Short Communication

Molecular detection of *Mycoplasma* spp. and its associated risk factors in domestic cats in the city of Ibagué, Tolima

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Abstract

Feline hemoplasmosis is a disease with worldwide distribution, mainly in tropical countries, where its transmission is favored by the presence of vectors such as fleas and ticks. In Colombia, the diagnosis of the disease in small animals has been limited to the identification of the pathogen in blood smears, which have low sensitivity and specificity, demanding methods of higher diagnostic value, such as those based on molecular techniques. The objective of the present study was to investigate the prevalence of *Mycoplasma* spp. in felines from the city of Ibagué using the PCR and the associated risk factors. Briefly, blood samples were taken from cats (n=150), DNA was extracted, and *Mycoplasma* spp. DNA was detected by PCR. Additionally, a survey was carried out on the owners to determine possible risk factors associated with the presence of the pathogen. The study revealed a prevalence of 16.6% of cats positive for *Mycoplasma* spp. The results showed a higher incidence in cats older than one year compared to males and a greater predisposition when the cats were positive for FIV and FeLV. It is noteworthy that most of the cats have outdoor access, do not have a vaccination for FeLV, or have not carried out diagnostic tests for viral diseases, which may underestimate the health status of the cats. This first epidemiological report in the city of Ibague highlights the demand for molecular diagnosis for hemotropic pathogens and includes hemoplasma in the differential diagnosis scheme for cats.

Keywords: PCR, prevalence, *Mycoplasma* spp., feline

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Received June 27, 2024
Accepted August 18, 2024

Introduction

Feline hemoplasmosis is caused by hemotropic mycoplasmas; it is considered an emerging zoonotic disease that should be considered when observing a fever of unknown origin or anemia in a cat (Tasker, 2018). As with other hemotropic infections, vectors play an important role in their transmission and tend to be abundant in tropical and subtropical countries (Do *et al*., 2020).

Clinical signs include anemia, jaundice, paleness, anorexia, and weakness (Díaz-Regañón *et al*., 2018). As a treatment, antibiotic therapy protocols reduce hemoplasma loads in the blood and improve the clinical outcome; however, the infection tends to be persistent in asymptomatic carrier animals (Tasker, 2022).

Diagnosis has been limited to demonstrating structures compatible with *Mycoplasma* spp. in blood smears, showing low specificity and sensitivity (Caballero *et al*., 2022). However, molecular diagnostic techniques have been described, including PCR and real-time PCR (Melo *et al*., 2023).

In Colombia, there is no national data available; however, prevalences as high as 80% have been reported in some regions (Caballero *et al*., 2022). Hemoplasma can only survive by parasitizing red blood cells, limiting the *in vitro* culture and the investigation of their epidemiology and pathogenesis (do Nascimento *et al*., 2012). Additionally, Willi *et al*. (2009) reported a case of feline hemoplasma molecularly detected in a human, supporting the zoonotic potential of these agents. The aim of this study is to describe the prevalence of *Mycoplasma* spp. in the city of Ibagué, Tolima (Colombia), and the risk factors associated with its presence in domestic cats.

Materials and Methods

The study was conducted in the city of Ibagué, Tolima (Colombia), with a sample size estimated using the formula described by Thrusfield & Christley (2018) based on an expected prevalence for *Mycoplasma* spp. of 80% (Caballero *et al*., 2022). For this study, 150 blood samples were collected by puncture of the cephalic vein from cats, with EDTA as an anticoagulant. The owners signed an informed consent form and completed an epidemiological survey regarding demographic factors, environmental factors, and lifestyle based on the study carried out by AVEPA (2006). Furthermore, information about the vaccination or serological test for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) was also requested.

DNA was extracted by PCI method, and its quality was assessed by spectrophotometry (Nano500, Allsheng, China) and an endpoint PCR amplifying *actb* gene using a specific primer set (F-GGCTACAGCTTCACCACCAC; R-TACTCCTGCTTG CTGATCCACA) in a ProFlex PCR System (Applied Biosystems™, MA, USA). For the specific detection of *Mycoplasma* spp., a set of specific primers for *16S rRNA* were used (F-ACGAAAGTCTGATGGAGCAATA; R-ACGCCCAATAAATCCGCATAAT) following protocols previously described (Mayorga *et al*., 2019).

Data were analyzed using descriptive statistics, and the survey information was used to assess the association between the presence of *Mycoplasma* spp. and the epidemiological data by using the Chi-square test, establishing odds ratio values accordingly. A p-value ≤ 0.05 was considered statistically significant for all cases. The data were processed using Epi Info 7.2.6.0 software (CDC, USA).

Result and Discussion

Among sampled individuals, no cats showed severe clinical condition or death. Twenty-five out of 150 cats were positive for *Mycoplasma* spp. DNA corresponding to 16.6% was detected by amplification of the *16S rRNA* gene (Figure 1), which was the first population report for this pathogen in the city of Ibagué. Nevertheless, Mayorga *et al*. (2019) reported the presence of *Mycoplasma haemominutum* as the predominant species through molecular identification in a case series, which established a correlation with seroreactivity and co-infection with viral pathogens.

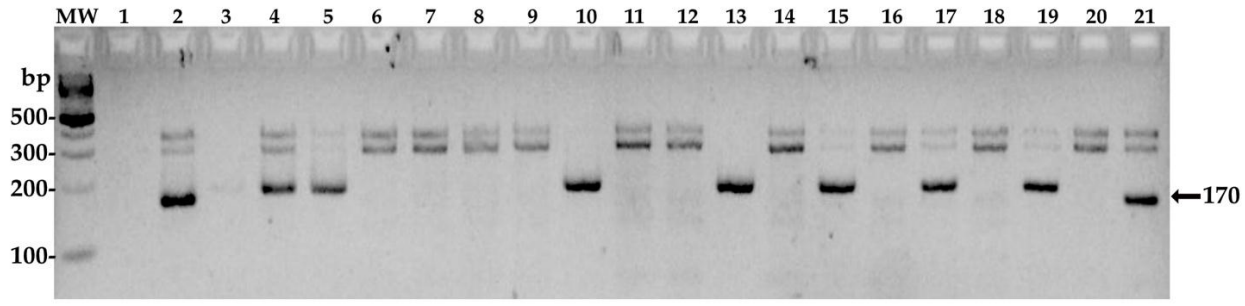

Figure 1 Agarose gel electrophoresis of 170 bp amplicon of *16S rRNA* from *Mycoplasma* spp. from domestic felines. MW: Molecular weight marker, 100bp DNA ladder (New England Biolabs, USA). Lane 1 negative control, Lanes 2 - 21: representative samples.

In Colombia, there are few epidemiological reports about *Mycoplasma* infection in small animals, being common individual case reports or case series (Mayorga *et al*., 2019), which may underestimate the importance of the infection. The prevalence of 16.6% in our study is lower than reported in Colombian cities (>80%) by Caballero *et al.* (2022) and Echeverri-De la Hoz *et al.* (2022). However, it is close to Martínez *et al.*

(2018) in Medellín (Antioquia) with 27.8% as well as worldwide reports as Demkin & Kazakov (2021) who reported 13.8 % of prevalence in cats from Moscow (Russia), 12.2% in the east region of Germany (Schäfer *et al*., 2023), Álvarez-Fernández *et al.* (2022) reported 14% in Spain, in Italy from 11.6 % to 18.9% (Latrofa *et al*., 2020; Ravagnan *et al*., 2017), in countries from east and southeast of Asia (Indonesia, Malaysia, Philippines, Taiwan and Thailand) of 16.13% (Zarea *et al*., 2022), specifically in Thailand from 9.6% to 38.05% (Do *et al*., 2020; Luong *et al*., 2023), in USA from 15 to 18% (Manvell *et al*., 2021), in Chile of 13.2% (Walker-Vergara *et al*., 2016) and in Brazil of 13.28% (Melo *et al*., 2023), which support *Mycoplasma* spp. as a cosmopolitan pathogen.

From the sampled population, females showed a prevalence of 8.33% (5/60), and males had a prevalence of 22.2% (20/90). Regarding the sex, females showed less probability (p=0.04) of being infected compared with males (Table 1), which can be explained by the transmission of the hemoplasma through aggressive behavior, which is more common in male cats with outdoor access, with higher probabilities of fights and bites (Zhang *et al*., 2021; Willi *et al*., 2010), in addition, to the higher risk of infestation with ticks and fleas, which can be vectors of *Mycoplasma* spp. as described by Razgūnaitė *et al.* (2024). However, recently, Moore *et al*. (2024) described that the detection of *Mycoplasma* in these vectors could be affected by sample processing and primer design and may overestimate the detection because of the lack of data depuration, and *Spiroplasma* spp. has been detected instead of hemoplasma species.

In addition, a significant correlation (p=0.028) between age and the presence of *Mycoplasma* spp. was found, with cats older than 1 year more predisposed to the infection (Table 1). These findings agree with previous reports (Do *et al*., 2020; Tasker *et al*., 2018) and may be the result of an accumulative risk of exposure since *Mycoplasma* spp. often persist in the organism after infection.

Furthermore, a high predisposition was found in animals positive to FeLV (p=0.04) and feline immunodeficiency (FIV) (p=0.0032) to be positive for *Mycoplasma* spp. which agrees with Ravagnan *et al.* (2017) and Marcondes *et al.* (2018).

Table 1 Risk factors associated to the presence of *Mycoplasma* spp. in domestic felines in the city of Ibagué*

	Odds ratio	IC 95%	*p*
Age	**3.73**	1.21-11.54	**0.028**
Sex	**0.31**	0.11-0.90	**0.04**
Positive FeLV	**3.77**	0.98-14.53	**0.04**
Positive FIV	**7.74**	1.61-37.11	**0.015**
Test FeLV-FIV	1.98	0.76-5.12	0.24
Vaccination feline 3	0.26	0.03-2.08	0.311
Vaccination FeLV	5.16	0.31-85.48	0.75
Live with more cats	1.09	0.39-3.00	1.0
Outdoor Access	1.97	0.68-5.57	0.20

*Values with significant association are bolded.

In this study, no correlation was found between vaccination status, living with more cats, or outdoor access with the presence of the bacteria (Table 1). However, 69.33% of the cat´s owners reported that pets have outdoor access, 98.67% did not report vaccination against FeLV, and 78.67 % did not check the health status for viral pathogens by ELISA or PCR, which may imply an underdiagnosis or undernotification. These results demand the establishment of informative campaigns for pet owners about animal care and zoonosis (Tasker, 2022).

Recently, families tend to have several pets, including cats, without caring about health status, including vaccination and regular attention by veterinary clinicians, which allow the animals to get infected, recover, and become a carrier state. In the city of Ibague, there are a significant number of free-roaming cats, which demand politics to preserve their wellness.

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