losses as a result of these issues. In the US, respiratory illnesses are thought to result in yearly economic losses of between $800 million and $900 million due to treatment expenses, decreased feed efficiency, and mortality (Chirase and Greene, 2001; Gorden et al., 2010).

The diagnosis of BRD may be difficult, especially in subclinical or chronic stages, and also because typical signs, including both increased rectal temperature and alteration of the respiratory function (tachypnea, dyspnea, or nasal discharge, etc..), lack both specificity and sensitivity (Buczinski 2013 short communication). However, auscultation of the lungs shows a low sensitivity in detecting lung pathologies (Buczinski, 2014). TU is very useful in the antemortem determination of lung lesions and has a positive correlation with postmortem lesions (Flöck, 2004). And besides that, it is very useful in revealing the lesions in bronchopneumonia in calves (Adams, 2016; Buczinski, 2016). Also, TU can reveal pleural disorders, comet-tail artifacts, lung consolidation, hepatization, and thoracic effusions. The frequency of these lesions has been investigated in previous studies (Braun, 2018; 2020; Tharwat 2011), but the relationship between the right and left sides of the thorax has not been evaluated. This study aimed to determine whether TU findings in calves with clinical pneumonia and also investigate the difference between right and left thoracic region lesions.

Materials and methods

The study's animal material consisted of calves that were brought to Ondokuz Mayıs University Faculty of Veterinary Medicine Training-Application and Research Hospital with respiratory problems. Calves less than six months old were included in the study. The animals underwent clinical respiratory scoring (CA BRD3 scoring system) and clinical examinations to confirm the complaint. Calves with a score of five or above were diagnosed with pneumonia. Between August 2017 and August 2021, a total of 84 calves were brought to the hospital and included in the study.

Thoracic Ultrasonography Application

Ultrasonography was performed by the same physician to minimize application differences and errors. The Esaote MyLab Five Vet ultrasonography device with a 2.5-6.6 MHz micro convex probe was used, and the depth was adjusted between 5-15 cm based on the lesions. The area was not shaved or clipped, and 70% isopropyl alcohol and ultrasound gel were applied before performing thoracic ultrasonography (TU).

The ultrasonography procedure was standardized for all animals. The systematic scanning of the thorax began at the processus transversus in the right/left 10th intercostal spaces. The probe was positioned parallel to the ribs within each intercostal space and moved ventrally toward the costal arch. The scanning continued sequentially through the caudal intercostal spaces towards the cranial region. The examination extended up to the 1st intercostal space on the right side and the 2nd intercostal space on the left side.

Pneumonic ultrasonographic lesions were defined by Detrich et al. (2015) in humans and by Rabeling et al. (1998) and Flöck (2004) in cattle. These lesions were classified as comet-tail artifact, diffuse comet-tail artifact, thoracic effusions, pleural irregularities, consolidation, and hepatization of the lung tissue. The identified pathologies were recorded separately for the right and left lung lobes. All animals included in the study were hospitalized, and treatment was initiated. For treatment purposes, all calves received subcutaneous florfenicol at a dose of 40 mg per kg and flunixin meglumine at a dose of 2.2 mg/kg. If necessary, fluid therapy was administered to dehydrated calves. Each calf was housed in an allocated calf box with heating lamps and provided with 24-hour access to water and feed. Necropsy was performed on deceased animals.

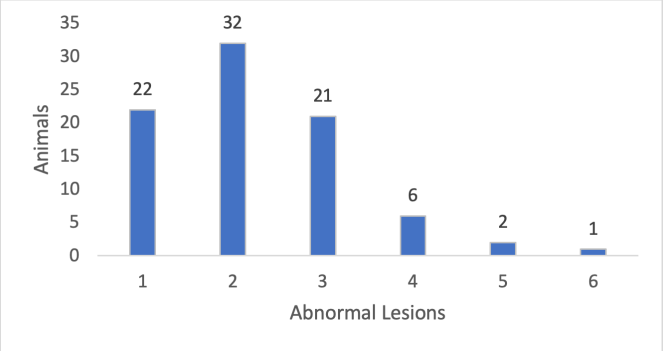
Statistical analysis

The data obtained in the study were subjected to statistical analysis using SPSS 21v. software. For this purpose, the t-test was employed to determine whether there was a difference in the number of lesions between the right and left lung lobes. Additionally, the chi-square test was used to assess the presence of the lesions.

Results

An average of 2.25 pathological thoracic ultrasonographic lesions were determined per animal. The most common abnormal ultrasonographic images were comet-tail artifacts (48), diffuse comet-tail artifacts (31), and consolidation (71). Pleural disorders (55), hepatization (16), and effusion (11) were observed less frequently. The number of abnormal ultrasound lesions in each animal resulting from ultrasonography is summarized in Figure 1.

Figure 1. The number of abnormal ultrasound lesions in each animal



Considering the lesions detected in the lungs, the most common lesions encountered were comet-tail artifacts and pleural irregularities in 20 animals. No difference was found between the right or left lung lobes in terms of comet-tail artifacts. Pleural irregularities were more common in the right thoracic region than the left ($p<0.05$). Among the animals with pleural irregularities (27 regions), the most common accompanying lesion was lung consolidation (19 regions). The ultrasound findings obtained in the right and left thorax are presented in Table 1.

Table 1. Differences in abnormal ultrasonographic findins at right and left lungs.

Lesions	Right Thorax	Left Thorax	Total	P
KOMT	13 (12.14%)	12 (11.21%)	25	P<0.05
Diffuse KOMT	7 (6,54%)	9 (8.41%)	16	
Pleural Irregulaties	17 (15.88%)	10 (9.34%)	27	
Consolida-tion	12 (11.21%)	9 (8.41%)	21	
Thoracal efusions	4 (3.73%)	3 (2.80%)	7	
Hepatisa-tion	6 (5.60%)	5 (4.67%)	11	
Total	56	51	107	

KOMT: Comet-tail artifact, Diffuse KOMT: Diffuse Comet-tail artifact.

All calves in which thoracic effusion and hepatized lung lesions were detected died despite the treatments applied. Necropsy was performed on these animals, and the thoracic ultrasonography (TUS) findings were compared to the necropsy findings.

Discussion

The ultrasonographic appearance of a healthy respiratory system in calves and the ultrasonographic findings of lesions have been revealed in various studies (Babkine, 2009; Jung and Bosted, 2003). Additionally, the effectiveness of thoracic radiography in diagnosing calf pneumonia has been investigated (Shimbo, 2019). Comparisons have been made between thoracic ultrasonography and thoracic radiography (Jung and Bosted, 2003), as well as between thoracic ultrasonography and bronchoalveolar lavage (Olivett et al., 2015), and it has been determined that thoracic ultrasonography is effective in detecting both clinical and subclinical pneumonia in calves.

Studies have been conducted to assess the specificity and sensitivity of thoracic ultrasonography in diagnosing pneumonia, and a strong positive correlation between necropsy findings and thoracic ultrasonography has been observed (Reinhold, 2002). Subclinical lung lesions detected by thoracic ultrasonography have been evaluated in terms of their impact on disease susceptibility and daily weight gain in calves. It has been found that average daily weight gain decreases as the number of lesions increases (Francoz, 2015). Furthermore, studies have indicated that a higher number of lesions increases the likelihood of mortality (Buczinski et al., 2014).

It has been determined that all animals diagnosed with pneumonia through clinical scoring systems and clinical examination exhibited pneumonia lesions on thoracic ultrasonography. While auscultation can help in identifying thoracic and/or lung diseases, it requires significant expertise, and the characteristics of lung lesions cannot be determined through auscultation alone. Buczinski et al. (2014) reported that only 5.9% of lung consolidations could be identified through auscultation, and this rate increased to 71.4% when clinical scoring and auscultation were combined, but the success of thoracic ultrasonography could

not be achieved.

Although radiography allows for the evaluation of lung tissue, it is not practical for use in field conditions in large animal medicine. On the other hand, thoracic ultrasonography has gained importance as a valuable adjunctive clinical diagnostic tool in pneumonia cases due to its applicability under field conditions, providing insights into the nature and size of lung lesions, and offering objective findings.

Comet-tail artifacts, which appear as bright, closely spaced echo bands starting at the lung surface and running perpendicular to the pleura in the lung tissue, have been observed in ultrasonography. Comet artifacts are not specific to a particular pathology but indicate interstitial or alveolar diseases in the lungs. These findings are commonly seen in pulmonary emphysema affecting the surface alveoli (Tharwat and Oikawa, 2011). Even small changes in the lung can reveal comet artifacts. In human medicine and calves, these findings are always considered indicative of lung parenchymal diseases (Lichtenstein and Meziere, 1998). The most common lesions detected in calves diagnosed with pneumonia were comet artifacts, which were found in 38% of the right and left lungs of calves, regardless of their extent or size. It has been mentioned in a study that sporadic comet artifacts may also be observed in healthy calves (Buczinski et al., 2014).

It has been reported that bovine respiratory disease (BRD) often starts in the cranial part of the right cranial lobe (Reinhold, 2002). Studies have reported the presence of consolidation areas in calves with clinical or subclinical pneumonia in this region, and ultrasonography was not sufficient for detecting consolidations smaller than 1 cm. However, severe and widespread comet tail artifacts were observed in these regions (Ollivett, 2015).

Furthermore, it has been found that lesions can occur in both the cranial and caudal aspects of the right cranial lobe, as well as in the right medial lobe and left cranial lobe. In this study, Pravettoni et al. (2021) concluded that scanning the entire lung tissue is superior to diagnosing BRD compared to ultrasonography of specific lung areas. Consistent with these studies, the higher occurrence of pleural irregularities in the right thoracic region compared to the left thoracic region suggests that scanning the right lung contributes to earlier diagnosis of pneumonia compared to the left side. Another study revealed that calves treated for pneumonia while still young experienced slower growth, delayed age at first calving, and shorter survival compared to animals without pneumonia (Stanton, 2012).

Pleural irregularities and thickening were noted when the pleural line appeared serrated with an irregular shape and a thickness greater than 1 mm, as opposed to the normal thin, smooth hyperechoic line (Fig. 2). Reef (1998) mentioned that the sonographic diagnosis of pulmonary parenchymal consolidation is based on detecting hypoechoic pulmonary parenchyma with visible bronchograms or vessels. Extensive consolidation appears as wedge-shaped hypoechoic and often heterogeneous zones, with anechoic areas representing fluid-filled or necrotic regions. Studies have linked the diagnosis of active inflammation or BRD in the lungs to areas of consolidation (Flöck, 2004; Jung, 2004). The presence of lung consolidation in 71 calves with pleural irregularities observed in 55 calves indicates a progressive disease associated with active pneumonia. The number and depth of consolidation areas, as well as the presence of pleural disorders, provide insights into the survival rate of calves (Braun, 2020). Braun suggests that these lung lesions in livestock have prognostic value. Ultrasonographic consolidation corresponded to firm, red lung lesions observed during gross examination at necropsy (Fig. 3, 4, and 5).

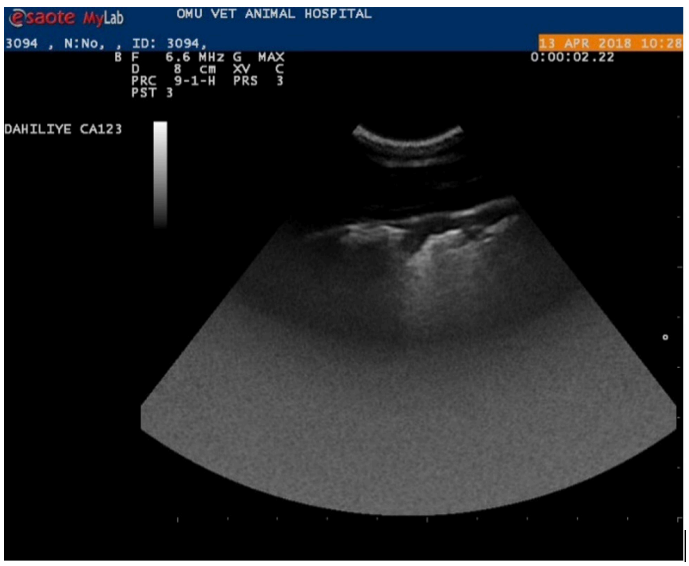


Figure 2. Calf No 10, calf pleural irregularities

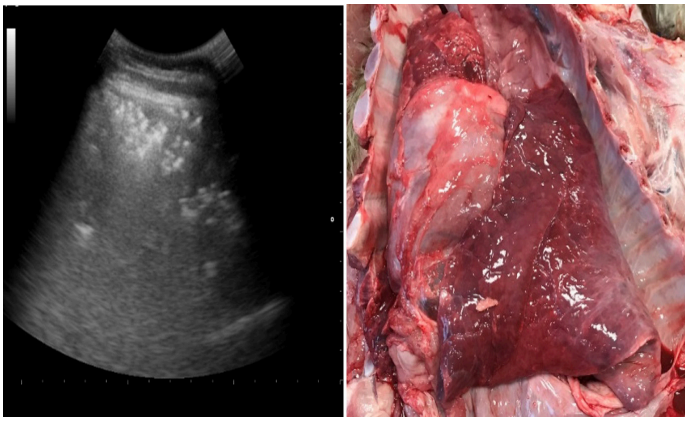


Fig 3. Calf No 6, consolidation

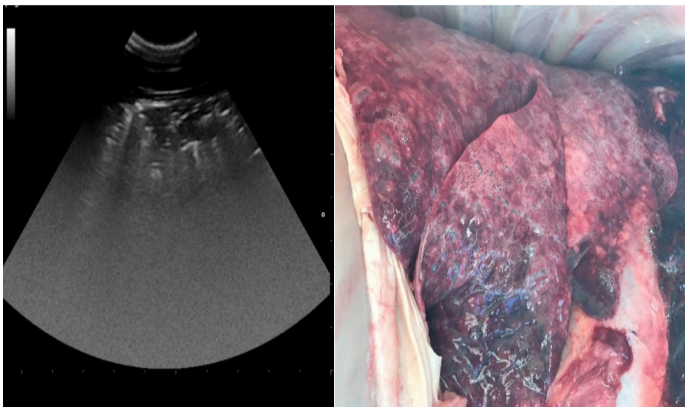


Fig 4. Calf No 7, superficial alveolar emphysema and consolidation

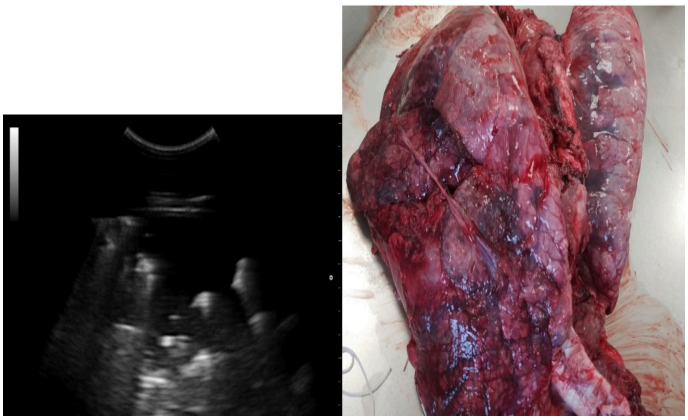


Fig 5. Calf No 18, consalidation

Hepatization of lung parenchyma occurs with severe and widespread consolidation, while effusion is a sign of severe pneumonia, giving rise to an ultrasonographic appearance similar to that of a liver (Rabeling et al., 1998; Reef, 1998). Pleural effusions appear as anechoic spaces between the lung and thoracic wall. The fibrin image formed as a result of pneumonia can be detected within the anechoic fluid in this area, indicating very severe pneumonia (Fig. 6). All calves with hepatization of lung tissue and/or thoracic effusion died despite treatment. Considering that all animals with these two lesions died despite treatment, it can be concluded that the prognosis for calves with these lesions is quite poor. Assessing the severity of pneumonia by identifying lung lesions with ultrasonography can contribute to decision-making regarding the continuation or completion of treatment, prevention of excessive medication usage or costs, and prognostic evaluations. When used as described in this study, thoracic ultrasonography can provide a rapid and objective assessment of lung health, improve the classification of BRD status, and should be considered as a primary method for detecting lung lesions in both clinical and research settings (Ollivett, 2015).

In cases of severe respiratory symptoms, extensive lung consolidation, and lack of response to treatment, it is crucial to be able to inform the animal owner about the prognosis in terms of breeding costs, treatment costs, and the future of the herd. Ultrasonography has been found to be a suitable complementary tool to clinical examination, with each method providing complementary information for recognizing and quantifying respiratory diseases in pneumonic calves.

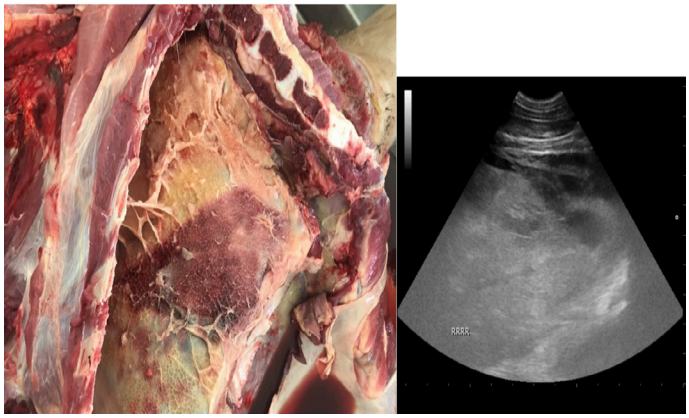


Fig 6. Calf No 19, diffuse KOMT, fibrin ve effusion.

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Review Article

Icterus in Cats

Ali Can ÖZCAN*, Mustafa Sinan AKTAŞ

Atatürk University, Veterinary Faculty, Internal Medicine Department, Erzurum/ Türkiye

ORCID : 0000-0001-8636-0610
ORCID : 0000-0002-7206-5757

*Corresponce:
Ali Can Özcan
Atatürk University, Veterinary Faculty, Internal Medicine Department, Erzurum/ Türkiye

Phone : +90 546 762 91 62
E- mail : **vetalicanozcan@gmail.com**
Doi : **10.5281/zenodo.14754415**

Abstract

Icterus in cats is characterized by the yellowing of the skin, eyes, and mucous membranes due to an increase in bilirubin levels in the blood. It is a serious symptom that necessitates a thorough evaluation to determine the underlying cause. It has been reported that icterus in cats does not have any age, breed, or gender predisposition. Also known as hyperbilirubinemia, icterus can result from various conditions such as liver damage, infections, bile duct obstructions, and hemolysis. Icterus is classified into three types based on its mechanism of formation: prehepatic, hepatic, and posthepatic icterus. Clinical signs, ultrasonography, radiography, and various laboratory tests are crucial for the diagnosis of icterus. Since icterus can develop due to various factors, accurate diagnosis forms the basis of treatment, and treatment procedures are developed separately for each cause.

Keywords: Cat, Icterus, pathophysiology

Introduction

The term “icterus” is derived from the French word “Jaune,” and it refers to a significant clinical finding characterized by yellowish pigmentation of the skin, oral mucosa, and sclera due to elevated bilirubin levels. This condition is also commonly known as “jaundice.” Icterus is a frequently observed symptom in cats (Saraiva et al., 2019). Known as hyperbilirubinemia, icterus has been reported to occur as a result of various conditions such as liver damage, infections, bile duct obstructions, and hemolysis. It is understood that icterus is not associated with any breed or age predisposition (Abbas, Shamshad, Ashraf, & Javaid, 2016).

Pathophysiology of Icterus

Bilirubin is an organic anion metabolized by the liver. It is produced as a result of the metabolism of various heme-containing proteins, including hemoglobin, myoglobin, cytochromes, catalase, and peroxidase. Approximately 80-85% of bilirubin synthesized from heme proteins originates from hemoglobin. In the reticuloendothelial system, macrophages present in organs such as the liver and spleen phagocytize aged or damaged erythrocytes. During this phagocytosis process, heme is released (Sherding, 2000). Heme oxygenases (HOs), encoded by the heme oxygenase 1 gene (HMOX1), catalyze a reaction in which heme is converted into carbon monoxide (CO), free iron (Fe), and biliverdin, a green pigment (Gozzelino, Jeney, & Soares, 2010).

Biliverdin is reduced to bilirubin IXa by biliverdin reductase (BVR), an enzyme found in various cellular components, including cell membranes. Unconjugated bilirubin binds to albumin molecules and is transported to hepatocytes via the cardiovascular system. This protein-bound unconjugated bilirubin cannot be excreted by the kidneys. Once transported to hepatocytes, unconjugated bilirubin is retained and carried by two proteins, Y (ligandin) and Z, which bind and transport bilirubin. Similar compounds, such as sulfobromophthalein and indocyanine green, also bind to these proteins, while bile acids do not. The binding of bilirubin to Y and Z proteins within hepatocytes limits its diffusion back into the plasma. Subsequently, bilirubin is transported to the endoplasmic reticulum, where it is conjugated with glucuronic acid, forming bilirubin glucuronide or bilirubin diglucuronide (conjugated bilirubin) via the enzyme uridine diphosphate-glucuronosyltransferase (Dandrieux, 2022; Joon et al., 2018; Turgut, 2000). This conjugation process renders bilirubin water-soluble (Bosma, 2003; Dandrieux, 2022).

Following conjugation, bilirubin is excreted into bile canaliculi and stored in the gallbladder until it is released into the duodenum. In the small intestine, conjugated bilirubin is first converted back to unconjugated bilirubin by bacteria and then further metabolized into tetrapyrrole compounds such as urobilinogen and stercobilinogen. Most urobilinogen (90%) is oxidized to urobilin and excreted in feces, while the remainder is absorbed, either reused in bilirubin synthesis via the enterohepatic circulation or excreted by the kidneys as urochrome. Stercobilinogen, a colorless compound, is oxidized to stercobilin, which gives feces its characteristic color (Dandrieux, 2022; Méndez-Sánchez et al., 2019; Sherding, 2000; Turgut, 2000).

The formation of urobilinogen is influenced by the amount of conjugated bilirubin entering the intestine, intestinal flora, and intestinal transit time. Therefore, measuring urobilinogen in urine samples is not considered a reliable diagnostic tool (Sherding, 2000).

Figure 1 illustrates the schematic mechanism of jaundice formation (Vandana Pushpendra, & Saket Singh, 2019).

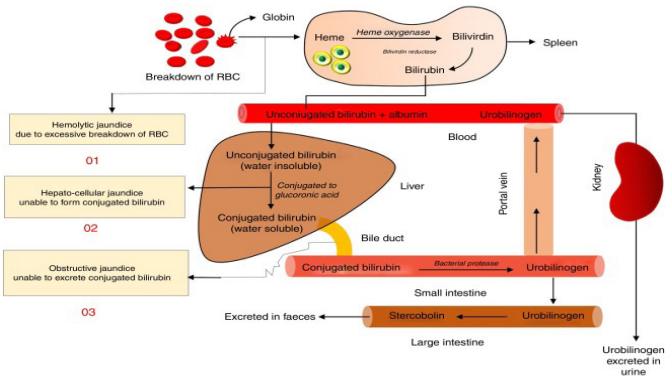

Figure 1. Mechanism of icterus formation in cats.

Classification of Icterus

Jaundice, as a significant clinical finding, is classified into three types based on its mechanism of formation.

Prehepatic Icterus (Hemolytic/Hyperfunctional)

Prehepatic icterus occurs as a result of excessive erythrocyte destruction (Abbas et al., 2016). Cats have a high red blood cell (RBC) turnover rate, and RBC destruction can occur both in lesions and in circulation. The breakdown of RBCs leads to the release of various hemoglobin products, including bilirubin and biliverdin. Due to their limited glucuronidation capacity, cats are known to have a restricted ability to metabolize and recycle bilirubin and biliverdin. Glucuronidation is a process that converts bilirubin into a water-soluble form, facilitating its excretion. The limited glucuronidation capacity in cats results in the accumulation of bilirubin and biliverdin, contributing to the development of icterus (Niels, 2014).

Prehepatic icterus in cats can be caused by various factors. Infectious diseases are a relatively common cause of prehepatic hemolysis in cats. Identifying the specific cause of prehepatic icterus in cats is critical for accurate diagnosis and appropriate treatment.

Hepatic Icterus (Toxic/Retention)

Hepatic icterus develops due to the effects of toxic substances on liver epithelial cells, leading to hydropic degeneration, fatty infiltration, or necrosis. In such cases, liver cells either undergo excessive damage and fail to process bile, resulting in the accumulation of unconjugated bilirubin in the bloodstream, as seen in hemolytic icterus, or the swollen liver cells obstruct the bile canaliculi. Bile is excreted by the bile epithelial cells but cannot reach the gallbladder or intestines. Consequently, conjugated bilirubin accumulates in the liver and is reabsorbed into the bloodstream. In this type of icterus, both conjugated and unconjugated bilirubin levels increase in the blood (Şennazlı, 2016).

Posthepatic Icterus (Obstruction/Reabsorption)

Posthepatic icterus is characterized by the accumulation of bilirubin resulting from the obstruction of bile flow from the liver to the intestines. This type of icterus arises from a range of pathophysiological processes involving cholestasis mechanisms. Cholestasis can develop due to various factors, including bile duct obstruction, hepatobiliary diseases, or dysfunction of bile acid transporters (Zollner & Trauner, 2008)..

Clinical Findings

In cases of icterus resulting from prehepatic icterus, urine color can vary from dark brown to yellow. Additionally, anemia, yellowish discoloration of the sclera and skin, and elevated bilirubin levels are commonly observed clinical findings (Bektaş et al., 2010).

Common diseases causing prehepatic icterus and the clinical signs associated with these conditions are summarized in the table below (Table 1) (Webb, 2016).

Table 1. Causes and Clinical Signs of Prehepatic Icterus

Causes of Prehepatic Icterus	Anorexia	Lethargy	Fever	Lymphadenopathy	Vomiting and Diarrhea	Weight Loss	Anemia	Abdominal Pain	Other Clinical Signs
Mycoplasma Species	X	X	X						Hypothermia, Physiological murmur, Increased heart rate/ respiratory distress secondary to anemia
Cytauxzoon felis		X	X	X					Dehydration, Shock signs, Respiratory distress, Hypothermia
FIP	X	X	X			X			Effusive (wet) FIP: Ascites, pleural effusion; Non-effusive (dry) FIP: Ocular and neurological signs
Babesia Species	X	X	X			X			
FeLV							X		Immunosuppression, Secondary infections
FIV			X	X		X			Oral inflammation, Secondary infections, Lymphoma
IMHA	X	X			X				Symptoms of IMHA, Pica
Erythrocyte Pyruvate Kinase Deficiency/ Osmotic Fragility	X	X				X			Pica
Neonatal Isoerythrolysis		X							Stopping nursing, Disseminated intravascular coagulation, Pigmenturia, Acute kidney injury, Death
Transfusion Reactions			X		X				Erythema or pruritus, Dyspnea or tachypnea, Tachycardia or bradycardia, Tremors, Convulsions, Shock, Cardiopulmonary arrest
Hypophosphatemia	X	X				X			Acute hemolysis, Weakness, Tachypnea, Tachycardia

Micro-angio-pathic Hemol-ytic Anemia	X	X	X				X		Hyperthermia, Ob-tundation, Cardio-pulmonary arrest
Drugs, Toxins, Poiso-ning, Oxi-dative Stress									Non-specific clinical findings depending on the underlying etiology, May include signs associated with he-molytic anemia and allergic reactions

The clinical signs of hepatic icterus include yellow discoloration of the mucous membranes and sclera, along with symptoms such as abdominal pain, high fever, vomiting, eating disorders, gastrointestinal bleeding, diarrhea, anemia, edema, and loss of appetite. If left uncontrolled, hepatic icterus can lead to severe conditions such as kernicterus, coma, and even death (Mathew, 2008).

Common diseases causing hepatic icterus and the clinical signs associated with these conditions are summarized in the table below (Table 2) (Webb, 2016).

Table 2. Causes and Clinical Signs of Hepatic Icterus

Cau-ses of Hepatic Icterus	Anorexia	Lethargy	Fever	Lymphadenopathy	Vomiting and Diarrhea	Weight Loss	Anemia	Abdominal Pain	Other Clinical Signs
Hepatic Lipido-sis	X	X			X				Idiopathic or secondary hepatic lipidosis. Clinical signs vary based on the underlying etiology.
Cholan-gitis	X	X	X		X			X	Bacterial, acute or chronic neutrophi-lic, lymphocytic. Dehydration, hepa-tomegaly.
FIP									Clinical signs of FIP.
FCV (Feline Calici-virus)			X						Oral ulceration, upper respiratory tract signs, edema, ulcerative dermati-tis, conjunctivitis.
Fran-cisella tularen-sis	X	X	X						Tachypnea, tachy-cardia.
Drugs, Toxins									Non-specific poi-soning symptoms, acute liver failure, death.
Amylo-idosis		X							Genetic and other types of amylo-idosis. Sudden death, acute gastric bleeding.
Sepsis / SIRS	X		X		X				Specific underlying etiology, clinical signs associated with collapse, bradycardia, and hypotension.

The clinical signs of posthepatic icterus include lethargy, loss of appetite, weight loss, vomiting, dehydration, excessive urination, excessive water consumption, palpable masses in the abdominal region, abdominal pain, abdominal distension, diarrhea, and icterus (Harvey, Holt, Barr, Rizzo, & Tasker, 2007; Jensen & Chan, 2014; Jifcovici, Caraty, Vincken, & Bongartz, 2021; Linton et al., 2015). Additionally, symptoms such as brownish urine, pale-colored stools, generalized pruritus, high fever, biliary colic, and significant weight loss can occur. Figures 2, 3, 4, and 5 show various images of cats with icterus.

Common diseases causing posthepatic icterus and the clinical signs associated with these conditions are summarized in the table below (Table 3) (Webb, 2016).

Table 3. Causes and Clinical Signs of Posthepatic Icterus

Causes of Post-hepatic Icterus	Anorexia	Lethargy	Fever	Lymphadenopathy	Vomiting and Diarrhea	Weight Loss	Anemia	Abdominal Pain	Other Clinical Signs

Chole-lithiasis									Clinical signs associated with extrahepatic bile duct obstruction (EHBO).
EHBO		X	X		X	X	X	X	Coagulopathy, hypotension, and shock.
Triadi-tis	X	X	X		X	X		X	Clinical signs may progress in cases of EHBO and hepato-megaly.
Liver Para-sites (Platy-noso-mum concin-num)	X	X			X		X		

In cats, bilirubinuria often occurs alongside hyperbilirubinemia before the clinical symptoms of icterus become apparent. In most cats with hyperbilirubinemia (72%), liver disease is present. A significant proportion of these conditions are hepatic problems that develop secondarily to diseases affecting other body systems (Turgut, 2000).

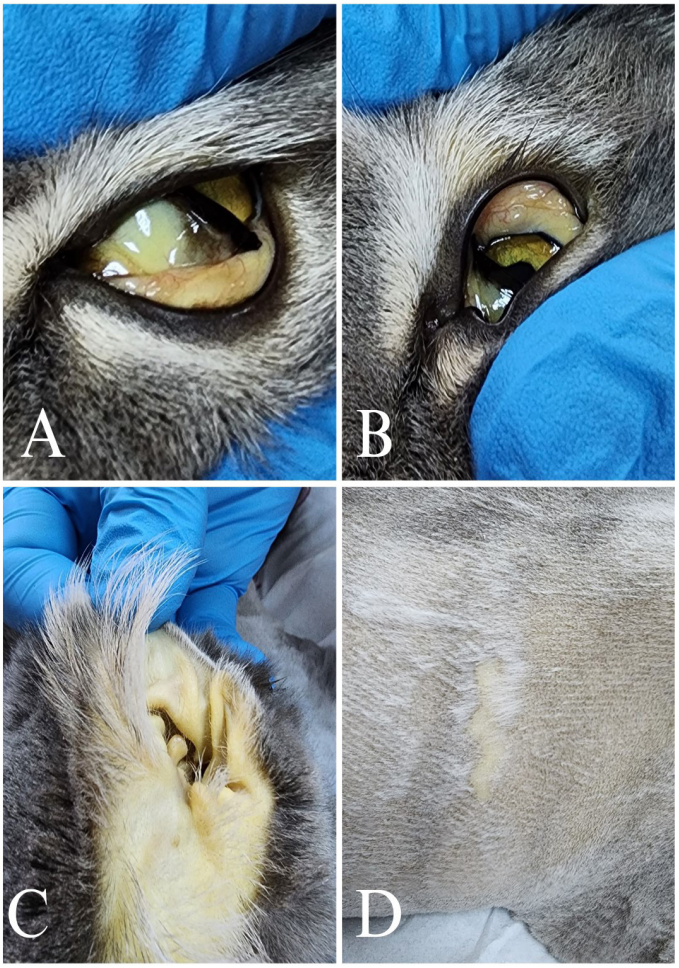

Figure 2. Clinical Signs of Icterus in Cats (Feline infectious peritonitis in a cat, sourced from the archives of Atatürk University, Faculty of Veterinary Medicine, Animal Hospital.) A and B: Noticeable yellow discoloration in the ocular mucosa. C: Yellow discoloration in and around the ears. D: Yellowing of the skin.

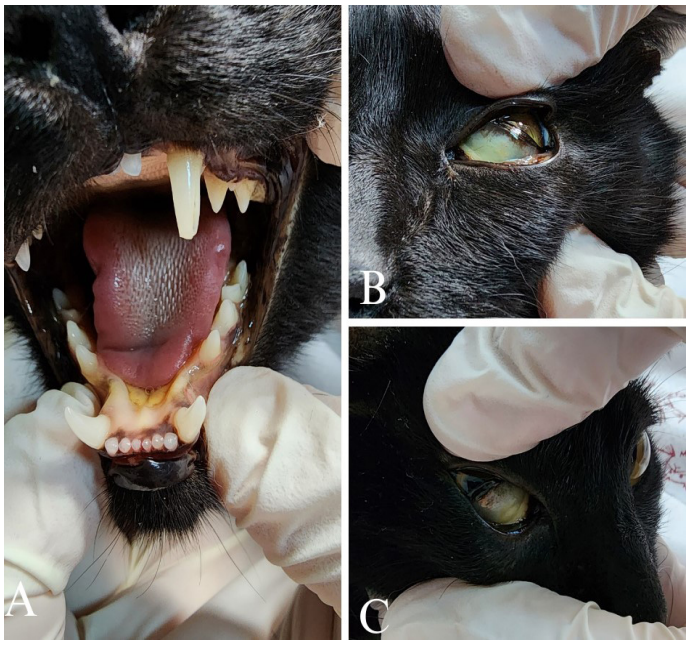

Figure 3. Clinical Signs of Icterus in Cats (A cat with hepatic lipidosis, sourced from the archives of Atatürk University, Faculty of Veterinary Medicine, Animal Hospital.) A: Oral mucosa of a cat with icterus. B and C: Ocular mucosa of a cat with icterus.

Figure 4. Clinical Signs of Icterus in Cats (Icterus resulting from Babesia infestation, sourced from the archives of Atatürk University, Faculty of Veterinary Medicine, Animal Hospital.) A and B: Noticeable yellow discoloration around the ears of a cat with icterus. C: Ocular mucosa of a cat with icterus. D: A cat with icterus.

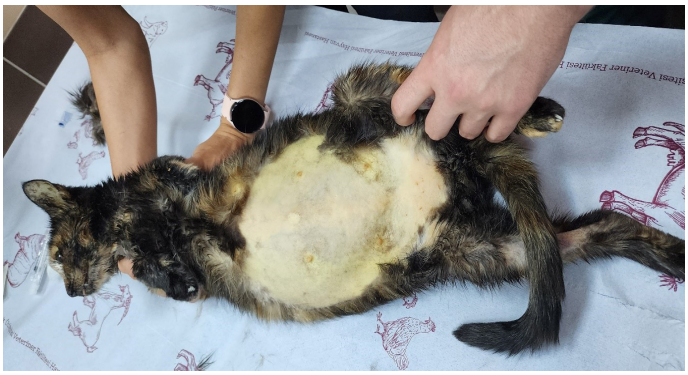

Figure 5. Clinical Signs of Icterus in Cats (A cat diagnosed with feline infectious peritonitis, sourced from the archives of Atatürk University, Faculty of Veterinary Medicine, Animal Hospital.)

In addition to clinical signs, the diagnosis and severity of icterus can also be determined using various laboratory parameters.

Diagnostic Approach in Icteric Cats

Diagnostic Approach to Prehepatic Icterus

In any case of prehepatic icterus, hemolytic anemia should first be investigated. Routine hematology analysis (+/- manual packed cell volume (PCV)) can quickly identify the presence of anemia, followed by blood smear examination to investigate hemolysis. Blood smears should be screened for polychromasia and anisocytosis to confirm regeneration; aggregate reticulocyte counts can be performed to further measure the degree of regeneration. However, it is important to note that in the early stages of the disease (the first 3–4 days), regeneration may be absent or minimal (Huang et al., 2021; Practice, 2020).

In icteric dogs and cats, if the erythrocyte count is low, with reticulocytosis and bilirubinemia, hemolytic diseases should be considered. In hemolytic anemias (regenerative anemias; hemorrhagic, autoimmune hemolytic anemia), reticulocytosis, hemoglobinemia, hemoglobinuria, erythrocytic autoagglutination, spherocytosis, positive Coombs' test results, splenomegaly, and/or hepatomegaly are commonly observed. Determining conjugated and unconjugated bilirubin concentrations is not significant in differentiating hemolytic icterus from hepatic icterus and may lead to misinterpretation. In hemolytic cases, unconjugated bilirubin may initially predominate. However, over time, conjugated bilirubin may replace unconjugated bilirubin. Even if unconjugated bilirubin predominates, the patient may still have hepatic disease. Clinicians should not be misled by elevated ALT activity, as severe acute hemolytic anemia can also cause high ALT levels due to acute hypoxia (Turgut, 2000).

In icterus caused by hemolysis, bilirubin concentration rarely exceeds 3–4 mg/dL in dogs and cats. Bilirubin concentrations above 3–4 mg/dL may indicate the presence of hepatic and/or posthepatic disease in addition to hemolysis (Turgut, 2000).

Diagnostic Approach to Hepatic Icterus

In the absence of hemolysis and bile duct obstruction, icterus should be suspected to have a primary hepatic origin. Liver disease does not always result in icterus, and when it does, it typically causes only mild to moderate increases in bilirubin levels rather than significant elevations. This is often accompanied by elevated liver enzyme levels. Elevated bilirubin levels due to liver disease confirm the presence of hepatic dysfunction. A detailed clinical history is essential to rule out the possibility of toxin or drug exposure, and additional serological testing may be beneficial to investigate infectious causes (e.g., FIP, toxoplasmosis) (Practice, 2020; Rothuizen, 2020).

Diagnostic Approach to Posthepatic Icterus

After ruling out the possibility of hemolysis, the likelihood of posthepatic icterus should be investigated. Posthepatic icterus is often associated with elevated hepatic enzyme levels, with ALP typically being higher than ALT. However, ultrasonography is always the most useful diagnostic tool. In normal patients, the intrahepatic bile ducts are not visible, while in normal cats, the common bile duct can be easily identified and should measure less than 4 mm in diameter. Bile duct obstruction leads to dilation of the bile ducts and the biliary tree, which will become evident during ultrasound examination. The gallbladder itself

may or may not appear enlarged. Comprehensive investigations for mass lesions as potential causes of biliary obstruction should be conducted, as these are often of pancreatic origin (e.g., pancreatitis, pancreatic carcinoma) or hepatic origin (e.g., primary neoplasia, hepatic metastases, liver cysts) (Griffin et al., 2021; Practice, 2020). Additionally, bilirubin concentrations can rise significantly in cases of posthepatic icterus (>20 mg/dL) (Turgut, 2000).

Therapeutic Approach to Icterus

In cats, icterus arises from various causes, and treatment protocols are developed specifically for each underlying etiology. Accurate diagnosis is the cornerstone of effective treatment. Diagnosis established through a combination of anamnesis, systemic examination, laboratory findings, and imaging techniques is crucial for determining the prognosis and appropriate treatment of prehepatic, hepatic, and posthepatic icterus.

However, as a general treatment protocol, fluid therapy based on hydration status, pain management (e.g., buprenorphine, 0.01 mg/kg sublingually every 8 hours), and antiemetic therapy (e.g., maropitant, 1 mg/kg subcutaneously every 24 hours) can be addressed relatively effectively and significantly impact clinical outcomes. Ursodeoxycholic acid (5−15 mg/kg once daily) has been used in cases of bilirubin cholelithiasis, EHBO, and PK deficiency but should not replace antibiotics or prednisolone in lymphocytic or neutrophilic cholangitis. Supportive therapy may include S-adenosylmethionine (90 mg/cat once daily), silymarin (2−5 mg/kg once daily), and/or vitamin E (50 IU once daily) (Center, Randolph, Warner, Flanders, & Harvey, 2022; Webb, 2016).

Treatment Options for Prehepatic Icterus

As previously mentioned, prehepatic icterus can arise from various causes. Identifying the underlying cause and implementing a specific treatment protocol is of utmost importance. The primary goal of treatment is to eliminate hemoplasma infection and restore normal RBC function.

The diagnostic and treatment options for prehepatic icterus are presented in the table below (Table 4) (Webb, 2016).

Table 4. Diagnostic and Treatment Options for Prehepatic Icterus

Differential Diagnosis	Diagnosis Methods	Treatment Options
Diagnostic and Treatment Options for Prehepatic Icterus		
Mycoplasma Species	CBC, Blood smear test, Serum biochemistry profile, Polymerase chain reaction (PCR)	Doxycycline, 5 mg/kg PO every 12 hours for 14 days Pradofloxacin, 5 mg/kg PO every 24 hours for 14 days Enrofloxacin, 5 mg/kg PO every 24 hours for 14 days
Cytauxzoon felis	CBC, Blood smear test, PCR	Atovaquone, 15 mg/kg PO every 8 hours Azithromycin, 10 mg/kg PO every 24 hours
FIP / Babesia Species	CBC, Blood smear test, PCR	FIP: Supportive therapy, Polyprenyl immunostimulant, Pentoxifylline, 10 mg/kg PO every 12 hours Prednisolone, 2–4 mg/kg PO every 24 hours Babesiosis: Imidocarb dipropionate, 2.5 mg/kg IM Doxycycline, 10 mg/kg/day PO for 21 days
FeLV / FIV	FeLV: p27 antigen test FIV: Antibody test	Blood transfusion, Supportive therapy, Antiviral therapy Medical therapy: Prednisolone, 2.2 mg/kg PO every 12 hours Cyclosporine, 5 mg/kg PO every 24 hours Chlorambucil, 2 mg/cat every 3 days Mycophenolate mofetil, 10 mg/kg PO every 12 hours
Immune-Mediated Hemolytic Anemia (Primary)	Saline agglutination Coombs test	Prednisolone, 2.2 mg/kg PO every 12 hours Mycophenolate mofetil, 10 mg/kg PO every 12 hours

Erythrocyte PK Deficiency / Increased Erythrocyte Osmotic Fragility	Genetic testing	Breeding management Supportive therapy Ursodeoxycholic acid, 5–15 mg/kg PO every 24 hours
Neonatal Isoerythrolysis	Appropriate blood typing before breeding	Supportive therapy, Cardiovascular support
Transfusion Reaction	Pre-testing of donors, Blood typing, Cross-matching	Supportive therapy, Cardiovascular support
Hypophosphatemia	Electrolyte testing	Supportive therapy, Treatment of the underlying disease
Microangiopathic Hemolytic Anemia	CBC with platelet count Coagulation times	Intensive care, Cardiovascular support, Treatment of the underlying disease
Drugs, Toxins, Poisoning, Oxidative Stress	Various toxin assays Blood smear test	Plasmapheresis Elimination of exposure Support for affected organs Symptomatic treatment

Therapeutic Options for Hepatic Icterus

The treatment of hepatic icterus resulting from liver diseases in cats often requires careful selection and administration of medications. A comprehensive evaluation should be conducted to identify the underlying causes of icterus, and specific treatment methods tailored to liver diseases should be implemented. The diagnostic and therapeutic options for hepatic icterus are provided in Table 5 (Webb, 2016).

Table 5. Diagnostic and Therapeutic Options for Hepatic Icterus

Differential Diagnosis	Diagnosis	Therapeutic Options
Hepatic Icterus Diagnostic and Treatment Options		
Hepatic Lipidosis (Idiopathic or Secondary)	CBC, Serum biochemistry profile, Urinalysis, FeLV/FIP testing, Bile acids, Feline pancreatic lipase (fPLI) blood test, Ultrasound-guided fine needle aspiration (FNA) of the liver	Vitamin K1, 1 mg SC every 12 hours E-tube feeding
Sepsis / Systemic Inflammatory Response Syndrome	Various diagnostic methods	Supportive therapy Symptomatic treatment
Cholangitis (Bacterial, Acute or Chronic Neutrophilic, Lymphocytic)	Ultrasound-guided or Laparoscopy-assisted cholecystocentesis and liver FNA Cytology Culture and antibiogram	Amoxicillin-clavulanic acid, 62.5 mg/cat every 12 hours, Enrofloxacin, 5 mg/kg every 24 hours, Metronidazole, 7.5 mg/kg every 12 hours, Prednisolone, 2 mg/kg every 24 hours, reduced to 0.5–1 mg/kg every 24 hours, Cyclosporine, 5 mg/kg PO every 24 hours, Ursodeoxycholic acid, 10–15 mg/kg every 24 hours
Drugs and Toxins	Various toxin tests	S-Adenosylmethionine, 90 mg/cat every 24 hours, Silymarin, 2–5 mg/kg every 24 hours, Vitamin E, 50 IU every 24 hours
Infectious Diseases (FIP, FCV)	Infectious disease testing	Supportive therapy Symptomatic treatment
Amyloidosis (Genetic, Other)	Liver FNA Cytology	Supportive therapy

Therapeutic Options for Posthepatic Icterus

Posthepatic icterus in cats is commonly a result of bile duct obstruction caused by various potential factors. It is crucial to identify the underlying cause of this condition as the first step, followed by specific treatment aimed at resolving the primary issue. The diagnostic and therapeutic options for posthepatic icterus are detailed in the table below (Table 6) (Webb, 2016).

Table 6. Diagnostic and Therapeutic Options for Posthepatic Icterus

Differential Diagnosis	Diagnosis	Treatment Options
Diagnostic and Therapeutic Options for Posthepatic Icterus		
Cholelithiasis (Cholelithiasis)	Abdominal ultrasound	Ursodeoxycholic acid (5-15 mg/kg once daily), surgical intervention

Extrahepatic Bile Duct Obstruction (EHBO)	Abdominal ultrasound, fine needle aspiration (FNA) of the affected tissue	Ursodeoxycholic acid (5-15 mg/kg once daily), systematic therapy, surgical or medical intervention
Triaditis	fPLI blood test, abdominal ultrasound, liver FNA, cholecystocentesis, cytology, culture and antibiogram, endoscopic biopsy of the small intestine	Supportive care: hydration, perfusion, acid-base balance; Buprenorphine (0.01 mg/kg sublingual every 8 hours), Maropitant (1 mg/kg SC once daily), antibiotics, E-tube feeding, single-dose anti-inflammatory glucocorticoids
Liver Parasites (Platynosomum concinnum)	Fecal examination, ultrasound-guided cholecystocentesis, bile cytology, abdominal ultrasound	Praziquantel (10-30 mg/kg once daily for 3 days), surgical intervention if EHBO is present

Conclusion

In conclusion, icterus in cats is often the result of various underlying pathologies, including hepatobiliary diseases, hematological disorders, toxic agents, and parasitic infestations. Clinical signs play a crucial role in the diagnosis of icterus in cats. Additionally, laboratory parameters are essential for identifying the cause of icterus, assessing the patient's condition, establishing a definitive diagnosis, and formulating an effective treatment plan. Treatment approaches may vary depending on the underlying cause of icterus. An individualized treatment plan should be developed for each patient.

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Review Article

Management of Non-Regenerative Anaemia in Cats

Selin Sinem Sümbül LACİN[1*], Mustafa Sinan AKTAS[2], Sümeyye BAYSAL[3]

[1] Sağlık Bilimleri Enstitüsü, İç Hastalıkları Anabilim Dalı, Atatürk Üniversitesi, Erzurum, Türkiye
[2] İç Hastalıkları Anabilim Dalı, Atatürk Üniversitesi, Erzurum, Türkiye
[3] İç Hastalıkları Anabilim Dalı, Atatürk Üniversitesi, Erzurum, Türkiye

ORCID : 0000-0002-8680-5402
ORCID : 0000-0002-7206-5757
ORCID : 0000-0003-1464-4993

***Corresponce:**
Selin Sinem Sümbül Laçin
Atatürk Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, Erzurum, Türkiye

Phone : 0544 320 25 07
E- mail : selinsinemsum@gmail.com
Doi : 10.5281/zenodo.14754560

Abstract

Non-regenerative anemia is a common condition in cats. Non-regenerative anemia in cats is a condition caused by insufficient erythrocyte production or impaired erythropoiesis. Non-regenerative anemia occurs in cats of all ages , which is characterized by a low number of erythrocytes in the blood and, as a result, a decrease in oxygen-carrying capacity. The etiology of non-regenerative anemia in cats is a complex issue and includes a variety of conditions such as acute blood loss, infectious diseases such as Feline leukemia virus (FeLV), Feline infectious peritonitis, and immune hemolytic anemia. It is usually associated with a variety of causes, such as iron deficiency, chronic diseases, toxin exposure, or genetic factors. Symptoms include fatigue, pale gums, loss of appetite, and weakness. The diagnosis is made by laboratory tests and a complete blood count. Treatment varies depending on the underlying cause and may include iron supplements, blood transfusions, immunosuppressive agents, or specific drug therapies, primarily with the elimination of the underlying cause. Non-regenerative anemia is a very serious condition and requires timely intervention. Non-regenerative anemia in cats is an important issue in clinical practice and it is important for veterinarians to maintain their knowledge and skills in this area.

Keywords: Anemia, cat, non-regenerative anemia

Introductıon

Anaemia is a condition characterized by a decrease in haemoglobin (Hb), haematocrit (HCT), or red blood cell (RBC) count. It is caused by an underlying condition and can be classified into macrocytic, microcytic or normocytic subgroups based on morphology (Turner and Parsi, 2024). Anaemia is categorised If the bone marrow is functioning normally, this is regenerative anaemia. This type of anaemia is due to the removal of red blood cells from the body, for example in trauma, or the degradation of red blood cells in the system, for example in haemolytic anaemia or internal haemorrhage. Regenerative anaemia is in general easier to diagnose than non-regenerative anaemia (Tvedten, 2022). The incapacity of the bone marrow to react adequately to peripheral erythrocyte deprivation results in non-regenerative anaemia in cats. The causes of non-regenerative anaemia include both primary bone marrow diseases and

systemic diseases with secondary involvement of the bone marrow. Prognosis varies, with some conditions being short-lived while others may become chronic or fatal (White and Reine, 2009). Non-regenerative anaemia occurs when the bone marrow responds ineffectively to the increasing demand for red blood cells. Anaemias that result from a decrease in the hormone that stimulates red blood cell production or an abnormality in the bone marrow are called non-regenerative anaemias. Non-regenerative anaemia is a common condition in cats (Winzelberg Olson and Hohenhaus, 2019). The cause of feline non-regenerative anaemia is unknown, but it is probably due to the fact that cats become anaemic more often than dogs, and cats are susceptible to a number of chronic diseases that lead to predisposition to anaemia (Lynch et al., 2016).

Etiology ff Non-Regenerative Anaemia

Non-regenerative anaemia in cats can have various causes. The most commonly occurring are acute blood loss, infectious diseases, renal failure and immune mediated haemolytic anaemia (Winzelberg Olson and Hohenhaus, 2019).

Acute Blood Loss

During the early phase of the loss of blood, before there is a peripheral reticulocyte reaction, the anaemia may not be regenerative (White and Reine, 2009).

Infectious Diseases

Non-regenerative anaemia can be caused by infectious diseases. Feline leukaemia virus (FeLV) is one such disease (Gleich and Hartmann, 2009), Feline Immunodeficiency Virus (FIV) (Shelton et al., 1990; Gleich and Hartmann, 2009), Feline Infectious Peritonitis (FIP) (Paltrinieri et al., 2001), *Cytauxzoon felis* (MacNeill et al., 2015), Haemotrophic *Mycoplasma species* (Weingart et al., 2016), *Leishmania infantum* (Pennisi et al., 2015), and *Ehrlichia canis* (Braga et al., 2013). There are many causes of infection-related anaemia, but the most important is hepcidin, a type 2 acute phase protein which is produced by the liver (Verga Falzacappa and Muckenthaler, 2005). Hepcidin synthesis is induced by interleukin-6, which is produced early in host defence (Ganz, 2006). Hepcidin interferes with iron uptake by duodenal enterocytes and macrophages, which results in reduced iron uptake and iron deposition in macrophages (Verga Falzacappa and Muckenthaler, 2005). Hypoxia or iron deficiency reduces hepcidin production (Pietrangelo and Trautwein, 2004). Erythropoietin levels rise or fall depending on the severity of anaemia in infectious diseases. (White and Reine, 2009). Cats may develop anaemia within 2 to 3 days of the beginning of the infectious process and the haematocrit drops by up to 8% on average (Waner and Harrus, 2000).

Kidney Failures

Erythropoietin is predominantly secreted by the kidney into the peritubular interstitial cells of the inner renal cortex and outer medulla. Renal failure impairs the kidneys' ability to increase erythropoietin secretion in responding to hypoxia (White and Reine, 2009). Both acute and chronic etiologies of renal failure reduce erythropoietin secretion, and chronic renal failure is known to a primary cause of non-regenerative anaemia in cats (Furman et al., 2014). Renal failure commonly leads to normocytic, normochromic, non-regenerative anaemia (White and Reine, 2009).

Immune Mediated Haemolytic Anaemia (Imha)

IMHA is less frequent in cats than in dogs. The disease can be primary (idiopathic) or secondary to infections including Feline Leukemia Virus (FeLV), toxin, medication, blood parasite, neoplasia and systemic scleroderma (Little et al., 2018). Complement-mediated and extravascular haemolysis is observed in cats with IMHA. To date, no cases of intravascular haemolysis have been reported in cats (Kohn et al., 2006).

Pure Red Cell Aplasia (Prca)

Pure red cell aplasia (PRCA) is a unique syndrome that is characterised by severely nonregenerative anaemia with a deficit of erythrocyte precursors in the bone marrow in spite of normal leukocyte and platelet counts. PRCA can be either primary, or secondary to FeLV infection (White and Reine, 2009).

Myeloproliferative Syndromes

Myeloproliferative conditions are a group of associated neoplasms which result from the clonal

transformation of non-lymphoid stem cells and their derivatives (Feldman et al., 2000). Dysmyelopoiesis is a term used to describe bone marrow disorders that originate from haematopoietic stem cells and result in a decrease in one or more species of circulating blood cells. It is not known whether FeLV infection causes myeloproliferative syndrome in cats; an estimated 80% of cats with myeloproliferative syndrome test positive for FeLV (Feldman et al., 2000).

Iron Deficiency

Iron is naturally present in the body as haemoglobin (the most abundant form), myoglobin, labile iron, tissue iron and transport iron. A haemoglobin molecule carries four iron atoms, representing 0.34% of its total weight. Every millilitre of red blood cells carries 1.1 mg of iron (Feldman et al., 2000). Iron depletion is typically linked to chronic blood loss in the cat (gastrointestinal, parasitic through heavy flea infestation, or noted on anecdotal evidence in cases of chronic haematuria) (Winzelberg Olson and Hohenhaus, 2019). Iron measurement can also help to differentiate between anaemia resulting from iron deficiency and anaemia due to inflammation, but the difference can still be difficult to make. In both situations, serum iron levels are depressed. Ferritin, the tissue soluble form of iron storage, is typically reduced in iron deficiency anaemia and elevated in inflammatory anaemia (Winzelberg Olson and Hohenhaus, 2019).

Inadequate Nutrition

Nutritional deficit anaemia is now uncommon in veterinary practice as a result of the improved nutritional quality of commercial pet foods and the greater knowledge. These anaemias are typically caused by errors such as feeding an incorrectly formulated homemade diet, or by a digestive problem that inhibits nutrient absorption (White and Reine, 2009). Malnourished cats may exhibit normocytic, normochromic, non-regenerative anaemia resulting from protein, calorie, vitamin or mineral depletion (Watson and Canfield, 2000).

Clinical Findings

The clinical findings that indicate a need for the cat owner to visit the clinic include symptoms such as malaise, muscle weakness, pale mucous membrane colour, icterus, haemoglobinuria, or haematuria (Tvedten, 2022) A study was conducted on 15 cats with IMHA disease, which found that all cats experienced malaise for a duration ranging from 5 days to 3 months (mean 7 days). Other clinical signs reported were decreased appetite (n=12), weight loss (n=4) and pica (n=3). Pallor of mucous membranes was observed in all cats. Clinical exam findings were: tachypnea (n=13), galloping heart rhythm (n=9) and grade II-III/VI systolic murmur (n=9) (Black et al., 2016). In our clinic, pale mucous membrane is a common clinical sign in cats with IMHA (Figure 1-a and Figure 1-b).

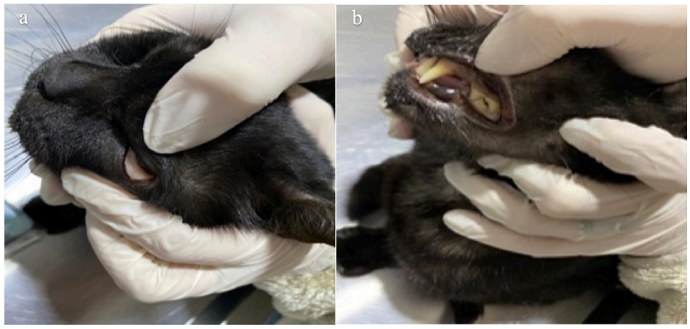

Figure 1-a, b: Anemia seen in the eye mucosa of a cat with IMA

Diagnosis And Classification of Anaemias

When diagnosing and classifying anaemia in cats, it is important to primarily assess RBC, HGB, HCT, MCH and MVHC. A HCT less than 29% is seen in cats with anaemia. Differential diagnosis of anaemia is performed on the bases of RBC size and haemoglobin capacity. The classification of anaemias is based on the parameters of mean corpuscular volume (MCV) and reticulocytes. To identify anaemia, different tests are carried out on blood samples. These tests are normally carried out as part of the complete blood count (CBC). The most widely used test to diagnose anaemia is the packed cell volume (PCV), also called the haematocrit. In a healthy cat, 25-45% of the blood is red blood cells. If the HCT falls below 25%, the cat is considered anaemic (Weir, 2024).

Reticulocyte Count

A count of reticulocytes is required to distinguish regenerative anaemia from non-regenerative anaemia, and a high reticulocyte count is suggestive of regenerative anaemia. The cat definition of reticulocytosis varies between values >0.045 x 1012/l and >0.060 x 1012/l. (Fielder, 2024). Feline WBCs can be counted by manual and automated techniques. WBC count by automated techniques

(blood counting machines) is slightly higher, but correlates with manual total reticulocyte count (Fujino et al., 2013).

Mcv

RBC markers are MCV and mean corpuscular haemoglobin concentration (MCHC). These can help to identify the type of anaemia by examining cell volume and haemoglobin concentration, e.g. microcytic hypochromic anaemias (regenerative or otherwise) are generally caused by iron deficiency (Eclinpath, 2024).

Other Tests

If the cause of non-regenerative anaemia cannot be determined through haematology, biochemistry, or infectious disease testing, additional tests such as blood smear, bone marrow cytology, or organ aspiration cytology may identify cytopenias, abnormal blood cells, Heinz bodies, organ hypertrophy, or diagnostic imaging abnormalities (Winzelberg Olson and Hohenhaus, 2019).

Treatment

Non-regenerative anaemia should be managed by identifying the underlying condition and developing an appropriate treatment plan. Supportive therapies are also commonly used to treat non-regenerative anaemia. (Winzelberg Olson and Hohenhaus, 2019).

Supportive Treatment

Agents Stimulating Erythropoiesis

In cats, these drugs are used to manage non-regenerative anaemia due to chronic renal failure. Studies have shown that these drugs, used in aplastic anaemia, FIP and FIV-related non-regenerative anaemia, cause an increase in HCT (Tanaka et al., 2015). Several recombinant human erythropoiesis-stimulating products are currently on the market, such as epoetin alfa, epoetin beta and darbepoetin alfa. Darbepoetin alfa is hyperglycosylated compared to epoetin alfa, which results in a three time longer circulating half-life and a reduction in mean elimination rate of over 70% (ml/kg x h) (Tanaka et al., 2015). Darbepoetin is given only once a week. initial dose of 1.0 µg/kg in cats (Marks, 2023). As the target HCT is achieved, the dosing interval is decreased every 2-3 weeks and the dose is adapted to maintain the HCT within the target range (25-35%). It is important to note that epoetin should be dosed three times a week (Chalhoub et al., 2012). For cats, the suggested starting dose is 100 units/kg subcutaneously three times per week. Once the target hematocrit (HCT) level of 30-40% is achieved, the dosing frequency can be reduced to twice a week for ongoing treatment (Chalhoub et al., 2012).

Iron Supplementation

Cats being treated for iron deficiency anaemia or after the administration of erythropoietin-stimulating agents may require iron supplementation (Winzelberg Olson and Hohenhaus, 2019). Iron dextran, iron gluconate, and iron sucrose are all used for parenteral supplementation. In veterinary medicine, iron dextran is the most frequently used. The recommended dose for cats is 10 mg/kg, administered intramuscularly every 3-4 weeks. To minimize the risk of anaphylaxis, intramuscular administration is recommended rather than intravenous administration (Winzelberg Olson and Hohenhaus, 2019).

Vitamin B12

Anaemia due to hypocobalaminemia may require vitamin B12 supplementation (Winzelberg Olson and Hohenhaus, 2019). For parenteral administration, vitamin B12 is given as cyanocobalamin. Dosing protocols may vary, but the most recent recommendation is to administer 250 µg subcutaneously once a week for six weeks, followed by an initial dose 30 days later; serum cobalamin levels should be reassessed 30 days after the last injection (Kempf et al., 2017). Research indicates that oral cyanocobalamin supplementation can increase serum cobalamin levels in cobalamin-deficient cats, above the normal reference range. The recommended dose is 250 µg administered orally (PO) every 24 hours with continuous supplementation (Toresson et al., 2017).

Glucocorticoids

Glucocorticoids are the preferred initial treatment for immune-mediated haemolytic anaemia. They slow down the destruction of erythrocytes by altering Fc receptors (Wang et al., 2013). Long-term use of corticosteroids may result in several side effects, including gastrointestinal irritation, hyperadrenocorticism, repeated infections, sepsis, weight gain, polyuria/polydipsy, delayed wound healing and alopecia (Wang et al., 2013; Manev

and Marincheva, 2018).

Prednisolone and Prednisone

The immunosuppressive dose will vary between drugs, but for prednisolone and prednisone it is 2 mg/kg given twice daily for 2 weeks or until HTC is stabilised (McCullough, 2003).

Cyclosporine

Cyclosporine is the most studied and widely used supplement to glucocorticoids and is used in the treatment of IMHA in cats (Black et al., 2016). To obtain the desired clinical results, oral microemulsion cyclosporine is recommended at a dose of 5-20 mg/kg given at 24-hour cycles for 2-7 weeks (Swann et al., 2016).

Blood Transfusion

Blood transfusion is indicated for cats with severely anaemic conditions and HCT values between 12-14%. (Castellanos et al., 2004). Cats have three blood groups: A, B, and AB. Type A is the most widely used type in the world, particularly in shorthaired domestic cats, type B is less common and type AB is believed to be rare (Barfield and Adamantos, 2011). Blood group identification can be carried out in any standard laboratory from a whole blood sample anticoagulated with EDTA, or by using rapid diagnostic test kits (Barfield and Adamantos, 2011). Fresh whole blood is currently the most widely used product in cats, but transfusions also include stored whole blood, packed red cells and fresh frozen plasma (FFP) (Roux et al., 2008). It is preferable for the donor cat to weigh more than 4 kg (Castellanos et al., 2004). Cats that are on long-term medication, including non-steroidal anti-inflammatory agents, should not be used as donors. Cats should be fully vaccinated as required in your region (Castellanos et al., 2004). To draw a full unit of blood (50-60 ml), it is recommended to pre-fill three 20 ml syringes with 3 ml each of acid citrate dextrose or citrate phosphate dextrose anticoagulant. It is important to avoid heparin as an anticoagulant due to its potential to inhibit platelet aggregation and clotting factors. However, heparin can be used as an alternative anticoagulant in an emergency at a rate of 5-10 units of heparin/ml of blood (Dippenaar, 1999). The jugular vein is punctured using a 19-21G butterfly needle, and blood is collected over a period of 10-15 minutes. To ensure sufficient anticoagulant distribution in the collected blood, each syringe is slowly rotated during and after donation. A maximum of 10-12 ml/kg of blood can be transfused at one time (Castellanos et al., 2004). The primary objective of this procedure is to raise the patient's HCT levels adequately to alleviate the symptoms of anaemia. The target should be to achieve a 20% increase in HCT (Castellanos et al., 2004).

Conclusion

Non regenerative anaemia is common in cats. There are many different causes in the aetiology and differential diagnosis is of great importance in its treatment. Therefore, the underlying disease should be treated first. There are many options for supportive treatment of non-regenerative anaemia in cats. Blood transfusion is indicated depending on the haematocrit level.

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Research Article

Evaluation of Hematological, Biochemical, and Echocardiographic Findings in Dogs Infected with *Dirofilaria spp.*

Hasan ERDOGAN*, Serdar PAŞA, Ali AYDIN, Ilayda TENDAR, Tahir OZALP, Songül ERDOGAN, Kerem URAL

Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine, 09100, Aydın, Turkey

* ORCID:0000-0001-5141-5108
ORCID:0000-0003-4957-9263
ORCID:0009-0005-9303-1336
ORCID:0000-0003-4039-6460
ORCID:0000-0002-9873-0364
ORCID:0000-0002-7833-5519
ORCID:0000-0003-1867-7143

***Corresponce:**
Hasan ERDOGAN
Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine, 09100, Aydın, Turkiye

Phone : 0256 220 62 49
E- mail : hasan.erdogan@adu.edu.tr
Doi : 10.5281/zenodo.14754657

Abstract

Canine dirofilariasis is a significant disease that associate with heart and cardiopulmonary complications. In endemic regions, the co-occurrence of other vector-borne infections further complicates diagnosis and treatment. Additionally, the lack of standardized diagnostic tools to assess disease progression represents a critical gap in veterinary literature. In this study echocardiographic, hematological, and biochemical findings of dogs infected with heartworm alone and those co-infected with other vector-borne diseases were compared. Furthermore, the study aimed to evaluate the usability of these parameters in determining the prognosis and severity of the disease. This study included 12 dogs diagnosed with *Dirofilaria spp.* infection, categorized into two groups: mono-infected (n=7) and co-infected (n=5). Dirofilaria antigens and additional co-infecting agents were detected using the Knott test and SNAP 4Dx Plus, blood samples were collected for complete blood count (CBC) and serum biochemistry analysis. Each dog underwent an echocardiographic evaluation. While most parameters were similar between the mono-infected and co-infected groups, platelet (PLT) counts and mean corpuscular hemoglobin concentration (MCHC) values were lower, and liver enzyme levels were higher in the co-infected group. Although echocardiographic parameters were generally similar, the mono-infected group showed higher left atrial dimensions and ventricular volumes, while the co-infected group exhibited slightly elevated fractional shortening (FS) and ejection fraction (EF) values. These findings suggest that co-infection may influence both platelet counts and liver enzyme levels. This study indicates that co-infections in dogs with dirofilariasis may lead to lower PLT and MCHC levels, accompanied by higher liver enzyme levels, which could impact disease management approaches.

Keywords: Canine dirofilariasis, Echocardiography, Vector borne

Introduction

Canine dirofilariasis, commonly known as heartworm infection, could cause cardiac disorder and has also been mentioned as one of the the top ten causes of mortality, particularly in tropical and temperate regions (Kim, 2011; Rath et al., 2014). Caused by the nematode *D.immitis*, this parasitic disease primarily targets the cardiopulmonary system, leading to structural and functional impairments such as cardiomegaly, pulmonary artery enlargement, and congestive heart failure. Clinical manifestations may range from asymptomatic to severe conditions with symptoms including weight loss, lethargy, exercise intolerance, and chronic cough, depending on the infection load (Maxwell et al., 2014).

In advanced stages, the disease often results in right ventricular enlargement, increased pulmonary artery pressure, and tricuspid regurgitation, as observed through echocardiography (Browne et al., 2005; Oldach et al., 2018). These structural changes complicate the progression of dirofilariasis and can lead to right ventricular insufficiency (atrioventricular canal dilation, tricuspid valve insufficiency, and subsequent right atrial enlargement) and other severe complications (Atkins et al., 1988; Venco et al., 2014; Falcón-Cordón et al., 2019).

The diagnosis of dirofilariasis is complex, often involving a combination of epizootiological data, clinical signs, pathoanatomical findings, and laboratory diagnostics (Strickland, 1998; Hoch & Strickland, 2008; Romano et al., 2021; Yermolenko et al., 2022). Hemolarvascopy and other blood tests are commonly employed to detect dirofilaria in blood samples; however, these approaches may have limited efficacy, particularly in cases where immature nematodes are present (Magnis et al., 2013; Ionica et al., 2017; Genchi et al., 2021). More advanced techniques, such as rapid immunochromatographic tests and genetic assays, offer enhanced sensitivity, enabling detection of both mature and immature dirofilaria species (Albonico et al., 2014; Borthakur et al., 2015). Imaging tools like radiography and echocardiography are invaluable in assessing cardiopulmonary complications related to dirofilariasis, providing crucial insights into the extent of cardiopulmonary involvement (Venco et al., 1996; Little et al., 2018; Corda et al., 2022).

In endemic regions, co-infections with other vector-borne diseases add further complexity to the diagnosis and management of dirofilariasis. Other vector-borne diseases, such as ehrlichiosis, babesiosis, and anaplasmosis, frequently occur alongside heartworm infections (Radzijevskaja et al., 2020; Ramos et al., 2022). The co-occurrence of these pathogens complicates both clinical presentation and treatment strategies, posing a unique challenge for veterinary practitioners, especially in areas with high prevalence rates of these vector-borne diseases (Otranto et al., 2009).

While *D. immitis* infections are prevalent in both domestic and wild carnivores, the morphological and functional impact of this parasite on cardiovascular structures remains insufficiently studied (Matos et al., 2023; Rafailov et al., 2022). Specifically, there is a lack of standardized echocardiographic criteria that can reliably assess the progression of dirofilariasis in relation to infection intensity, complicating disease monitoring and prognosis determination. Furthermore, the effects of concurrent vector-borne infections on the progression and clinical management of dirofilariasis are not fully understood, highlighting a critical gap in the current veterinary literature.

This study aims to address these gaps by comparing echocardiographic, hematological, and biochemical findings in dogs infected solely with heartworm and those co-infected with heartworm and other vector-borne diseases. Through this approach, we aim to identify specific echocardiographic parameters and hematological markers that can aid in the assessment of disease severity and progression, potentially leading to more tailored treatment protocols for canine dirofilariasis in endemic regions.

Materials and Methods

This study included 12 dogs diagnosed with *Dirofilaria spp.* infection, categorized into two groups: mono-infected (n=7) and co-infected (n=5). These animals were selected from clinical cases presented at the Aydın Adnan Menderes University Faculty of Veterinary Medicine Small Animal Clinic due to symptoms indicative of dirofilaria infection. The co-infected group consists of dogs that carry *D. immitis* along with single or multiple infections of *Leishmania spp., Anaplasma spp.,* or *Ehrlichia spp.*

Sample Collection and Blood Analysis

Peripheral blood samples were collected from the *Cephalic vein* of each dog using sterile 5 mL syringes, ensuring minimal stress and discomfort during handling. After collection, blood samples were immediately transferred to EDTA tubes for complete blood count (CBC) analysis and into plain tubes for serum biochemistry. CBC parameters, including White Blood Cell (WBC) count, Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), Red Blood Cell (RBC) count, Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), were assessed to identify any hematological abnormalities linked to dirofilaria infection. For serum biochemistry, samples were centrifuged at 1500 x g for 10 minutes to separate the plasma, which was then analyzed for key biochemical markers such as liver and kidney function tests (Blood Urea Nitrogen (BUN), Creatinine (CRE), Total Protein (TP), Albumin (ALB), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST)) to monitor organ involvement.

For *Dirofilaria spp.* antigen detection, Knott's modified test was employed to detect microfilariae in the blood, while a SNAP 4Dx Plus (IDEXX Laboratories, Westbrook, ME, USA) was used for serological confirmation. Both tests were conducted in line with the manufacturer's protocols and allowed the differentiation between single and co-infections.

Echocardiographic Examination

All dogs underwent an echocardiographic examination to evaluate the structural and functional cardiac changes associated with dirofilaria infection. Each animal was positioned in right lateral recumbency, with minimal restraint to reduce stress and avoid interference with cardiac measurements. The examinations were conducted using Mindray M5 (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) multifrequency ultrasound machine with spectral and color Doppler capabilities to capture real-time images and measurements. The left atrium-to-aorta ratio (LA/Ao) was measured in the right parasternal short-axis view, with values above the normal range (>1.6) flagged as indicative of cardiac enlargement. M-mode Echocardiography was performed on the right parasternal short-axis view at the level of the papillary muscles. The following parameters were measured for each dog: end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated to assess the chamber size and ventricular volumes during cardiac cycles; stroke volume (SV) was derived by subtracting ESV from EDV, indicating the blood volume ejected per beat; ejection fraction (EF) and fractional shortening (FS) values were computed to evaluate the contractile function of the left ventricle, with EF serving as an indicator of global systolic function and FS as a measure of left ventricular shortening.

Statistical Analysis

Statistical analyses were performed using SPSS version 26 (IBM Corp, Armonk, NY, USA). The data were first assessed for normality using the Shapiro-Wilk test. Based on the results, appropriate statistical tests were selected. For comparisons between mono-infected and co-infected groups regarding echocardiographic, hemogram, and biochemical parameters, the Independent Samples t-test was used when the data were normally distributed. In contrast, the Mann-Whitney U test was applied for non-normally distributed data. In cases where multiple groups were analyzed, One-Way Analysis of Variance (ANOVA) was conducted for normally distributed data, and the Kruskal-Wallis test was utilized for non-normally distributed data. Correlation analyses among echocardiographic, hemogram, and biochemical parameters were performed using Pearson correlation for normally distributed data, and Spearman correlation for non-normally distributed data. All statistical tests were considered significant at a p-value of less than 0.05.

Results

The hematological, biochemical, and echocardiographic parameters of the mono-infected and co-infected groups are detailed in Tables 1-3. In terms of hematological findings, there were no statistically significant differences between the two groups for WBC, NEU, LYM, MON, EOS, RBC, HGB, HCT, MCV, MCH, or RDW. However, MCHC and PLT values were significantly lower in the co-infected group compared to the mono-

infected group, with p-values of 0.030 and 0.003, respectively. This suggests that co-infected animals may have alterations in coagulation and oxygen transport capacities.

Table 1: Hematological parameters in mono-infected and co-infected dogs with dirofilariasis.

	Group	Mean ± Std. Deviation	P value
WBC	*Mono-infected*	12.80 ± 2.40	0.639
	Co-infected	18.39 ± 17.72	
NEU	*Mono-infected*	8.87 ± 2.23	0.432
	Co-infected	12.85 ± 14.08	
LYM	*Mono-infected*	2.25 ± 0.75	0.876
	Co-infected	2.87 ± 1.88	
MON	*Mono-infected*	0.79 ± 0.39	0.755
	Co-infected	1.58 ± 1.85	
EOS	*Mono-infected*	0.81 ± 0.71	0.876
	Co-infected	1.07 ± 0.89	
RBC	*Mono-infected*	6.07 ± 0.78	1.000
	Co-infected	6.03 ± 1.38	
HGB	*Mono-infected*	14.72 ± 2.56	0.530
	Co-infected	13.62 ± 2.15	
HCT	*Mono-infected*	40.81 ± 4.58	0.876
	Co-infected	40.09 ± 8.35	
MCV	*Mono-infected*	67.42 ± 3.15	1.000
	Co-infected	66.68 ± 3.98	
MCH	*Mono-infected*	24.32 ± 3.70	0.268
	Co-infected	22.92 ± 2.70	
MCHC	*Mono-infected*	360.85 ± 48.99	0.030
	Co-infected	290.32 ± 146.04	
RDW	*Mono-infected*	13.62 ± 1.13	0.268
	Co-infected	14.46 ± 1.00	
PLT	*Mono-infected*	301.42 ± 165.90	0.003
	Co-infected	90.20 ± 35.61	
MPV	*Mono-infected*	10.02 ± 3.20	0.202
	Co-infected	11.30 ± 2.18	
PDW	*Mono-infected*	14.17 ± 2.23	0.343
	Co-infected	19.68 ± 9.64	
PCT	*Mono-infected*	0.28 ± 0.09	0.106
	Co-infected	0.77 ± 0.44	

Table 2: Comparison of Biochemical Parameters in Mono- and Co-Infected Dogs with Dirofilariasis

	Group	Mean ± Std. Deviation	P value
BUN	*Mono-infected*	39.01 ± 16.14	0.755
	Co-infected	49.78 ± 51.83	
CRE	*Mono-infected*	1.85 ± 0.45	0.639
	Co-infected	1.45 ± 0.81	
TP	*Mono-infected*	6.59 ± 1.01	0.432
	Co-infected	6.96 ± 0.54	
ALB	*Mono-infected*	2.84 ± 0.68	0.149
	Co-infected	3.36 ± 0.11	
ALT	*Mono-infected*	87.14 ± 63.36	0.149
	Co-infected	128.00 ± 65.39	
ALP	*Mono-infected*	242.85 ± 190.46	0.202
	Co-infected	892.20 ± 1464.36	
AST	*Mono-infected*	68.14 ± 44.51	0.432
	Co-infected	109.80 ± 87.73	

Table 3: Comparison of Echocardiographic Parameters in Mono-Infected and Co-Infected Dogs with Dirofilariasis

	Group	Mean ± Std. Deviation	P value
LA	*Mono-infected*	2.54 ± 0.54	0.755
	Co-infected	2.3 ± 0.43	
AO	*Mono-infected*	1.82 ± 0.51	0.639
	Co-infected	2.00 ± 0.59	
LA/AO	*Mono-infected*	1.42 ± 0.14	0.149
	Co-infected	1.23 ± 0.26	
IVSd	*Mono-infected*	0.91 ± 0.22	0.432
	Co-infected	1.08 ± 0.24	
LVPWd	*Mono-infected*	0.85 ± 0.07	0.530
	Co-infected	0.95 ± 0.37	
LVIDs	*Mono-infected*	2.17 ± 0.48	0.106
	Co-infected	1.59 ± 0.29	
EDV	*Mono-infected*	52.53 ± 22.14	0.073
	Co-infected	30.95 ± 14.39	
SV	*Mono-infected*	35.25 ± 14.18	0.106
	Co-infected	23.98 ± 10.74	
FS	*Mono-infected*	37.61 ± 2.45	0.149
	Co-infected	43.84 ± 7.29	
LVIDd	*Mono-infected*	3.47 ± 0.69	0.073
	Co-infected	2.85 ± 0.54	
IVSs	*Mono-infected*	1.53 ± 0.33	0.149
	Co-infected	1.13 ± 0.29	
LVPWs	*Mono-infected*	1.38 ± 0.27	0.755
	Co-infected	1.31 ± 0.31	
ESV	*Mono-infected*	17.28 ± 8.10	0.073
	Co-infected	6.95 ± 4.43	
EF	*Mono-infected*	70.15 ± 2.78	0.268
	Co-infected	77.09 ± 9.08	

For the biochemical parameters, there were no significant differences between the groups for BUN, CRE, TP, ALB, ALT, ALP, AST. However, it

is noteworthy that the mean values of ALT and ALP tended to be higher in co-infected dogs, suggesting a trend towards increased liver enzyme levels, which could imply greater hepatic involvement in these animals.

Echocardiographic parameters also showed mostly overlapping results between the two groups. The LA Dimension, AO Dimension , and the LA/AO ratio did not differ significantly. However, the mono-infected group displayed a trend towards a higher LA/AO ratio, which may suggest a degree of atrial enlargement in these individuals. Furthermore, both the EDV and ESV were generally higher in mono-infected dogs, although these differences did not reach statistical significance ($p = 0.073$). Similarly, the FS and EF were slightly elevated in the co-infected group, though these were not statistically significant. These findings highlighted that while the majority of parameters did not show significant differences between the mono-infected and co-infected groups, the observed reductions in PLT and MCHC in co-infected dogs might be clinicallv relevant indicators of co-infection.

Figure 1. Detection of Dirofilaria spp. using Microscopic and Rapid Test Kits

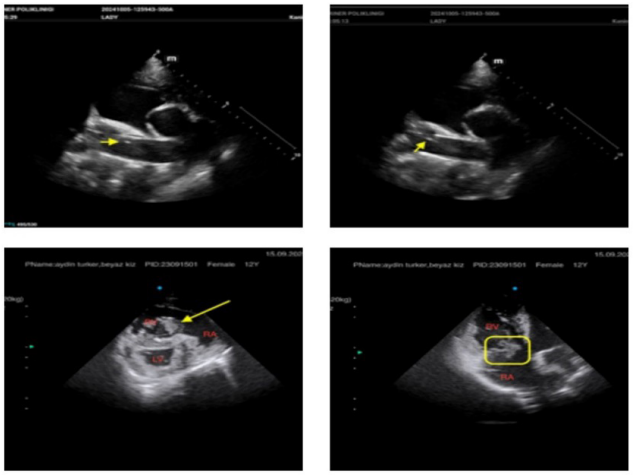

Figure 2. Visualization of Dirofilaria spp. parasite Using Echocardiography

Discussion

Canine dirofilariasis, commonly known as heartworm disease, is a significant cause of cardiovascular disorders and one of the leading causes of death in dogs (Kim, 2011). Dirofilariasis is a parasitic disease caused by the nematode *D. immitis*, which targets the cardiopulmonary system and leads to structural and functional abnormalities such as cardiomegaly, pulmonary artery dilation, and congestive heart failure.

Clinical signs can vary widely depending on the parasite load, ranging from asymptomatic to severe symptoms including weight loss, lethargy, exercise intolerance, and chronic cough (Maxwell et al., 2014). In this study, dogs were divided into mono-infected and co-infected groups. In the mono-infected dogs, clinical signs such as coughing (n=5), lethargy and anorexia (n=6), and exercise intolerance (n=4) were observed, while in the co-infected group, additional signs such as nasal bleeding (n=2), mucosal pallor (n=3), hematuria (n=1), and ascites (n=1) were noted.

In dogs with dirofilariasis, CBC plays a crucial role in detecting the clinical effects of the infection. Eosinopenia is considered an indicator of acute infection, while eosinophilia, particularly in cases involving pulmonary involvement, is associated with chronic infections (Lilliehöök et al., 2000). Thrombocytopenia may be related to increased platelet activity or immune-mediated platelet destruction in heartworm infections (Niwetpathomwat et al., 2007). Neutrophilia,

presence of monocytes, and activated monocytes are common findings in dogs with heartworm infections (Nelson & Couto, 2015). Anemia, commonly observed in heartworm infections, is associated with the movement of the parasite through red blood cells and blood vessel walls, induced by trauma (Attiyah & Alani, 2016; Madril et al., 2020). In our study, no significant differences were found between mono-infected and co-infected groups in hematological parameters such as WBC, NEU, LYM, MON, EOS, RBC, HGB, HCT, MCV, MCH, and RDW. However, a significant decrease in MCHC and PLT values was observed in the co-infected group compared to the mono-infected group ($p=0.030$ and $p=0.003$), indicating potential changes in clotting and oxygen-carrying capacity in co-infected dogs. Thrombocytopenia observed in other studies is believed to result from increased platelet consumption due to damage caused by the parasite to vascular endothelial cells (Su et al., 2004). The lower PLT count in the co-infected dogs, compared to mono-infected dogs, may be associated with additional stress on the hematopoietic system, leading to clotting problems. The decrease in MCHC suggests a reduction in hemoglobin concentration in red blood cells, weakening oxygen-carrying capacity. Eosinophils play a role in the immune response, surrounding the parasites and metabolizing infection-related substances. Although eosinophilia is rarely seen in pulmonary dirofilariasis, it may increase as the infection progresses, especially with metazoan parasites, including heartworms (Behm & Ovington, 2000; Ciferri, 1982; Werner et al., 1984). Consistent with these observations, higher eosinophil counts were seen in co-infected cases, indicating an increased immune response.

In the pathogenesis of the infection, intravascular hemolytic anemia is observed due to the mobility of microfilariae and the damage they cause to red blood cells (Kitagawa et al., 1989). Contrary to our findings, a study conducted in 2023 comparing the hematological and clinical findings of dogs co-infected with dirofilaria, babesia, or both did not report a significant decrease in hematological parameters in co-infected animals (Wężyk et al., 2023). This discrepancy may stem from host immune modulation, differences in the virulence of co-pathogens, the severity of infections, and the timing of sample collection (Wężyk et al., 2023). Naturally, in microfilaremic dogs, mild to moderate anemia, thrombocytopenia, leukocytosis, neutrophilia, eosinophilia, and monocyte elevation are common hematological abnormalities (Bowman, 2003).

Considering the natural course of infections, it is difficult to determine the duration for which these animals have been infected. However, clinical signs of the disease, physical activity, host immune response, parasite load, and infection duration can all lead to changes in hematological parameters (Nelson et al., 2014). Studies have highlighted significant kidney damage in dogs infected with *D. immitis* (Abramowsky et al., 1981; Morchón et al., 2012; Simón et al., 2012). In these infections, ALP elevation is reported as the only altered parameter in dogs (Niwetpathomwat et al., 2007). The increased ALP activity, coupled with normal AST and ALT levels, minimizes the likelihood of hepatocellular damage and instead points to chronic stress induced by elevated endogenous glucocorticoid levels (Fernandez, 2007). Nevertheless, concurrent increases in AST, ALT, and ALP activities may indicate potential liver damage. In our study, no significant differences were found between the two groups in biochemical parameters such as BUN, CRE, TP, ALB, ALT, ALP, and AST. However, the higher average ALT and ALP values in the co-infected group are noteworthy, suggesting that liver function may be more severely affected in co-infected dogs. This trend in liver enzyme elevation could indicate mild liver damage caused by the infection, leading to higher enzyme levels, and aligns with previous studies reporting elevated enzyme levels in dogs with concurrent infections (Niwetpathomwat et al., 2006).

In the diagnostic approach to heartworm disease, the importance of echocardiographic examination has been emphasized, highlighting its value in assessing pulmonary pressures and secondary effects on the right heart (Venco et al., 2014). In our study, most echocardiographic parameters were similar between the two groups, and no statistically significant differences were observed. This suggests that echocardiographic changes due to dirofilaria infection may be more subtle and may become more pronounced as the disease progresses. Additionally, the similarity in echocardiographic findings may reflect the variability in the clinical presentations of the animals. Other studies have shown a significant relationship between the severity of dirofilariasis

and echocardiographic findings (Pajas & Acorda, 2018; Su et al., 2004). However, our findings showed that concurrent infections have a clearer impact on cardiac health. In mono-infected dogs, the mean interventricular septal diastolic thickness (IVSd) was 0.91 ± 0.22 cm, while in co-infected dogs, this value was 1.08 ± 0.24 cm. Although no statistically significant difference was found (p = 0.432), the increase in IVSd in co-infected individuals may indicate a myocardial response to hemodynamic stress, which could lead to early myocardial remodeling. In mono-infected dogs, the mean stroke volume (SV) was 35.25 ± 14.18 ml, while in co-infected dogs, this value was 23.98 ± 10.74 ml (p=0.106). This reduction may indicate a decrease in cardiac output due to the increased workload caused by concurrent infections. Specifically, the significant reduction in SV and the increase in IVSd in co-infected dogs are in line with changes observed in advanced dirofilaria cases (Pajas & Acorda, 2018). This correlation underscores that the severity of dirofilaria infection, combined with the presence of co-infections, leads to significant hemodynamic changes, emphasizing the need for careful monitoring of cardiac function in dogs with concurrent infections

This study has several important limitations. First, the inability to measure the pulmonary artery and right ventricular outflow tract during echocardiography limits the comprehensive assessment of the cardiovascular status of patients with suspected dirofilaria infection. This limitation complicates the understanding of the systemic effects of the disease. Additionally, the sample size used in this study is limited, which introduces uncertainty in the generalizability of the findings. The limited sample size may have reduced the statistical power of this study, potentially obscuring subtle yet clinically relevant changes in echocardiographic parameters.

In conclusion, this study demonstrates that co-infections can lead to significant systemic effects in dogs with dirofilariasis. Hematological, biochemical, and echocardiographic evaluations reveal differences in certain parameters, particularly in the co-infected group. The lower PLT and MCHC values, along with the observed increase in liver enzymes, suggest that the disease may have broader systemic effects in this group. Therefore, considering co-infection in dogs diagnosed with dirofilariasis is crucial for managing the disease and preventing potential complications.

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Research Article

Investigation of The Prevalence of Respiratoric Coronavirus in Dogs in Kirikkale and Surrounding Provinces

Ahmet MUSLU[1*], Buğrahan Bekir YAGCI[2]

[1]Kırıkkale University, Veterinary Faculty, Internal Medicine Department, Kırıkkale/ Türkiye,
[2] Kırıkkale University, Veterinary Faculty, Internal Medicine Department, Kırıkkale/ Türkiye, ORCID: 0000-0002-7473-3579

*ORCID: 0000-0003-2772-5223
ORCID: 0000-0002-7473-3579

***Corresponce:**
Ahmet Muslu
Kırıkkale University, Veterinary Faculty, Internal Medicine Department, Kırıkkale/ Türkiye, 71000

Phone : 05315741853
E- mail : ahmet43muslu@gmail.com
Doi : 10.5281/zenodo.14754691

Abstract

Diseases in the lungs and other respiratory organs of dogs are usually cured with appropriate treatment. If left untreated, serious problems such as hypoxemia and sepsis occur. Canine respiratory coronavirus (CRCoV) was first identified in the United Kingdom in 2003 in a group of shelter dogs with respiratory disease. It is now detected in dogs worldwide. The disease can be found in dogs with and without clinical respiratory signs. This study aims to investigate to what extent the respiratory coronavirus is effective in the formation of respiratory complaints and to determine its prevalence in dogs. Within the scope of the thesis, 100 dogs with respiratory problems were included in the study. These were dogs brought to Kırıkkale University Veterinary Faculty Hospital, municipal shelters, and private veterinary clinics in Kırıkkale and its surroundings. Nasal and pharyngeal swab samples were analyzed using the RT-PCR technique to detect the presence of CRCoV. Analyzes showed that the incidence of CRCoV in dogs in Kırıkkale and its surroundings was 2% (2/100). This is the first study conducted in Turkey to detect the presence of canine respiratory coronavirus (CRCoV). Therefore, it will form a basis for future studies and provide an insight to the researchers.

Keywords: Dogs, espiratory coronavirus, respiratory system.

Introduction

Respiratory system diseases in dogs rank high in prognostic terms due to their potential to cause death. Therefore, early diagnosis of respiratory system diseases is important. Some respiratory system diseases can be easily diagnosed with simpler diagnostic methods, however, in others, advanced examinations are necessary. Upper respiratory system diseases usually occur in the nasal cavities and frontal sinuses, nasopharynx and laryngopharynx, larynx and extra thoracic trachea. Lower respiratory system diseases occur in the organs in the thorax.

Respiratory disease in dogs is generally observed in places where dogs live or are kept in groups, such as shelters, daycare facilities and animal hospitals. Infectious respiratory disease in dogs has a multifactorial etiology. The most accurate definition is canine infectious respiratory disease complex (CIRDC), formerly known as kennel cough, shelter disease. Many bacteria and viruses may cause CIRDC.

Canine respiratory coronavirus (CRCoV) is a member of the Coronaviridae family and was identified in 2003. It is classified by 4 separate genera depending on their characteristics. These are alpha, beta, delta and gamma. This virus is a betacoronavirus and it is closely related to human coronavirus HCoV-OC43 and bovine coronavirus (BCoV). CRCoV showed 96% amino acid identity with BCoV in its variable spike protein. The presence of CRCoV in dogs was first detected in detailed studies conducted on dogs with CIRDC. It was also frequently identified in samples taken from the trachea of dogs with mild clinical signs (Erles K., 2003). The causative agent cannot be determined by looking at clinical symptoms because respiratory disease has a multifactorial etiology. There are many effective methods and techniques for early diagnosis of the disease. Many serological methods are difficult and costly to use in practice. The use of PCR and RT-PCR methods in rapid serological identification provides benefits in terms of both convenience and cost (Lai MYY, 2005).

Materials and Methods

Material of Animal

This study was conducted with the approval of Kırıkkale University Animal Experiments Local Ethics Committee (Date 29.09.2022, Meeting No: 2022/05, Decision No: 24). 100 dogs with respiratory problems of different ages, breeds and genders in Kırıkkale and its surroundings were used in the study. The dogs were examined at Kırıkkale University Veterinary Faculty Research and Application Hospital. Then, samples were collected to determine the presence of Canine Respiratory Coronavirus, which is involved in the etiology of the Infectious Respiratory Diseases Complex of Dogs.

Clinical Examination

The dogs were brought to Kırıkkale University Veterinary Faculty Research and Application Hospital with respiratory system complaints such as cough, runny nose, sneezing and wheezy breathing. The dogs were clinically examined and recorded in terms of body temperature, pulse, respiratory rate, nasal discharge, tear discharge, and lung sounds.

Blood Count

2 ml of blood was taken into each tube with EDTA (Ethylene Diamine Tetra Acetic Acid) from Vena Cephalica Accecorius for hematological controls. Erythrocyte (RBC), Leukocyte (WBC), Hematocrit (HCT), Average Erythrocyte Volume (MCV), Hemoglobin (Hb), Average Corpuscular Hemoglobin (MCH), Average Corpuscular Hemoglobin Concentration (MCHC) values were measured on the fully automatic blood counting device (Mindray BC-5000 Vet) at Kırıkkale University Veterinary Faculty Research and Application Hospital.

Radiographic Examination

Radiographic examinations of the dogs were performed using the Digital X-ray Device (International Medical Device IMD Basic 100-30) at Kırıkkale University Veterinary Faculty Research and Application Hospital. Direct thorax radiographs were taken in ventrodorsal and laterolateral positions by dosing 10-40 mAs, 40-80 kV, 0.3 seconds depending on the size of the dogs.

Molecular Tests

Collecting Samples

Nasal and pharyngeal swabs (n = 100) were collected from dogs. A total of 100 samples, including nasal swabs and pharyngeal swabs, were simultaneously collected from 100 dogs. The collected samples were transferred to Molecular Transport an Lysis Reagent (NucleoGene Biotechnology Company, Istanbul, Turkey) tubes. The tubes were vortexed and brought to the laboratory. They were stored at 4°C until RNA isolation process.

Nucleic Acid Extraction

Nucleic acid extraction was performed using the NucleoGene N32 nucleic acid automated extraction system (NucleoGene Biotechnology Company, Istanbul, Turkey) in compliance with the manufacturer's recommendation. 200 µl was taken from the samples, which were brought to the laboratory and stored in Molecular Transport an Lysis Reagent (NucleoGene Biotechnology Company, Istanbul, Turkey) tubes at 4°C. They were transferred to Well 1 of NucleoGene Viral NA Extraction Kit Plates. RNA isolation of 32 samples was completed in approximately 30 minutes by selecting the relevant program from the device. The RNAs of the samples were taken from Well 6 as 50 µl and transferred to new nuclease-free 1.5 ml tubes and they were named after RNA isolation was completed. The process continued until RNA isolation of all samples was completed. When each isolation process was completed, the RNAs were put into – 80 C and stored until the Real Time PCR process.

Real Time PCR Process

Real-time PCR is used to measure the absolute amount of a target sequence or to compare relative amounts of a target sequence among samples. This technique is monitored in real time by amplification of a specific fluorescent signal that binds to the target sequence.

NucleoGene Canine Respiratory Coronavirus One Step RT-qPCR Detection Kit (Research use only) (NucleoGene Biotechnology Company, Istanbul, Turkey) was used for the diagnosis of CRCoV infection. The kit contains primary probes that detect the virus with the CoV M gene. Synthetic positive and negative controls were used in each Real Time PCR process. Tests were performed in duplicate. Reading was made by selecting the FAM channel on the device. Curves were observed in the positive control and positive samples. No curves were observed in the negative control and other samples. All Real Time PCR processes were carried out using the AGS4800 Real Time PCR device. Preparation of Real Time PCR reagents and operating conditions in the Real Time PCR device are specified in the tables below.

Statistical Evaluation

The values in the study were digitalized in a computer environment and descriptive statistical information (average, standard deviation, etc.) was obtained. The data were shown as percentages as well as descriptive statistical information since they were not compared with any group.

Results

Clinical Examination Findings

The dogs were brought to Kırıkkale University Veterinary Faculty Research and Application Hospital with respiratory system complaints such as cough, runny nose, sneezing and wheezy breathing. The body temperatures of 16% (n=16) of the dogs were not within the normal range during clinical examinations. 11 of these dogs had high body temperatures (hyperthermia) and 5 had low body temperatures (hypothermia). The body temperatures of the remaining 84 animals were found to be normal. The respiratory rates of the dogs were examined. The respiratory rates of 36 dogs were not within the normal range. 34 of these dogs had hyperventilation and 2 had hypoventilation. The heart rate of 33 dogs was outside the normal range. 32 of these dogs had tachycardia and 1 had bradycardia. Other clinical examination findings were nasal discharge in 53 dogs, eye discharge in 13 dogs, cough in 16 dogs, wheezy breathing in 12 dogs and sneezing in 1 dog. The data obtained as a result of the clinical examination of the dogs are presented in table 3 and figure 1. It was determined that nasal discharge was a common clinical finding in positive animals, and no other clinical findings were observed.

Hematological Examination Findings

Asepsis-antisepsis was performed in the application environment. 2 ml of blood was taken into each tube with EDTA (Ethylene Diamine Tetra Acetic Acid)

from Vena Cephalica Accecorius for hematological controls. Blood samples were measured on the fully automatic blood counting device (Mindray BC-5000 Vet) at Kırıkkale University Veterinary Faculty Research and Application Hospital. Complete blood analyzes of the dogs were measured. Measurements showed minimal changes in leukocyte counts. The most significant finding was in leukocyte counts although it is not specific. Leukocytosis was observed in some of the cases. The presence of leukopenia was also detected in some others. Hematological data are shown in Table 4.

Radiographic examinations of the dogs were performed using the Digital X-ray Device (International Medical Device IMD Basic 100-30) at Kırıkkale University Veterinary Faculty Research and Application Hospital. Direct thorax radiographs were taken in ventrodorsal and laterolateral positions by dosing 10-40 mAs, 40-80 kV, 0.3 seconds depending on the size of the dogs. The radiological examinations were performed. Non-specific findings for CRCoV infection were observed, such as prominence in the trachea and branching in the lungs (prominence of the bronchi).

RT-PCR Findings

Real-time PCR is used to measure the absolute amount of a target sequence or to compare relative amounts of a target sequence among samples. This technique is monitored in real time by amplification of a specific fluorescent signal that binds to the target sequence. The Ct values observed in the graph are the values that indicate the amount of the target gene in the cell. Low Ct values indicate high amounts of targeted nucleic acid. High Ct values indicate lower (or even very small) amounts of the target nucleic acid. Ct values below 29 cycles typically indicate abundant nucleic acid and Ct values above 38 cycles indicate minimum amounts. Nasal and pharyngeal swab samples taken from 100 dogs in the study were analyzed for the M gene by RT-PCR for the diagnosis of CRCoV infection. 98 nasal swab samples were negative while 2 of them were positive for the M gene.

RT-PCR Results and Assessment of Individual Data (gender, age, etc.)

In this study, the CRCoV infection was investigated in samples collected from dogs of different breeds and ages in Kırıkkale and its surroundings. 84% (n=100) of the dogs were mixed breed and the remaining 16% (n=100) consisted of various races. The samples that gave positive results in RT-PCR examinations and detected to have CRCoV were from mixed breed dogs.

The presence of CRCoV infection was identified in dogs aged 1 year and above rather than in younger dogs unlike CCoV according to studies in the literature. The average age of the samples in the project study in Turkey was 2.36 (n=100). The average age of positive samples in the RT-PCR study was 1.50 (n=2). Studies with more samples are required to clearly determine the age predisposition. There is no information in the literature studies about the gender predisposition of CRCoV infection. 49% (n=100) of the samples were from the female dogs and the remaining 51% (n=100) were from the male dogs in this study.

Table 1. RT-PCR Component Table

Components	Volume
NucleoGene Canine Respiratory Coronavirus Reaction Mix	5 µl
NucleoGene Canine Respiratory Coronavirus Oligo Mix	10 µl
Sample, Negative or Positive Control	5 µl
Total Final Volume 20 µl	

Table 2. RT-PCR Operating Conditions

Steps	Cycle Numbers	Temperature	Duration
Application of UNG	1	25 °C	1 minute
Reverse Transcriptase	1	50 °C	10 minutes
Pre-denaturation	1	95 °C	10 minutes
Amplification	45	95 °C 60 °C1	15 seconds 30 seconds
1 Data was collected by taking readings from the FAM channel.			

Table 3. Classification of clinical examination data of the samples used in the study

Clinical Findings		Number of Animals (n)
Body Temperature	Hyperthermia	11
	Normothermia	84
(38,3 – 39,2 oC)	Hypothermia	5
Respiratory Rate	Hyperventilation	34
	Normal	64
(18 -24 respiration/ min)	Hypoventilation	2
Heart Rate	Tachycardia	32
	Normal	67
(70-120 heartbeat/min)	Bradycardia	1
Nasal Discharge		53
Eye Discharge		13
Cough		16
Wheezy Breathing		12
Sneezing		1

Table 4. Hematological data of samples

	n	x ± Sx min-max
RBC (106/mm3)	100	7,46 ± 0,36 5,82 - 8,90
WBC (10^3/µL)	100	9,66 ± 0,42 5,12 - 19,26
HCT (%)	100	45.12 ± 1,92 34,30 - 53,50
Hb (g/dL)	100	14,42 ± 0,68 11,44 - 17,50
MCV (fL)	100	64,86 ± 0,84 60,10 - 68,90
MCH (pg)	100	20,60 ± 0,32 20,10 - 21,80
MCHC (g/dL)	100	33,24 ± 0,26 31,70 - 34,10

Figure 1. Percentage distribution of examination

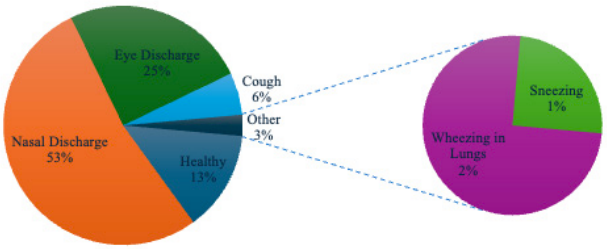


Figure 2. 22-24. Real-Time PCR graph of canine coronavirus detected in cycles (ct: 23.26)

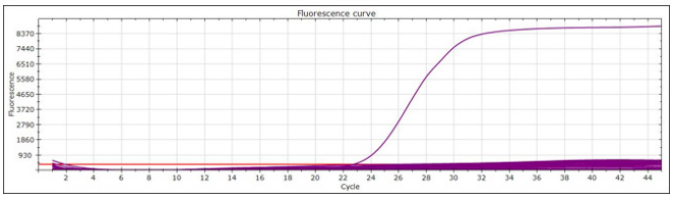
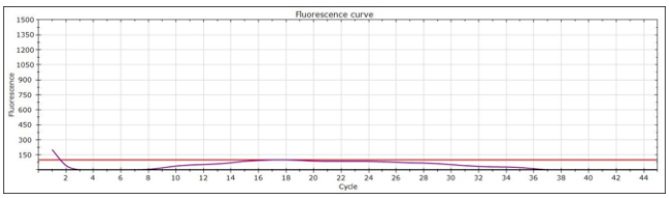


Figure 2. Example of a negative result with no peak (Case 33)



Discussion

Dogs became friendly animals as a result of domestication dating back approximately 14,000 years ago. People have benefited from both hunting and tracking abilities of dogs. The first documents of dog domestication in Anatolia were found in the neolithic murals in Çatalhöyük and they date back to 9000 BCE. The phenomenon of pet farming has become increasingly important since then. There is a dog population of over 500 million in the world. Therefore, issues such as the coexistence of dogs and humans and animal health come forward. The direct effects of contagious viral infections on animal health and the fact that they will also affect human health due to their zoonotic potential has made the research of these types of viruses popular. CRCoV infection is the subject of this study. It has infectious potential and is one of the viral diseases that affect the respiratory system. Nasal and pharyngeal swab samples were taken from 100 dogs and they were examined virologically. The presence of CRCoV nucleic acid was identified in 2 dogs (2%) via RT-PCR. These results constitute the first data on CRCoV infection in Turkey.

There are different reports about the importance and incidence of CRCoV infection in dogs in the world in terms of population and multiple living conditions (Arsevska, 2018). Many researchers believe that transmission of CRCoV infection is quite easy in

multiple living conditions and in conditions where more than one animal shares the same habitat, it may be possible to encounter CRCoV infection more frequently than in animals living alone because the virus spreads with viremia lasting up to 2 weeks and it has a high probability of spreading by oral transmission (Erles K., Toomey, 2003). CRCoV is a new canine coronavirus that is different from CCoV. CRCoV is frequently detected in dogs with clinical respiratory signs and it may cause CIRD complex (Erles K., and Brownlie J., 2005).

Many bacteria and viruses can be involved in CIRD. Bordetella bronchiseptica (Bb), canine adenovirus type 2 (CAV-2), canine distemper virus (CDV), canine herpesvirus (CHV) and canine parainfluenza virus (CPiV) are traditionally the main agents. Canine influenza virus (CIV), canine respiratory coronavirus (CRCoV), canine pneumovirus (CnPnV), Mycoplasma cynos and Streptococcus equi subspecies zooepidemicus (S. zooepidemicus) are new pathogens that have recently emerged in the development of CIRDC. Canine bocavirus and canine hepacivirus have been weakly associated with respiratory disease in isolated dogs (Priestnall, 2014).

Canine respiratory coronavirus (CRCoV) is a member of the Coronaviridae family and was identified in 2003. It is classified by 4 separate genera depending on their characteristics. These are alpha, beta, delta and gamma. This virus is a betacoronavirus and it is closely related to human coronavirus HCoV-OC43 and bovine coronavirus (BCoV). CRCoV showed 96% amino acid identity with BCoV in its variable spike protein. The presence of CRCoV in dogs was first detected in detailed studies conducted on dogs with CIRDC (Erles K., 2003). It was also frequently identified in samples taken from the trachea of dogs with mild clinical signs. The causative agent cannot be determined by looking at clinical symptoms because respiratory disease has a multifactorial etiology. There are many effective methods and techniques for early diagnosis of the disease. Many serological methods are difficult and costly to use in practice. The use of PCR and RT-PCR methods in rapid serological identification provides benefits in terms of both convenience and cost (Lai MYY, 2005). Control of CRCoV includes vaccination and improvement of kennel conditions, taking into account factors such as sanitation, population density, ventilation and quarantine procedures (Kerstin Erles and Joe Brownlie, 2008).

Unlike CECoV (Canine Enteric Coronavirus), CRCoV (Canine Respiratory Coronavirus) has been detected more frequently in dogs aged 1 year and above. Serum samples were collected from dogs in North American states in a prevalence study conducted by Simon L. in 2006. CRCoV infection has been investigated. It was a comprehensive study and it allowed the age sensitivity of the virus to be examined. Seropositive dogs were mostly 7-8 years old 68% (n=1000) (Simon L., 2006). 100 dogs in different age groups, over 1 year old, were tested with the RT-PCR technique to detect the CRCoV infection in Turkey. The positive samples in the study were between the ages of 2-4. The dogs under 1 year old will be less likely to be CRCoV positive compared to older dogs (>1) according to the study.

Canine respiratory coronavirus (CRCoV) was discovered in an extensive study conducted by Erles to identify the source of persistent respiratory complaints despite vaccinations in dog shelters in the United Kingdom. Erles collected blood/serum samples from a dog shelter in London. The number of CRCoV seropositive samples in the shelter was 30% (n = 123) as a result of Elisa antigen tests (Erles K., 2003). The main cause of respiratory complaints in dog shelters was considered to be Bordetella Bronchiseptica until recently. Advanced techniques such as Elisa and RT-PCR were used to resolve complaints that persist despite vaccinations in shelters. Blood/serum samples, nasal-pharyngeal swabs and lung samples taken from dogs were examined. Multiple pathogens have been identified to cause respiratory complaints (A. Mitchell, 2013). Erles and Brownlie collected blood serum samples from dog shelters in London and Warwickshire in another study conducted in 2005. They examined blood serums. They found a CRCoV antibody seropositivity rate of 22.2% (n=54) in London and 54.2% (n=59) in Warwickshire (Erles K., Brownlie J., 2005). Apart from the United Kingdom, Ellis conducted a study in Canada in 2005 and examined 126 dogs with respiratory complaints. 2 dogs were CRCoV positive (Erles K., Brownlie J., 2005). CRCoV was investigated in a different region for the first time in 2005. The positive results found in Canada showed that the new type of coronavirus was not only limited to the United Kingdom.

In 2006, Simon L. conducted a study to determine the prevalence of canine respiratory coronavirus in North America. Serum samples of 1000 dogs were collected from various states. They were examined using the Elisa technique. CRCoV seropositivity prevalence was 54.7% (n=1000) (Simon L., Priestnall, 2006). CRCoV prevalence studies conducted in different regions indicate that canine respiratory coronavirus has a high morbidity (spread) rate. Canine respiratory coronavirus (CRCoV) infection was detected at a prevalence rate of 2% (n=100) in Kırıkkale, Turkey and its surroundings after countries such as the United Kingdom, Canada and North America.

Although the rate is seen as low as 2% in the study, it is actually quite important. Because this ratio indicates the presence of antigen, that is, the agent itself. However, the reason why the rate is high in other studies is that they are studies based on the presence of antibodies. If this study was based on the detection of the presence of antibodies in blood serum, the rate would be high, but it was thought that it would not be able to make an opinion about cross-reactions or would positively increase the rate in animals that survived the disease.

The CRCoV pathogen alone causes non-specific respiratory symptoms. However, when they form a mixed infection with other pathogens, the symptoms are more severe and the risk of pneumonia increases (T. LeRoith, 2012). 2 CRCoV positive dogs had no symptoms other than mild nasal discharge in this study. Information was given about the clinical findings only at the time of sampling in these animals because at what stage of disease the animals were not known, and also only 2 animals had CRCoV.

CRCoV has a viremia period of two weeks in the living body ; for this reason, no obvious markers are seen on lung radiography. Studies have proven that evaluating CRCoV infection with CIRCD is clinically a more accurate approach than addressing CRCoV infection alone. Animals with respiratory problems had also similar radiographic findings in this study.

It is not possible to comment on the determination of racial predisposition of the CRCoV virus due to the narrow scope of the study on racial sensitivity and the lack of genomic analysis. The CRCoV pathogen has a high spread rate when evaluated in terms of morbidity. There should be more sample groups and genetically advanced techniques should be used for racial predisposition studies.

Conclusion

There is a dog population of over 500 million in the world. World Health Organization data demonstrates that there are 3.5 million stray dogs in Turkey. The coexistence of dogs and humans has brought animal health issues to the fore from the earliest times to the present day.

The main cause of respiratory complaints in dog shelters was considered to be Bordetella Bronchiseptica until recently. Advanced techniques such as Elisa and RT-PCR were used to resolve complaints that persist despite vaccinations in shelters. Blood/serum samples, nasal-pharyngeal swabs and lung samples taken from dogs were examined. Multiple pathogens have been identified to cause respiratory complaints (A. Mitchell, 2013).

New studies show that there are many viruses that have not yet been discovered. Canine respiratory coronavirus is different from the canine enteric coronavirus, which has been known to cause enteric symptoms in puppies for years, in terms of the group it is included in and the system it affects. This is the first study conducted in Turkey to detect the presence of canine respiratory coronavirus (CRCoV). CRCoV prevalence studies conducted in different regions indicate that canine respiratory coronavirus has a high morbidity (spread) rate. Canine respiratory coronavirus (CRCoV) infection was detected at a prevalence rate of 2% (n=100) in Kırıkkale, Turkey and its surroundings after countries such as the United Kingdom, Canada and North America.

The study shows that there is a need for a more profound examination of CIRDC infection, which cannot be prevented with vaccines in shelters in Turkey, and that a more comprehensive vaccine is essential by monitoring current strains in vaccine studies.

It is necessary to collect more samples to determine the age, breed and gender predisposition of CRCoV infection. Advanced techniques should be used to obtain the relevant data. CRCoV infection has been proven to exist within the borders of Turkey with this study.

The study will constitute an example for future studies since it is the first study conducted to determine canine respiratory coronavirus (CRCoV) in Turkey. It is crucial to increase the number of samples and use advanced diagnostic techniques in future studies. More studies should be carried out to investigate the prevalence of CRCoV nationwide.

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Research Article

Semi-elemental diet Gut-cumin I is capable of switching fecal scoring to fabric settings to those of acceptable levels in dogs with inflammatory bowel disease: further evidence of proof for gut-brain axis

Kerem URAL*, Hasan ERDOGAN, Serdar PASA, Songul ERDOGAN

Deparment of Internal Medicine, Aydın Adnan Menderes University, veterinary Faculty, Efeler, Aydın, Türkiye

*ORCID: 0000-0003-1867-7143
ORCID:0000-0001-5141-5108
ORCID:0000-0003-4957-9263
ORCID:0000-0002-7833-5519

***Corresponce:**
Kerem Ural
Aydın Adnan Menderes University, Veterinary Faculty, Department of Internal Medicine, Aydın, Türkiye, 09900

Phone : +90 533 382 12 44
E- mail : uralkerem@gmail.com
Doi : 10.5281/zenodo.14754748

Abstract

Polypharmacy without precise diagnosis cause harmless effects to those with gastrointestinal issues. Moreover unnecessary antibiotic usage might hasten dysbiosis, which could be detected in several dogs with gastroenteritis. Use of a novel and completely natural nutraceutical [Gut-cumin I Liquid Fomulation] against dogs with inflammatory bowel disease (ibD) whether if this natural trophic aminoacid and nutraceutical complex was capable of restoration for gastrointestinal health as detected by fecal scoring and clinical recovery/remission. A total of 59 dogs with ibD referred to the Intestinal Permeability Measurement Center (İPÖM) were entered into the study. All dogs participated, were completed the nutritional intervention protocole. Gut-cumin I was prescribed for each participant dog at a dose of 2 to 5ml/dogs based on weight of the animals for 1 week. Prior to treatment mean, median, quartile 1 (25%) and 3 (75%) fecal scoring values were deemed 2.9, 2.5, 2.0 whereas semi-elemental diet Gut-cumin I was capable of switching fecal scoring post-treatment values for mean, median, quartile 1 (25%) and 3 (75%) ranges as 3.5, 3.5, 3.,0 and 4.0, respectively. It should not be unwise to draw preliminary conclusion that semi-elemental diet Gut-cumin I was throughly altered gastrointestinal health conditions, as determined by limited access data throughly via inspeciton of fecal indices.

Keywords: Dog, Fecal consistency, Nutraceutical, Inflamatory bowel disease

Introduction

Regarding dogs, the quality of fecal matter could be changeable due to self-individual characteristics (age, size etc.), pathogenic microorganisms at the gastrointestinal system, dietary habbits and residing environment (stress) (Hernot et al., 2006; Sokolow et al., 2005; Stavisky et al., 2011; Weber et al., 2002, 2003). Several different manure interpretations were suggested for evaluating the quality of fecal matter among adult dogs (Allenspach et al., 2007; Hernot et al., 2006; Meyer et al., 1999; Rolfe et al., 2002a,b; Propst et al., 2003,). Aforementioned scoring systems were subclassified into 1 to 7 (Lappin et al 2022) or 4 to 10 points, with lower end points came accross to dry feces or diarrhea, along with an optimal scoring altered from 2 to 7.5 Allenspach et al., 2007; Hernot et al., 2006; Rolfe et al., 2002a,b). In the present study the purpose was to clearly define whether if semi elemantal diet Gut-cumin I, could reverse fecal scoring results in dogs with ibD.

Materials and methods

Data collection from dogs involves all relevant facilities were deemed available for all participant dogs: (i) İPÖM standard owner questionnaire gathering data from owners about case demographics, quality of life, feeding habits, water consumption, environmental exposures as detected by Quantum Pet Analysis Device, ii) the weekly/ monthly veterinary visit (medical past background, vaccination, anti-parasitic medication, data for disease or health conditions, entire physical examination, iii) and collection and submission of clinical samples from the participating dog (whole blood/serum).

Diagnostic interpretation and inclusion criterias

Dogs were enrolled based on presence of IbD (Cave, 2003) [chronic enteropathy with evidence of existence for more than 21 days (Washabau et al 2005, exclusion of other differential diagnosis for enteritis (Hall and German, 2009; Kleinschmidt et al., 2007; Schreiner et al., 2008; Washabau et al., 2005), colonoscopic examination] along with World Small Animal Veterinary Association (Washabu et al., 2010) guidance. Furthermore clinical disease activity (Jergens et al 2003), through scoring system, well recognized as the canine IbD activity index, CIBDAI was deemed available for first occasion criteria, similar to Ural et al. (2024) study.

Fecal indices

Fecal consistency, the vast majority interpretation of manure moisture and could thus be determining alterations in colon health and other relevant issues (Lappin et al 2022). In a healthy dog, a stool sample preferably be firm, not solid, flexible, segmented and easily handled (score 2) (Lappin et al 2022).

At the present study daily, fecal scores were detected into a simple score sheet at beginning (day 0) and thereafterwards on day 10 (finishing day). At physical examination all dogs underwent gastrointestinal interpretation (i.e. haematochezia/ melena, mucus, appetite etc.).

Statistical analytes

All statistical analytes were performed by use of R Studio (R Core Team, Vienna, Austria). In an attempt to understand data distribution prior to and thereafter treatment, descriptive and comparative statistical methodologies were deemed available. Given prior to and thereafter treatment data, mean, standart deviation, minimum and maximum values within interquartile range (IQR). For better detection of area with data dansity first quartile (Q1) and third quartile (Q3) values were found, in which aperture among those values were presented as IQR. Interquertiles aperture was used for better understanding distribution and central tendence of prior to and after treatment data.

Results

As the present author group would have the idea that this manuscript could have been used a brief atlas of fecal pool for diseased animals, in which in turn to healthy fabric settling following Gut-Cumin I liquid semi-elemental diet. Therefore, selected cases were deemed available for photographing for fecal matter prior to (day 0) and after treatment (day 10).

Fecal scoring based on individual cases

Herein at photographic records of case no 1 to 10 (Figure 1-10), were also involved at Table 1, respectively, left ones were prior to treatment and the right ones were following 10 days of Gut-cumin I treatment of selected cases (Table 2).

Table 1. Selected 12 cases, that were photographically shown below, out of 59 dogs enrolled entirely at this study. This table simply showed prior to and thereafter values for fecal scoring in relationship with Gut-cumin I semi elemental liquid diet administration.

Time	Fecal Scoring											
Prior to Gut-cumin I treatment	3	2	4	3	2	3	5	2	4	2	2	2
Following Gut-cumin I treatment	4	3	5	4	2	4	4	3	2	4	3	3

Table 2. For better detection of area with data density first quartile (Q1) and third quarttile (Q3) values were found, in which aperture among those values were presented as IQR. Interquertiles aperture was used for better understanding distribution and central tendence of prior to and after treatment data

	Prior to treatment	After treatment
Mean	2.9	3.5
Median	2.5	3.5
Q1 (25%)	2.0	3.0
Q3 (75%)	3.75	4.00
IQR (Q3 - Q1)	1.75	1.00

Discussion

All dogs enrolled at this study were born and grown in several different ecological and geographical locations. None of them were exposed to similar management, nourishment, nor microechological pathogens. Briefly this might be criticized making it impossible to differentiate influence of nutrition or breed size on the fecal score. Comparatively a prior study evaluated the efficacy of breed size on fecal scoring among puppies (Grellet et al 2012). Given large breed puppies exhibited soft and unformed feces, influence of breed size has already been detected among adult canines. Some selected large breed dogs [i.e. German shepherds/ Great Danes], moisture of fecal ingredient was elevated, soft uniformity was commonly observed and defecation frequency was increased in contrast to in smaller breeds (Hernot et al., 2006; Weber et al., 2001, 2002, 2003). The latter dissimilarity might be linked to diminished mineral absorption and/or to elevated fermentation activity associated with increased colic permeability/elevated colic transit time (Kirkwood, 1985; Herschel et al., 1981; Meyer et al., 1993, 1999; Rolfe et al., 2002a; Weber et al., 2004).

In a prior study aimed at determining feasibility of 2 previously realized (Purina and Waltham) fecal scoring systems, 126 dogs were evaluated by 3 veterinarians. In that study the uniformity of fecal scoring upgraded through veterinary surgeons experience level. Overall comparative analytes showed that there was inconsistency in fecal scoring which might have implications for veterinarians managing diarrhoeic canine patients (Cavett et al., 2021). Further studies are needed to better investigate how fecal scoring can be optimised for use in clinical and research settings.

What could have been changed with semi-elental Gut-cumin I diet, regarding gastrointestinal system at this study? Amino acids modify genes expression, and the creation of molecules [i.e. polyamines and nitric oxide etc.] (Fernandes and Murakami, 2010), all required for welfare of gastrointestinal system (Li et al., 2007). Specifically threonine, arginine and glutamine entirely have been explored for their participation with mucin production (Fernandez et al., 1994), immune respond (Tan et al., 2014a,b; Chen et al., 2016), and proliferation of epithelial tissue (Scheppach et al., 1996). Glutamine as a significant fuel for epithelial cells/leukocytes residing at the small intestine, took pivotal roles in several significant metabolic issues i) protein synthesis, ii) gluconeogenesis, iii) transfer of inter-organ nitrogen, iv) biosynthesis of nucleic acid, v) immune respond, and vi) modification of tissue redox conditions (Wu et al., 2007). Moreover, glutamine added onto diet, diminished enterocytes/ lymphatic cells apoptosis (Domeneghini et al., 2006), whereas altering anti-oxidative utilization and cell proliferation through small intestine (Wang et al. 2008). Hence circulating/luminal glutamine was capable of promoting intestinal functioning and integrity of mucosae (Baskerville et al. 1980). As a matter of fact glutamine included as a significant part of semi-elemental diet component Gut-cumin I, used at this study should have helped promotion of intestinal functioning as reported above.

There has been growing body of evidence and arousing interest for participation of nitric oxide for inducing health promoting efficacy of arginine for intestinal functioning. Arginine induces secretion of gut fluids via nitric oxide pathways (Alican and Kubes 1996). On the other hand, even if

nitric oxide synthase (NOS) is inhibited, intestinal secretion diminishes, causing intestinal ischemia (Kanwar et al., 1994). As a matter of fact arginine prescription has been efficacious for modification of intestinal barrier functioning and vascular issues (Wang et al., 2009). Oral arginine deduced mucosal injury linked to lipopolysaccharide endotoxemia in animals (Sukhotnik et al., 2004), as was determined by altered mucosal morphology and elevated proliferation of cells.

Threonine has been selectively participated for mucin synthesis and preservation of intestinal barrier integrity (Bertolo et al. 1998). Regarding gut mucosal lining, significant role for threonine is consolidation into mucins, to those of foremost glycoproteins preventing the epithelial injury (Le Floc'h and Se`ve, 2005; Schaart et al., 2005). In rats dietary threonine restricted diet caused altered synthesis of mucins among small intestine, with the largest deduced rate for 40% within the duodenum (Faure et al., 2005).

In conclusion trophic amino acids (Bortoluzzi et al., 2018; O'Connell, 2017; Wang et al 2009), namely, threonine, arginine and glutamine, might be of beneficial for altering gut physiology/ immunology/microbiology (Bortoluzzi et al., 2018) of dogs, which needs to be further supported by valuable biomarkers, which were lacking in this study herein reported. broilers. Trophic amino acids threonine, arginine, and glutamine are quite crucial for intestinal mucosal lining and intestinal recovery (Bortoluzzi et al., 2018; O'Connell, 2017, Wang et al., 2009), in which Gut-cumin I have helped diminishing fecal scoring results obtained at this study indicating that the intestinal health promoting.

Case I: Fecal scoring : 3 Fecal scoring:4

Case II: Fecal scoring: 2 Fecal scoring:3

Case III: Fecal scoring: 4 Fecal scoring:5

Case IV Fecal scoring: 1 Fecal scoring:2

Case V Fecal scoring:3 Fecal scoring: 4

Case VI Fecal scoring:5 Fecal scoring:4

Figure 1. Fecal scoring images of cats at prior and following of Gut-cumin I semi elemental liquid diet administration.


Case VII Fecal scoring: 2 Fecal scoring:3


Case IIX Fecal scoring: 2 Fecal scoring:3


Case IX Fecal scoring: 1 Fecal scoring: 3


Case X Fecal scoring:1 Fecal scoring:4

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