



Mass spectrometry-based identification of 26 *Pasteurella* species and *in vitro* antimicrobial susceptibility pattern of isolates recovered from diseased domestic cats¹

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ABSTRACT.- Rodrigues C.A., Arabe-Filho M.F., Bello T.S., Portilho F.V.R., Listoni F.J.P., Possebon F.S., Paz P.Jr.L., Eliam P.C.L. & Ribeiro M.G. 2025. **Mass spectrometry-based identification of 26 *Pasteurella* species and *in vitro* antimicrobial susceptibility pattern of isolates recovered from diseased domestic cats.** *Pesquisa Veterinária Brasileira* 45:e07540, 2025. Departamento de Produção Animal e Medicina Veterinária Preventiva, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu, SP 18818-681, Brazil. E-mail: marcio.ribeiro@unesp.br

Pasteurella species are well-known opportunistic bacteria that inhabit the microbiota of the oral cavity and upper respiratory tract of cats and have been related to a set of pet-associated diseases in addition to humans. Most studies involving feline pasteurellosis have been described as case reports and species identification based on classic phenotypic methods. In turn, a lack of comprehensive studies involving a great number of cats with pasteurellosis has been described, especially where diagnosis at the species level has been performed by molecular-based methods. In this scenario, we investigated the molecular identification of *Pasteurella* species isolated from 26 diseased domestic cats (*i.e.*, cutaneous abscesses, pneumonia, conjunctivitis, open wounds, urinary tract infections, pleural effusion, pyometra, and infection secondary to neoplasia) based on proteomic diagnosis, using mass spectrometry (matrix-assisted laser desorption ionization time-of-flight mass spectrometry – MALDI-TOF MS). The *in vitro* antimicrobial susceptibility patterns of isolates and selected epidemiological data (with emphasis on the outcome) were assessed as well. MALDI-TOF MS identified predominantly *P. multocida* (23/26=88.5%), followed by *P. dagmatis* (2/26=7.7%) and *P. canis* (1/26=3.8%). The isolates revealed 100% susceptibility to beta-lactams (amoxicillin/clavulanic acid, ampicillin, cephalixin, ceftriaxone), tetracyclines (tetracycline, doxycycline) and fluoroquinolones (ciprofloxacin, levofloxacin, marbofloxacin) groups of antimicrobials. Conversely, the highest resistance of the isolates was observed for amikacin (10/26=38%). Data on outcomes were available for 61% (16/26) of cats, of which 50% (8/16) died or were subjected to euthanasia due to severe complications (*e.g.*, sepsis, pneumonia, and pleural effusion) secondary to disseminated/systemic infections, although no significant association was observed between *Pasteurella* species and the clinical-epidemiological findings studied. Our results contribute to the molecular identification of *Pasteurella* species and vigilance of multidrug-resistant bacteria that infect cats. Also, it highlights the need for the early diagnosis and therapy of feline pasteurellosis due to high mortality rates.

INDEX TERMS: Feline pasteurellosis, *Pasteurella multocida*, MALDI-TOF MS, multidrug-resistant bacteria.

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RESUMO.- [Identificação baseada em espectrometria de massas de 26 espécies de *Pasteurella* e perfil de sensibilidade microbiana *in vitro* de isolados obtidos de gatos domésticos com diferentes manifestações clínicas.] As espécies de *Pasteurella* são bactérias oportunistas que

habitam a microbiota da cavidade oral e do trato respiratório superior de gatos, relacionadas a vários sinais clínicos em animais de companhia e humanos. A maioria dos estudos envolvendo pasteurelose felina têm sido descritos como relatos de casos, e a identificação de espécies baseada em métodos clássicos de classificação fenotípica. No entanto, número restrito de estudos têm focado grande número de gatos com pasteurelose, nos quais métodos moleculares tenham sido utilizados para o diagnóstico dos patógenos em nível da espécie. Neste cenário, foi investigada a identificação molecular de espécies de *Pasteurella* isoladas de 26 gatos domésticos com diferentes manifestações clínicas (e.g., abscessos cutâneos, pneumonia, conjuntivite, feridas, infecções do trato urinário, derrame pleural, piometra e infecção secundária à neoplasia), com base no diagnóstico por proteômica, utilizando a espectrometria de massas (*Matrix-assisted laser desorption ionization time-of-flight mass spectrometry* – MALDI-TOF MS). O perfil de sensibilidade microbiana *in vitro* dos isolados e dados epidemiológicos dos animais (com ênfase no desfecho dos casos) também foram avaliados. MALDI-TOF MS identificou predominantemente *P. multocida* (23/26=88,5%), seguido por *P. dagmatis* (2/26=7,7%) e *P. canis* (1/26=3,8%). Os isolados revelaram 100% de sensibilidade aos grupos de antimicrobianos beta-lactâmicos (amoxicilina/ácido clavulânico, ampicilina, cefalexina, ceftriaxona), tetraciclina (tetraciclina, doxiciclina) e fluoroquinolonas (ciprofloxacino, levofloxacino, marbofloxacino). Por outro lado, a maior resistência dos isolados foi observada para a amicacina (10/26=38%). Os dados de desfecho estavam disponíveis para 61% (16/26) dos gatos, dos quais 50% (8/16) morreram ou foram submetidos à eutanásia devido a graves complicações secundárias a infecções disseminadas/sistêmicas, embora nenhuma associação estatística tenha sido observada entre as espécies de *Pasteurella* e os achados clínico-epidemiológicos estudados. Os resultados do presente estudo contribuem para a identificação molecular de espécies de *Pasteurella* e para a vigilância de bactérias multirresistentes que infectam gatos, indicando a necessidade de diagnóstico e tratamento precoces da pasteurelose felina devido às altas taxas de mortalidade.

TERMOS DE INDEXAÇÃO: Pasteurelose felina, *P. multocida*, MALDI-TOF MS, bactérias multirresistentes.

INTRODUCTION

It is roughly estimated that > 600 million cats live across the globe, of which over two-thirds (480 million) probably live as stray cats (Iapwa 2021). Domestic, free-ranging, or feral cats possess a habit of competing for food, for females during the reproductive period, or for the hierarchy of the environment, mainly among adult males (Stull 2022). It is common to experience skin infections secondary to bites and scratches, which can evolve into disseminated/systemic infections (De Cecco et al. 2021).

Pasteurella species are well-known bacteria that are opportunistic in nature, inhabit the oral cavity and upper respiratory tract of companions and livestock, and are related to a variety of clinical infections (Peng et al. 2019). It is estimated that the majority of cats can harbor *Pasteurella* species in the oral microbiota and may be potentially infectious for other animals (including cats). They are also the leading cause of

pet-associated infections in humans, secondary to bites and scratches (Bula-Rudas & Olcott 2018, Stull 2022).

Cutaneous-subcutaneous abscesses represent the most common clinical sign related to feline pasteurellosis. However, systemic spread of the pathogen is common, causing fever, nasal secretion, weight loss, organ abscesses, ocular and urinary tract infections, pneumonia, peritonitis, pleural effusions, and encephalitis, usually with a poor prognosis (Lloret et al. 2013, Stull 2022).

The routine diagnosis of pasteurellosis in cats has been based on clinical-epidemiological findings, hematological and biochemical tests, imaging, bacteriological culture and phenotypic identification, and *in vitro* susceptibility tests (Stull 2022). Cephalosporins, fluoroquinolones, tetracyclines, and amoxicillin/clavulanic acid have been the main groups of antimicrobials used in the therapy of infections caused by *Pasteurella* species in cats (Lloret et al. 2013, Stull 2022).

In humans, *Pasteurella* species represent one of the most common bacteria causing cutaneous (local) and disseminated lesions secondary to cat bites and scratches (Mirzai et al. 2019, Piorunek et al. 2023). In addition, thin teeth of cats may develop deep perforating injuries in the tissues secondary to bites, with inoculation of saliva containing *Pasteurella* species, which probably has been underestimated due to the minimal appearance of the lesions (Kheiran et al. 2019).

Despite the severity of systemic infections by *Pasteurella* species in cats and guarded prognosis, most studies involving feline pasteurellosis have been described as case reports (Dolieslager et al. 2011, Lloret et al. 2013) and/or identification of species based on traditional bacteriological (phenotypic) tests (Wang et al. 2009, Awosile et al. 2018). Considering this scenario, we investigated the identification of *Pasteurella* at the species level using mass spectrometry and the *in vitro* susceptibility pattern of isolates recovered from a variety of clinical infections in 26 diseased domestic cats, as well as selected epidemiological findings, with emphasis on the outcome.

MATERIALS AND METHODS

Ethics approval. This study was conducted under the Ethics Committee on Animal Use (CEUA) guidelines of the “Faculdade de Medicina Veterinária e Zootecnia” (School of Veterinary Medicine and Animal Science – FMVZ), “Universidade Estadual Paulista ‘Júlio de Mesquita Filho’” (Unesp), Botucatu/SP, Brazil (protocol number 0224/2021).

Animals and *Pasteurella* isolates. A convenient sampling of *Pasteurella* isolates obtained from diseased cats from the routine of a Teaching Veterinary Hospital and the “Laboratório de Diagnóstico Microbiológico” (Microbiological Diagnostic Laboratory) at FMVZ-Unesp, Botucatu/SP, Brazil, over 2019 and 2023, were used. The different clinical specimens were collected after rigorous antisepsis, kept refrigerated (4 to 8 °C), and immediately sent for microbiological culture in the aforementioned laboratory.

Bacteriological culture. A variety of clinical specimens (transtracheal wash, urine, organ fragments, and secretion from abscesses, ocular conjunctiva, pleural effusion, uterus, and wounds) were simultaneously cultured under aerobic conditions on bovine blood agar media (Oxoid™, São Paulo, Brazil) and selective MacConkey media (Oxoid™, São Paulo, Brazil), incubated at 37 °C, and kept for 72 hours. The isolated microorganisms were initially identified based on traditional morphotintorial and biochemical aspects

(Quinn et al. 2011). Colonies compatible with *Pasteurella* species were subsequently submitted for identification at the species level using mass spectrometry. Clinical infections by *Pasteurella* species were considered > 10 colony-forming units (CFU) of isolates.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Isolates compatible with *Pasteurella* species in bacteriological culture were subjected to mass spectrometry for diagnostic confirmation. The MALDI-TOF MS technique (Bruker and Daltonics™, Bremen, Germany) was used, following the manufacturer's recommendations. Three to four freshly isolated colonies (24 to 48 h) on blood agar were subjected to extraction with 20-40 µL of formic acid (70%) and centrifuged, aiming at bacterial lysis and release of proteins and formation of ions necessary for the formation of bacterial spectra. Approximately 15 minutes later, 20-40 µL of acetomil (100% P.A.) in the same proportion as formic acid (1:1) was added to each sample and centrifuged. Then, 1 µL of the solution for each sample was added to specific plates containing 96 wells (Bruker and Daltonics™, Bremen, Germany) and kept for approximately 20 minutes to dry at room temperature. The well with the dried samples was covered with 1 µL of matrix solution (2-cyano-4-hydroxycinnamic acid diluted with 50% acetonitrile and 2.5% trifluoroacetic acid). The plates were placed in the receptacle of the MALDI-TOF MS equipment (Bruker and Daltonics™, Bremen, Germany), operated with a 337-nm laser. Spectral data were analyzed between 2,000-20,000 m/z using FlexControl 3.3 software. Identifying microorganisms at the genus and species level was considered for isolates with spectra ≥ 1.7 and ≥ 2.0 , respectively (Gonçalves et al. 2014).

***In vitro* susceptibility test.** All isolates were subjected to *in vitro* susceptibility testing (disk diffusion method), according to the Clinical Laboratory Standards Institute (CLSI 2023, 2024) guidelines, using 11 antimicrobials belonging to five groups, predominantly indicated for the therapy of feline pasteurellosis, as follows: 1) aminoglycosides (amikacin, 30 µg), 2) beta-lactams and derivatives (amoxicillin/clavulanic acid, 30 µg; ampicillin, 10 µg; cephalixin, 30 µg; ceftriaxone, 30 µg), 3) fluoroquinolones (ciprofloxacin, 5 µg; levofloxacin 5 µg; marbofloxacin, 5 µg; norfloxacin, 10 µg), 4) macrolides (azithromycin, 15 µg), and 5) tetracyclines (doxycycline, 30 µg). Isolates resistant to ≥ 3 antimicrobials from different classes were considered multidrug-resistant ones (Magiorakos et al. 2012).

Epidemiological data. Selected epidemiological data, *i.e.*, breed, gender, outcome (cure or death), and breeding conditions (restricted indoors, semi-domesticated, stray or feral animals), were assessed. The history of recent diagnosis (< 30 days) of immunosuppressive retrovirus (feline immunodeficiency virus – FIV and feline leukemia virus – FeLV) was investigated among studied animals. The periods (seasons) of the year (considered in Brazil) in which cases occurred were also assessed as follows: spring (September to November), summer (December to February), autumn (March to May) and winter (June to August). The age of the sampled animals was stratified as follows: < 1 year of age, 1 to ≤ 5 years, > 5 to ≤ 10 years, and > 10 years [20], considered young, young adults, adults, and elderly, respectively.

Statistical analysis. Associations between categorical variables (sex and age of animals, seasons, access to the street, and outcome), different clinical infections and identification of *Pasteurella* species were evaluated based on the Chi-square (or Fisher's tests). The analyses were carried out using SAS OnDemand for Academics software (SAS Institute Inc., Cary/NC, USA), considering the level of significance results < 0.05.

RESULTS

Selected epidemiological data

Of the 26 cats sampled, 92.3% (24/26) did not have a defined breed. Data on age were available from 88.5% (23/26) of the cats sampled. The ages ranged from two months to 16 years (average of 5.5 years), and among cats with known age, 21.7% (5/23) were < 1 year old (young), 21.7% (5/23) were between one and ≤ 5 years old (young adults), 26.1% (6/23) were > 5 and ≤ 10 years old (adults), and 30.5% (7/23) were > 10 years old (elderly). Among eight animals studied that died, 62.5% (5/8) were > 10 years old (considered elderly).

Among all cats, 65.4% (17/26) were males and 34.6% (9/26) were females. In addition, 53.8% (14/26) had access to the street, 30.8% (8/26) did not have access to the street, and 15.4% (4/26) could not obtain this data. Of the 26 cats, 34.6% (9/26) were sampled in spring, 26.9% (7/26) in summer, 11.6% (3/26) in autumn, and 26.9% (7/26) in winter. Among the 26 cats studied, only 15.4% (4/26) had been recently tested (< 30 days) for FIV and FeLV, of which two males were reactive and, therefore, this information was not considered for further analyses.

Outcome

Data on outcomes were available in 61% (16/26) of animals. Of these animals, 50% (8/16) died or were subjected to euthanasia due to severe complications secondary to disseminated forms, including sepsis, pneumonia, and pleural effusion. In turn, among the remaining 50% (8/16) of animals with known outcomes, 75% (6/8 = 75%) recovered after treatment, mainly from skin lesions (or local infections).

Microbiological findings and mass spectrometry identification of *Pasteurella* species

Among the 26 cats sampled, rounded, mucoid, grayish, nonhemolytic colonies measuring ~1 mm in diameter were isolated between 24 and 48 hours in blood agar media (Fig.1), phenotypically compatible with *Pasteurella* species. All these isolates were subjected to Gram staining, revealing Gram-negative rods to coccobacillary bacteria with bipolar aspect tendency.

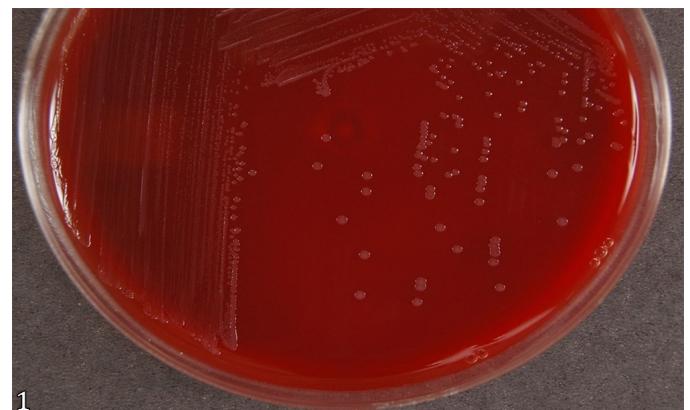


Fig.1. *Pasteurella multocida* isolated from a cat with pneumonia identified at the species level by mass spectrometry. Note rounded, mucoid, grayish, nonhemolytic colonies measuring ~1 mm in diameter isolated in blood agar media, after 48 hours of incubation, under aerobic conditions at 37 °C.

The isolates suggestive of *Pasteurella* species, subjected to mass spectrometry diagnosis, revealed a predominance of *P. multocida* (23/26 = 88.5%) identification, followed in a minor frequency by *P. dagmatis* (2/26 = 7.7%) and *P. canis* (1/26 = 3.8%) (Table 1).

In vitro antimicrobial susceptibility pattern

The isolates revealed 100% susceptibility to beta-lactam (amoxicillin/clavulanic acid, ampicillin, cephalexin, ceftriaxone), fluoroquinolones (ciprofloxacin, levofloxacin, marbofloxacin), and tetracyclines (tetracycline, doxycycline) groups of antimicrobials. In turn, the highest resistance of the isolates was observed for amikacin (10/26=38%) (Table 2). No multidrug-resistant isolates were identified.

Clinical signs

Abscesses (11/26 = 42.3%), pneumonia (3/26 = 11.5%), conjunctivitis (3/26 = 11.5%), open wounds (3/26 = 11.5%), urinary tract infections (2/26 = 7.7%), pleural effusion (2/26 = 7.7%), pyometra (1/26 = 3.9%), and infection secondary to neoplasia (1/26 = 3.9%) were the clinical signs observed among the 26 cats with identification of *Pasteurella* species (Table 1).

Statistical analysis

No significant association ($p > 0.05$) was observed between *Pasteurella* species and the clinical-epidemiological findings studied.

DISCUSSION

The population of domestic, free-ranging and feral cats has increased around the world (Iapwa 2021), including in Brazil, which possesses one of the greatest number of cats worldwide (Moutinho et al. 2019). Despite the psychosocial and well-being benefits to owners with the presence of domestic cats in households, their close contact with felines offers a risk of the transmission for humans of pet-associated diseases that have a zoonotic nature (Esch & Petersen 2013), a fact that deserves concern in the One Health concept (Kheiran et al. 2019, Mirzai et al. 2019), and may be considered a motivation of the current study.

Pasteurella constitute a group of bacteria that inhabit the oral microbiota and upper respiratory tract of domestic animals, whose infections in companion animals are strongly associated with the opportunistic behavior of the pathogen (Stull 2022). More than 15 *Pasteurella* species are well-known, although *P. multocida*, *P. dagmatis* and *P. stomatis* have been frequently isolated from a set of clinical infections in domestic animals (Quinn et al. 2011), particularly cats (Stull 2022). Among the 26 cats sampled, 88.5% of isolates were identified as *P. multocida* based on mass spectrometry diagnosis, followed in minor frequency by *P. dagmatis* and *P. canis*, reinforcing the predominance of *P. multocida* as a primary species in clinical infections in domestic cats (Lloret et al. 2013, Stull 2022).

Different epidemiological factors must be considered in *Pasteurella* infections in domestic cats, including sex, age and breed of the animals, territorial habits of the felines (disputes

Table 1. Identification of *Pasteurella* species by mass spectrometry* in 26 diseased domestic cats. Brazil (2019-2023)

Clinical infections	Cutaneous abscesses	Pneumonia	Conjunctivitis	Urinary tract infections	Wounds	Other**	Total
	N/Total (%)	N/Total (%)	N/Total (%)	N/Total (%)	N/Total (%)	N/Total (%)	N/Total (%)
Species							
<i>Pasteurella multocida</i>	10/11 (90.9)	3/3 (100)	1/3 (33.3)	2/2 (100%)	3/3 (100)	4/4 (100)	23/26 (88)
<i>Pasteurella canis</i>	0/11 (0)	0/3 (0)	1/3 (33.3)	0/2 (0%)	0/2 (0)	0/3 (0)	1/26 (3)
<i>Pasteurella dagmatis</i>	1/11 (9)	0/3 (0)	1/3 (33.3)	0/2 (0%)	0/2 (0)	0/3 (0)	2/26 (7)
TOTAL	11/26 (42.3)	3/26 (11.5)	3/26 (11.5)	2/26 (7.7)	3/26 (11.5)	4/26 (15.5)	26/26 (100)

N = number of samples; * Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), ** pleural effusions (2/26 = 7.7%), pyometra (1/26 = 3.9%), infection secondary to neoplasia (1/26 = 3.9%)

Table 2. In vitro antimicrobial susceptibility profile (disc diffusion method) in 26 *Pasteurella* isolates obtained from diseased domestic cats. Brazil (2019-2023)

Classes	Antimicrobials	Susceptibility		
		S (%)	PS (%)	R (%)
Aminoglycosides	Amikacin	9 (34)	7 (26)	10 (38)
Beta-lactam and derivatives	Amoxicillin/clavulanic acid	26 (100)	0 (0)	0 (0)
	Ampicillin	26 (100)	0 (0)	0 (0)
	Cefalexin	26 (100)	0 (0)	0 (0)
	Ceftriaxone	26 (100)	0 (0)	0 (0)
	Ciprofloxacin	26 (100)	0 (0)	0 (0)
Fluoroquinolones	Levofloxacin	26 (100)	0 (0)	0 (0)
	Marbofloxacin	26 (100)	0 (0)	0 (0)
	Norfloxacin	25 (96)	0 (0)	1 (3)
Macrolides	Azithromycin	21 (80)	3 (11.5)	2 (7.7)
Tetracyclines	Doxycycline	26 (100)	0 (0)	0 (0)
	Tetracycline	26 (100)	0 (0)	0 (0)

S = susceptible, PS = partially susceptible, R = resistant.

over dominance in the environment, food and females in estrus), underlying conditions (coinfection with FIV and FeLV), and the access to the external environment of the household (Love et al. 2000, Walker et al. 2000, Wang et al. 2009). Nonetheless, no significant associations were observed between these selected epidemiological variables, clinical signs, and proteomic identification of *Pasteurella* species.

Despite no statistical association of epidemiological data studied, the high prevalence of mixed-breed cats could be credited to an increase adoption of stray cats in Brazil (Moutinho et al. 2019) or to the low socioeconomic profile of the owners referred to the routine of the Teaching Veterinary Hospital studied.

A wide variation in the age of the cats sampled was observed (2 months to 16 years old), indicating that feline pasteurellosis can occur in any age group. Nonetheless, a highly lethal outcome was observed among cats studied considered elderly (> 10 years old), which could be attributed to the development of clinical complications (*i.e.*, sepsis, pneumonia, and pleural effusion) secondary to systemic spread of the pathogen in debilitated animals (Giordano et al. 2015, Stull 2022).

Among all cats studied, 65.4% (17/26) were males, and 53.8% (14/26) had access to the street. In addition, 34.6% (9/26) of *Pasteurella* infections occurred in spring and 26.9% in summer, considered hot periods for reproductive action. The free access of males to the street agrees with similar studies involving feline pasteurellosis (Freshwater 2008, Lloret et al. 2013), which could facilitate respiratory infections through direct contact between cats or result in cutaneous lesions secondary to bites or scratches due to the territorial habits of male cats (*i.e.*, disputes over food, females in estrus and/or dominance of the territory). The territorial behavior of cats could favor the traumatic inoculation of *Pasteurella* species in skin tissue and opportunistic infections of the pathogen (Johnston et al. 2001, Wang et al. 2009, Lloret et al. 2013, Giordano et al. 2015).

Traumatic inoculation in the skin of the pathogen by bites or scratches and inhalation of the agent represent frequent routes of feline infections by *Pasteurella* species. In addition, cat licking is also considered an infection route for *Pasteurella* species due to the grooming of felines, enabling contamination of previous lesions by owner saliva (Stull 2022). In these routes of infections, the animals commonly evolve to cutaneous abscesses, cellulitis or disseminated infections and, occasionally, sepsis and development of abscesses in organs (Quinn et al. 2011, De Cecco et al. 2021, Wei et al. 2021, Stull 2022). Likewise, in the current study, abscesses (42.3%) represented the most common clinical sign among 26 diseased cats, followed by a set of other clinical manifestations in minor frequency, *i.e.*, pneumonia, conjunctivitis, open wounds, urinary tract infections, pleural effusion, pyometra, and infection secondary to neoplasia. The predominance of cutaneous-subcutaneous lesions in cats studied agrees with similar studies, in which skin lesions have been considered the most common clinical sign of feline pasteurellosis, possibly due to traumatic inoculation of the agent secondary to bites and scratches (Lloret et al. 2013, Giordano et al. 2015, Stull 2022). In addition, the variety of clinical manifestations observed in 26 diseased cats studied reinforces the opportunistic behavior of *Pasteurella*-induced infections in domestic cats (Quinn et al. 2011, Stull 2022).

Except for amikacin, azithromycin and norfloxacin, all other antimicrobials showed > 95% *in vitro* efficacy against *Pasteurella* isolates among cats sampled. Conversely, the highest resistance of the isolates was observed to amikacin (10/26 = 38%), followed by azithromycin and norfloxacin, whereas no multidrug-resistant *Pasteurella* isolates were reported.

Beta-lactams and derivatives (amoxicillin/clavulanic acid, ampicillin, cephalexin, ceftriaxone) and tetracyclines (doxycycline and tetracycline) groups/antimicrobials showed 100% *in vitro* efficacy against the three *Pasteurella* species isolated in the current study. Furthermore, ciprofloxacin, levofloxacin and marbofloxacin (fluoroquinolone group) also showed 100% efficacy against the isolates, except for norfloxacin, which revealed one resistant *P. multocida* isolates. These results are consistent with similar *in vitro* studies of the susceptibility patterns of *Pasteurella* species isolated from cats (Freshwater 2008, De Cecco et al. 2021) and *P. multocida* isolated from humans bitten by cats in the USA (Westling et al. 2006). These findings indicate that, in general, antimicrobials belonging to the groups of beta-lactams, tetracyclines and fluoroquinolones represent good therapeutic options for the treatment of feline pasteurellosis, probably due to the broad spectrum of action (including Gram-negative bacteria), and the high therapeutic concentrations reached into cells and tissues (Lloret et al. 2013, Stull 2022).

Some groups of antimicrobials have shown effectiveness in treating *Pasteurella* infections in domestic cats. Nonetheless, it is recommended that, if possible, treatments be carried out with previous support of *in vitro* susceptibility tests of the isolates, which can increase the success of treatment (Guiguère et al. 2013), as well as avoid the nonrational or overusing of antimicrobials, which is related to increase of selective pressure for multidrug-resistant bacteria (Magiorakos et al. 2012), a global emerging concern (Wei et al. 2021).

Despite the clinical severity and poor prognosis of systemic disorders-related *Pasteurella* infections in domestic felines (Giordano et al. 2015), most studies involving feline pasteurellosis have been described as case reports (Dolieslager et al. 2011, De Cecco et al. 2021), and the diagnosis based on traditional phenotypic tests (Foster et al. 2004, Freshwater 2008, Wang et al. 2009, Giordano et al. 2015, Awosile et al. 2018). Conversely, in the current study, *Pasteurella* isolates obtained from 26 diseased domestic cats were diagnosed at the species level based on proteomics (MALDI-TOF MS), which has been revealed to be a reliable and fast technique with high discriminatory power for the identification of bacterial and yeast species, enabling etio-epidemiological studies with microorganisms from animal and human origin (Kuhnert et al. 2012, Zangenah et al. 2013).

Data on outcomes were available in 61% (16/26) of animals. Of these, 50% (8/16) died or were subjected to euthanasia due to severe complications, such as sepsis, pneumonia, and pleural effusion. This finding highlights the poor prognosis in cases of dissemination or systemic infections by *Pasteurella* species (Giordano et al. 2015, Stull 2022) and the need for early diagnosis and therapy approaches of feline pasteurellosis due to high mortality rates of disease.

Clinical pasteurellosis in cats has been related to debilitated animals or those coinfecting with an immunosuppressive retrovirus (Stull 2022). Nonetheless, among 26 cats sampled,

only two (7.7%) males showed reactions to FIV and FeLV tests, limiting the assessment to the impact of coinfection of *Pasteurella* species and these immunosuppressive diseases in cats studied.

Pasteurella species represent a predominant group of bacteria isolated from lesions in humans secondary to being bitten and scratched by cats (Mirzai et al. 2019, Lloret et al. 2013, Mu et al. 2020) due to inhabiting oral microbiota of normal cats (Portilho et al. 2024). In addition, *Pasteurella* isolates recovered from owners and cats that inhabit the same households have revealed similarities regarding the virulence and *in vitro* antimicrobial susceptibility profile, indicating that cats may be considered potential reservoirs of the pathogen from other animals and humans (Ujvári et al. 2019). Also, domestic cats possess a thin thickness of teeth that favor deep lesions in skin inoculated by their saliva, which may induce cutaneous-subcutaneous lesions and systemic dissemination of the pathogen (Love et al. 2000, Kheiran et al. 2019), which deserve relevance in human health.

Convenience sampling, a lack of identification of *Pasteurella* serogroups/subspecies, and no investigation of comorbidity conditions or immunosuppressive viral diseases (*i.e.*, FIV and FeLV) in a total of animals studied may be considered limiting factors of the current study.

CONCLUSION

Overall, the identification of *Pasteurella* species in 26 domestic cats with different clinical infections was investigated using proteomics (mass spectrometry), which revealed a predominance of *P. multocida* species and a high occurrence of skin lesions, mainly in male cats, in addition to a high fatal evolution of systemic or disseminated infections. Our results contribute to the molecular identification of *Pasteurella* species, the vigilance of multidrug-resistant isolates, and concerns regarding high mortality rates of disseminated/systemic feline pasteurellosis.

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Data availability statement.- The corresponding author is responsible for the data's maintenance, storage and accountability. All data were preserved on personal computers and in the cloud. The study data were published in the article at “Pesquisa Veterinária Brasileira” journal and are contained in the first author's dissertation (Carolina Aparecida Rodrigues) and the “Universidade Estadual Paulista ‘Júlio de Mesquita Filho’” (Unesp) repository.

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