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Research Article

A Molecular and Histopathological Study on Bronchopneumonia in Cats

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ABSTRACT

In this study, it was aimed to determine *Bordetella bronchiseptica, Mycoplasma felis, Staphylococcus aureus and Chlamydia felis*, which cause bronchopneumonia in cats, by Real-time PCR and to compare the pathological findings of the identified agents. The material of the study was constituted of paraffin blocks belonging to the lungs, of which 21 bronchopneumonia were detected in microscopic examination (with Hematoxylin Eosin (HE)) from a total of 78 cats samples brought to Selcuk University, Faculty of Veterinary Medicine, Department of Pathology for pathological diagnosis. Histopathologically, polymorphonuclear leukocytes (PMNL) and mononuclear cell infiltration (MCI) in the bronchi and bronchiolar lumens, desquamation in the bronchi and bronchiolar epithelium, PMNL infiltration with oedama in alveolar lumens and desquamated alveolar epithelium, PMNL infiltration in the interstitium, and peribronchi and peribronchiolar MCI, and pleuritis were detected. Real-time PCR analysis revealed *Bordetella bronchiseptica* in 3 (14.29%) cases, *Mycoplasma felis* in 3 (14.29%), *Staphylococcus aureus* in 5 (23.8%), and *Chlamydia felis* in 5 (23.8%). Morever, *Mycoplasma felis* and *Staphylococcus aureus* infection was detected in 1 case, and *Staphylococcus aureus* and *Chlamydia felis* mixed infection was observed in 1 case. Our results show that relevant agents can frequently be isolated in cases of feline bronchopneumonia.

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INTRODUCTION

Pneumonia in domestic animals may occur due to exposure to foreign substances fungi, viruses, parasites, irritating gases and, especially bacteria (Maxie, 2015; Çiftçi et al., 2021). Cases of bacterial pneumonia usually occur due to some viral infections, drugs that cause immunosuppression or neutropenia, uremia, hyperadrenocorticism, diabetes, diseases that impair immune responses or neutrophil function, including systemic mycoses or primary immunodeficiencies, ciliary dyskinesia and stress (Dear, 2014; Chauhan et al., 2024). Many bacterial agents play a role in the aetiology of bacterial pneumonia in cats, including species such as *Bordetella*, *Mycoplasma, Pasteurella, Francisella, Chlamydia, Mycobacterium*, and *Staphyloccocus* (Padrid et al., 1991; Dye et al., 1996; Chauhan et al., 2024).

Bordetella spp. are gram-negative, aerobic, encapsulated and small coccobacillus-shaped bacteria (Cornelissen et al., 2012). *Bordetella bronchiseptica* causes respiratory diseases in cats, rats, horses, pigs, marine mammals, humans, and especially dogs. Tracheobronchitis and pneumonia in cats have been associated with *B. bronchiseptica*. The disease progresses mildly unless complicated by agents such as *calicivirus* and *feline herpesvirus*, but it can cause fatal bronchopneumonia in kittens (Songer and Post, 2004; Walter et al., 2020).

Most *Mycoplasma* species are commensal organisms that colonise the respiratory tract mucosa. *Mycoplasma spp*. have a species-specific host organism relationship. They are facultative anaerobic microorganisms except *M. pneumoniae*. *Mycoplasma* microorganisms are the smallest prokaryotic cells lacking cell wall and able to multiply on their own (Cornelissen et al., 2012; Eissa, 2024). It has not been fully determined whether *Mycoplasma* is a primary pathogen or an opportunistic pathogen in cats and dogs. However, *M. cynos* is known to cause fibrinous pneumonia in dogs and *M. felis* cause fibrinous pneumonia in cats. Macroscopically, hepatised areas are identified in the lung. *M. felis* causes mostly pneumonia and conjunctivitis in young cats (Songer and Post, 2004; Çiftçi et al., 2021; Chauhan et al., 2024).

Staphylococci are gram-positive, cocci-shaped, nonmotile, sporeless, facultative anaerobic, catalase-positive bacteria that occur in chains, singly, and often in the appearance of a bunch of grapes (Songer and Post, 2004; Rasheed and Hussein, 2021). The presence of *Staphylococcus* in the natural flora of pet mucosa paves the way for *Staphylococcus* infections. Most infections occur as a result of damage to the integrity of the skin or mucous membranes due to various reasons. *S. aureus* is the causative agent of necrotic pneumonia. *S. aureus* causes suppurative infections and septicemia in all species (Songer and Post, 2004; Cornelissen et al., 2012).

Chlamydiae are gram-negative, obligate intracellular bacteria. Although small and oval-shaped, their shapes may vary during reproduction stages. *Chlamydia* is spread by direct contact or aerosol (Songer and Post, 2004; Cornelissen et al., 2012). *Chlamydia felis* is endemic among domestic cats worldwide and causes conjunctivitis, rhinitis and pneumonia (Songer and Post, 2004; Sykes, 2021). They cause serous, catarrhal, and sometimes purulent inflammation in the upper respiratory tract and, the most important lesion, interstitial pneumonia (Songer and Post, 2004; Çiftçi et al., 2021).

In this study, it was aimed to determine the presence of *Bordetella bronchiseptica, Mycoplasma felis, Staphylococcus aureus* and *Chlamydia felis*, which cause bronchopneumonia in cats, by Real-time PCR and to compare the pathological findings of the identified agents.

MATERIAL AND METHODS

Animal materials

The material of the study was paraffin blocks belonging to the lungs of which 21 bronchopneumonia were detected in microscopic examination (with Hematoxylin and Eosin (H&E)) from a total of 78 cat samples brought between 2022-2023 years for pathological diagnosis to Selcuk University Faculty of Veterinary Medicine, Department of Pathology. The sections were examined under a light microscope (Olympus BX51, Tokyo, Japan) and photographed. The age groups of the cats that comprised the study material were grouped according to Vogt et al.

(2010). Cases with histopathological findings of viral, parasitic and fungal agents, trauma and gunshot wounds, and primary tumour focus or metastasis were not evaluated as study materials.

Real-time PCR analysis

Sections from paraffin blocks of bronchopneumonia were taken into sterile eppendorf tubes. DNA isolation was performed using the commercial isolation kit (QIAamp® DNA FFPE Tissue Kit, Cat. No: 56404) following the instructions of the manufacturer. The isolated DNAs were stored at -20°C for use in Real-time PCR analyses. DNA copies of *Bordetella bronchiseptica*, *Mycoplasma felis*, *Staphylococcus aureus* and *Chlamydia felis* were investigated using the QIAGEN Rotor-Gene Q Real-time PCR device, using primers prepared by a private company in accordance with the instructions of the manufacturer. Deionised water was used as the negative control. The primer sequences used in Real-time PCR analysis are given in Table 1.

Table 1. *Primer sequences used in the study.*

Agents	Primer sequences
Mycoplasma felis	F: 5′-AGAAGCACTTGCGGGAGATA-3′
	R: 5′-CAACGATACGAGGAACACCA-3′
Staphylococcus aureus	F: 5′-GAT GAC CAA TAT TCT GGA TGG-3′
	R: 5′-TTA GAA CAG CAT CTC AAT GTG-3′
Bordetella bronchiseptica	F: 5′-CCCCCGCACATTTCCGAACTTC-3′
	R: 5′-AGGCTCCCAAGAGAGAAAGGCTT-3′
Chlamydia felis	F: 5′-GGCTGAAAGATGAGCTCGAGAG -3′
	R: 5′-TCTCAAAGCACAGCGGACTG-3′

F: Forward, R: Reverse

RESULTS

Of the cats with microscopically detected bronchopneumonia, 9 were female and 12 were male. Seven of these cases were under six months old (kitten), eight were between seven months and two years old (junior), two were between three and six years old (prime), three were between six and ten years old (mature), and one of them was 12 years old (senior). It was determined that 10 of the lung samples examined in the study belonged to cats staying in a shelter, 8 to stray cats brought by municipal authorities or animal lovers, and 3 to owned cats. The agents amplified by Real-time PCR from the lungs with microscopically detected bronchopneumonia and the distribution of these according to age and gender are given in Table 2.

Table 2. *Distribution of Bordetella bronchiseptica, Mycoplasma felis, Staphylococcus aureus and Chlamydia felis in lung tissues with bronchopneumonia by Real-time PCR according to age and gender.*

Real-time PCR result	N (21)	Male	Female	Kitten	Junior	Prime	Mature	Senior
Bordetella bronchiseptica	3	1	2	2			1	
Mycoplasma felis	2	1	1		2			
Staphylococcus aureus	5	4	1	1	2		1	1
Chlamydia felis	4	2	2	1	2	1		
Mycoplasma felis* + *Staphylococcus aureus	1	1		1				
Staphylococcus aureus* + *Chlamydia felis	1	1		1				
Samples where the agent could not be determined by Real-time PCR	5	2	3	1	2	1	1	

Macroscopic and microscopic results

Macroscopic findings of the lungs examined in the study are presented in Table 3. PMNL infiltration in the bronchi, bronchiole lumens and interstitium of the lung was observed microscopically in 3 of the lungs where *Bordetella bronchiseptica* was detected; while in 3 of them, alveolar, bronchi and bronchiolar epithelial desquamation was observed, MCI around the bronchi and bronchioles in 2 cases. Oedama in the alveoli and multifocal areas of necrosis scattered throughout the lung parenchyma were noted in 2 of these lungs, and pleuritis was noted in 1 of them (Figure 1-A).

Microscopically, PMNL infiltration in the bronchi and bronchiole lumens was observed in 1 of the lungs where *Chlamydia felis* was detected, MCI around the bronchi and bronchiole and in the lung interstitium in 2 cats, desquamation in the bronchi and bronchiolar epitheliums in 4 cats, and alveolar epithelial desquamation and oedama in 2 cats. Additionally, findings of interstitial pneumonia were detected in 1 case (Figure 1-B-D).

Microscopically, PMNL infiltration in the bronchi, bronchioles and alveolar lumens was observed in 2 of the lungs where *Mycoplasma felis* was detected, and bronchiectasis and desquamation of the bronchi and bronchiole epithelium were observed in 3 of them. MCI located around the bronchi and bronchioles were noted in 3 of these lungs. Pleuritis was detected in 3 of the lungs (Figure 1-C).

In the lungs where *Staphylococcus aureus* was detected, microscopically, it was determined that in 5 of them, the bronchi, bronchiole and alveoli lumens were filled with exudate, mostly consisting of neutrophil leukocytes, and there was desquamation in the bronchi, bronchiole and alveoli epithelium. PMNL infiltrates showing abscess formation scattered throughout the interstitium were noted in 5 cats, and pleuritis in 2 cats (Figure 1-C).

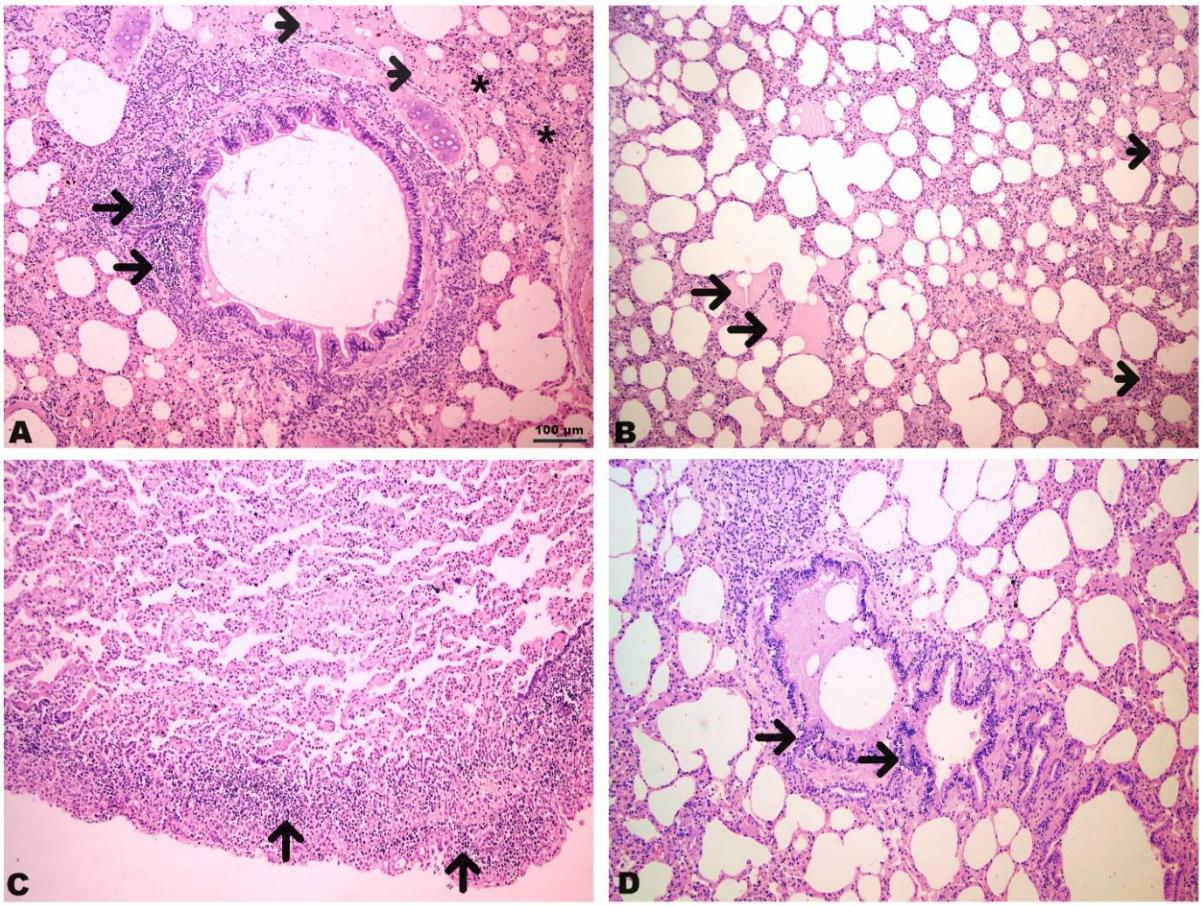

Figure 1. *Microscopic view of bronchopneumonia lung tissues, Hematoxylin&Eosin, x200.* ***A.*** *MCI around the bronchi (arrows), PMNL infiltration in the interstitium (stars) and alveolar oedama (arrowheads) in a case with Bordetella bronchiseptica.* ***B.*** *Alveolar oedama (arrows) and MCI (arrowheads) in the interalveolar septum in a case in which Chlamydia felis was detected.* ***C.*** *Microscopic appearance of pleuritis (arrows) in a case in which Mycoplasma felis and Staphylococcus aureus were detected.* ***D.*** *Peribronchiolar MCI (arrows) in the sample with Chlamydia felis.*

Table 3. *Macroscopic findings of lungs with bronchopneumonia*.

MACROSCOPIC FINDINGS	Real-time PCR result			
	Bordetella bronchiseptica	***Mycoplasma felis***	***Staphylococcus aureus***	***Chlamydia felis***
	(N=3)	**(N=3)**	**(N=5)**	**(N=5)**
Red, viscous areas in the lungs	**3**	**3**	**2**	**1**
The cross-sectional surface of the lung is swollen and wet.	**3**	**3**	**2**	**1**
Foamy content in trachea and bronchi	**3**	**1**	**4**	**4**
Fluid in the chest cavity	**-**	**2**	**1**	**-**

Real-time PCR results

Although bronchopneumonia was detected microscopically in the analyses performed with Real-time PCR, amplification could not be obtained with the primers used in five lung tissues. In cats where amplification was determined with primer sequences belonging to more than one agent in Real-time PCR analyses, the agent with the lowest "Cycle Threshold (CT)" value was accepted as the causative of bronchopneumonia. The detection status of DNA copies of *Bordetella bronchiseptica*, *Mycoplasma felis*, *Staphylococcus aureus* and *Chlamydia felis* by Real-time PCR from paraffin blocks of lungs with bronchopneumonia and their CT values are given in Table 4.

Table 4. *Determination status and CT values of Bordetella bronchiseptica, Mycoplasma felis, Staphylococcus aureus and Chlamydia felis DNA copies by Real-time PCR.*

NO	CT VALUES DETERMINED BY REAL TIME PCR			
	Bordetella bronchiseptica	***Mycoplasma felis***	***Staphylococcus aureus***	***Chlamydia felis***
1			28	
2*				
3	22			
4			24	
5				28
6	28			
7*				
8			28	
9			28	
10		30		
11				30
12		28		
13				24
14*				
15		24	27	
16*				
17				28
18*				
19			28	24
20			22	
21	28			
TP	3	2+1**	5	4+1**

* Samples where amplification could not be determined with the primers used in the study, ** In polymicrobial cases, the agent with a lower CT value was considered as the agent causing bronchopneumonia.

DISCUSSION

Determining the aetiology of respiratory system diseases in cats is one of the important problems encountered in veterinary clinics (Dear, 2020; Slaviero et al., 2021; Tural and Tuzcu, 2023). In this study, agents such as *Bordetella bronchiseptica, Mycoplasma felis, Staphylococcus aureus* and *Chlamydia felis* from paraffin blocks of cat lungs with bronchopneumonia were determined by Real-time PCR and their pathological findings were compared.

Identification of the causative agent is necessary to determine the aetiology of bacterial pneumonia in cats. However, usually, the success of identification decreases due to factors such as taking samples for bacteriological examinations, contamination during transport to the laboratory, and empirical antibiotic use (Garcia et al., 2015; Murray, 2015; Bonnet et al., 2020). In these cases, methods based on amplification of the genomic DNA of the agent appear to be an important option in determining the appropriate treatment. Real-time PCR has become an important diagnostic method in the field of veterinary medicine in recent years due to its rapid results, ability to detect microorganisms such as *Mycoplasma* and *Chlamydia* that are difficult to detect with conventional methods, and its high sensitivity (Hyeran et al., 2006; Pantchev et al., 2010; Sibitz et al., 2011; Tuzcu et al., 2021; Tuzcu et al., 2022; Akçakavak et al., 2023; Tuzcu et al., 2023).

There are reports that outline that bronchopneumonia in cats can occur at any age and that there is no preference of breed or gender (Tural and Tuzcu, 2023). Slaviero et al. (2021) in their study evaluating 1749 cat necropsies found that the cause of death was pneumonia in 78 of the cats. Of these cats, 14 were kittens (17.9%), 19 juniors (24.4%), 13 primes (16.7%), 14 matures (17.9%), 8 seniors (10.3%) and 5 geriatric (6.4%). They also reported the absence of age information in five cases (6.4%). In the current study, 7 of the cats with bronchopneumonia were younger than 6 months old (kitten) (33.3%), 8 were between 7 and 24 months old (junior) (38.09%), 2 were between 3 and 6 years old (prime) (9.52%), 3 were determined to be samples of cats between the ages of 6 and 10 (mature) (14.28%), and 1 of them was determined to be from a 12-year-old cat (senior) (21%). These results are compatible with the literature.

In retrospective studies aimed at determining the cause of death in cats, the mortality rate due to pneumonia has been reported to be between 1% and 6.5% (Egenvall et al., 2009; Togni et al., 2018; Slaviero et al., 2021). In this study, bronchopneumonia was detected in 21 of 78 cats necropsied. The determined bronchopneumonia rate is high compared to the literature. This may be related to the fact that the majority (18/21) of the cats included in the study were stray and shelter cats. Additionally, this study found that the rate of bronchopneumonia in male cats (12/9) was higher than in females. This is consistent with the findings of Foster et al. (2004) study, in which they reported that the prevalence of pneumonia in male cats was 2.4 times higher than in females.

In the current study, macroscopic findings recorded in cats with microscopically diagnosed bronchopneumonia were as follows: red, sunken, viscous areas in the lungs, the cross-sectional surface of the lungs appearing flooded and moist, a foamy content in the trachea and bronchi, and the presence of fluid in the chest cavity. These macroscopic findings were signs of bronchopneumonia, not specific to the agent.

Lappin et al. (2017), in their study on respiratory system diseases of cats, reported that the bacterial agents determined by PCR were *Mycoplasma* species (62.5%), *Bordetella* species (47.5%), *Staphylococcus* species (12.5%) and *Streptococcus* species (10%). Dear (2020), in a study examining pneumonia seen in cats and dogs, reported, based on literature data (Foster et al., 2004; Radhakrishnan et al., 2007; Proulx et al., 2014), that 22-71% of pneumonia in cats was caused by *Bordetella bronchiseptica*, 2-25% by *Klebsiella pneumoniae*, 30-70% by *Mycoplasma spp*. and 6-21% by *Streptococcus spp*. In this study, the data obtained by Real-time PCR are 3/21 (14.29%) for *Bordetella bronchiseptica*, 5/21 (23.8%) for *Staphylococcus aureus*, 3/21 (14.29%) for *Mycoplasma felis*, and 5/21 (23.8%) for *Chlamydia felis*. It has been determined that the rates found are compatible with literature data. In addition, in this study, polymicrobial infections were detected in 2 (9.5%) of the adult cats with respiratory tract infections, as reported in previous (Dear, 2014).

In this study, *Staphylococcus aureus* genomic DNA was detected by Real-time PCR in 7 of the cats examined. Researchers have reported that *Staphylococcus spp*. causes lung abscess, suppurative bronchopneumonia and pyothorax (Maxie, 2015; Reinero and Lee-Fowler, 2021; Sim et al., 2021). It has been reported that microscopically, neutrophil leukocyte infiltrations and desquamated epithelial cells in the alveoli, bronchi and bronchiolar lumens, as well as hyperemia and oedama, in cats with *Staphylococcus* spp. detected. It has also been reported that small abscesses are formed in the lung, most of which are surrounded by a fibrous capsule (Çiftçi et al., 2021; Sim et al., 2021; Slaviero et al., 2021; Chauhan et al., 2024). In this study, the fact that suppurative

pneumonia was detected in 5 of the cats in which *Staphylococcus aureus* was detected by Real-time PCR is consistent with the literature.

Present study, genomic DNA belonging to *Bordetella bronchiseptica* was amplified in 3 of the lung samples examined by Real-Time PCR. In this study, bacteria could not be detected microscopically in cilia. The fact that genomic DNAs belonging to *Bordetella bronchiseptica* were amplified by Real-Time PCR in these cases reveals that the diagnosis should be confirmed by PCR.

There are studies associating tracheobronchitis and pneumonia with *B. bronchiseptica* in cats (Bemis, 1992; Binns et al., 1999; Foster et al., 2004). *B. bronchiseptica* can cause fatal bronchopneumonia in kittens and, microscopically, neutrophils are detected in the bronchi lumen (Songer and Post, 2004; Maxie, 2015; Chauhan et al., 2024). *B. bronchicepta* causes catarrhal tracheitis and bronchitis, as well as catarrhal-purulent bronchopneumonia (Ettinger and Feldman, 2010). In this study, findings such as PMNL infiltration in the bronchus and bronchiolar lumens and interstitium of the lung, desquamation in the bronchi, bronchiolar and alveolar epithelium, and oedama in the alveoli, determined microscopically in the lung tissues where *Bordetella bronchiseptica* was detected, were found to be compatible with the literature.

Although it has not been precisely determined whether *Mycoplasma spp*. are the primary pathogen or an opportunistic pathogen in cats, there are reports that *M. felis* is the causative agent of fibrinous pneumonia in cats (Songer and Post, 2004; Çiftçi et al., 2021; Shil et al., 2022). There are reports that serous exudate, neutrophil leukocyte infiltration and desquamated alveolar epithelium are microscopically detected in the alveolar lumens in *Mycoplasma* pneumonia and that an exudate consisting of abundant neutrophil leukocytes, desquamated epithelium and mucus is found in the bronchi and bronchiolar lumens (Ettinger and Feldman, 2010; Swennes and Fox, 2014; Reinero and Lee-Fowler, 2021; Chauhan et al., 2024). In this study, the microscopic findings determined in the lungs in which *M. felis* was amplified by Real-time PCR were similar.

Chlamydia felis is endemic among domestic cats worldwide and is known to cause conjunctivitis, rhinitis and pneumonia (Browning, 2004; Songer and Post, 2004; Wasissa et al., 2021). It causes serous, catarrhal, and sometimes purulent inflammation in the upper respiratory tract, conjunctivitis, and interstitial pneumonia, which is its most important lesion (Kartashov et al., 2019; Schmal-Filius et al., 2020; Çiftçi et al., 2021; Wasissa et al., 2021). In this study, interstitial pneumonia was detected in 1 of the cases in which *Chlamydia felis* was detected, a finding which was similar to the literature.

CONCLUSION

As a result, in this study, *Bordetella bronchiseptica, Staphylococcus aureus, Mycoplasma felis* and *Chlamydia felis* agents were detected at rates of 14.29%, 23.8%, 14.29% and 23.8%, respectively, by Real-time PCR. Our results show that relevant agents can often be isolated in cases of feline bronchopneumonia, demonstrating the importance of polymicrobial agents in cases of feline bronchopneumonia and indicating that more than one bacterial species should be investigated.

AUTHOR CONTRİBUTİON

All authors contributed equally.

ETHICAL STATEMENT

Scientific rules, ethics and citation rules were followed during the writing process of the study titled "**A molecular and histopathological study on bronchopneumonia in cats**"; There was no tampering with the data collected and this study was not sent to any other academic publication environment for evaluation. The necessary ethics committee permissions were obtained by SUVDAMEK at its meeting dated 29.02.2024 and numbered 2024/35.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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