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Small Animal Diseases

Causes of death in domestic cats during COVID-19 pandemic (2020-2021): A multi-institutional necropsy study from Mato Grosso and Rio de Janeiro, Brazil[1]

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ABSTRACT.- Pereira G.O., Pereira A.H.B., Colodel E.M., Cruz T.P.P.S., Nakazato L., Dutra V. & Ubiali D.G. 2024. **Causes of death in domestic cats during COVID-19 pandemic (2020-2021): A multi-institutional necropsy study from Mato Grosso and Rio de Janeiro, Brazil**. *Pesquisa Veterinária Brasileira 44:e07420, 2024*. Setor de Anatomia Patológica, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: danielubiali@ufrrj.br

Retrospective studies that address the diseases in the feline species are scarce. Herein, we presented the cause of death or euthanasia of cats from January 2020 to December 2021, during the first and second years of the SARS-CoV-2 pandemic. The data were obtained from necropsies performed by the Federal Rural University of Rio de Janeiro and the Federal University of Mato Grosso. A total of 96 feline necropsies were performed. In 87 cases (90.6%), we established the reason for death, while in nine cases (9.4%), the diagnoses were inconclusive. We established the diagnostic groups: infectious and parasitic (37.5%), neoplasm (14.5%), malformation (7.3%), lower urinary tract disease (7.3%), degenerative (6.2%), traumas (6.2%), other causes (8.4%) and iatrogenic (3.1%). The most common cat diseases in Mato Grosso and Rio de Janeiro were infectious. The most common inflammatory lesions were bacterial and viral pneumonia. Alphaherpesvirus (FeHV), *Mycoplasma* sp., and *Pseudomonas* sp. were the main detected agents.

INDEX TERMS: Infectious diseases, diseases of feline, cats, pneumonia, immunohistochemistry, RT-PCR.

RESUMO.- [**Causas de morte e eutanásia em felinos domésticos durante os primeiros anos da pandemia de SARS-CoV-2 (2020 e 2021): um estudo multi-institucional de necropsias entre Rio de Janeiro e Mato Grosso.**] Estudos retrospectivos que abordam doenças em felinos domésticos são escassos. Apresentamos aqui a causa da morte ou razões para eutanásia de gatos domésticos entre janeiro de 2020 e dezembro de 2021, durante o primeiro e segundo ano da pandemia de SARS-CoV-2. Os dados foram obtidos em necropsias realizadas pela Universidade Federal Rural do Rio de Janeiro e Universidade Federal de Mato Grosso. Foram realizadas 96 necropsias de felinos. Em 87 casos (90,6%) foi estabelecido a causa da morte e em nove casos (9,4%) os diagnósticos foram inconclusivos. Estabelecemos os grupos diagnósticos: infecciosos e parasitários (37,5%), neoplasias (14,5%), malformações (7,3%), doenças do trato urinário inferior (7,3%), degenerativas (6,2%), traumas (6,2%), outras causas (8,4%) e iatrogênicas (3,1%). As doenças mais frequentes em gatos do Mato Grosso e Rio de Janeiro foram as infecciosas. As lesões inflamatórias mais frequentes foram as pneumonias bacterianas e virais. Os principais agentes detectados foram Alphaherpesvirus (FeHV), *Mycoplasma* sp. e *Pseudomonas* sp.

TERMOS DE INDEXAÇÃO: Doenças infecciosas, doenças de felinos, felinos, pneumonia, imuno-histoquímica, RT-qPCR.

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INTRODUCTION

Feline-human interaction has been described for about 11,000 years, initially its importance in controlling mice in grain warehouses (Vigne et al. 2012). Currently, the feline behavioral, clinical and diagnostic view has become a veterinary specialization worldwide, and studies in different areas of feline medicine are essential.

In the One Health approach, the close relationship of the feline and human allows for the transmission of pathogens (Vigne et al. 2004). There is a question on the risks of zoonotic diseases and the spread of pathogens from domesticated and feral cats to other species (Robertson 2008). The main zoonoses transmitted from cats to humans are sporotrichosis (Barnacle et al. 2023), leishmaniasis (Matralis et al. 2023), rabies (Ma et al. 2022), cryptococcosis (Costa et al. 2022) and toxoplasmosis (Inpankaew et al. 2021). Given the pandemic scenario of the present study, a fuss was triggered by the report of possible transmission of SARS-CoV-2 from feline to humans (Sila et al. 2022).

Retrospective studies that address the leading diseases in the feline species are scarce. There are surveys on feline disease in the United Kingdom (Murray et al. 2008), Canada (Olsen & Allen 2001), Portugal (Manteigas et al. 2013), United States (Kent et al. 2022) and Italy (Grieco et al. 2021). In Brazil, there are only surveys in the South (Trapp et al. 2010, Togni et al. 2018, Withoeft et al. 2019) and in the Northeast (Batista et al. 2016). The causes of death in cat populations in Brazil's Southeast and Midwest regions have not yet been investigated.

This two-year study, from 2020 to 2021, represents the first and second waves of the SARS-CoV-2 pandemic. This study aimed to identify and quantify *post-mortem* diagnoses of felines submitted to necropsy and ancillary examination in Mato Grosso and Rio de Janeiro.

MATERIALS AND METHODS

Animal Ethics. The domestic felines subjected to this study were referred by owners who signed a necropsy consent for research purposes. All feline tutors authorized the pathological study.

Case selection and case definition. This study was performed from January 2020 to December 2021. Necropsy was performed in 96 domestic felines (*Felis catus*) at the "Setor de Anatomia Patológica" from "Universidade Federal Rural do Rio de Janeiro" (SAP/UFRuralRJ) and the "Laboratório de Patologia Veterinária" from "Universidade Federal de Mato Grosso" (LPV/UFMT). The data collected, such as race, age, sex, origin, clinical history, and diagnostic suspicion, were compiled from the request form. The age was classified according to feline life stages guidelines (Quimby et al. 2021).

Pathologic examination. During the necropsy, tissue samples were systematically collected (skin, skeletal muscle, heart, liver, lungs, spleen, lymph nodes, kidneys, adrenals, thyroid, stomach, intestines, pancreas, brain, trachea, and nasal conchae) and fixed in 10% buffered formalin. The collected tissues were processed routinely, stained with hematoxylin and eosin (HE) and examined by optical microscopy. Gram Brown Hopps, Periodic acid-Schiff (PAS) reaction, and Grocott methanamine silver (GMS) histochemistry were performed in the cats' tissues with lesions compatible with bacterial or fungal infection. Besides, a set of tissues (skin, skeletal muscle, heart, liver, spleen, kidneys, brain and nasal conchae) were sampled by immersion in RNALater® solution. The etiologic diagnosis was determined by the association of the identified pathogen and compatible gross and or microscopic lesions (see section on diagnostic tests below). Cases with no causative agent identified were categorized as the morphological diagnosis. The cases in which it was not possible to establish the cause of death were classified as "inconclusive". After establishing a diagnosis, only one cause of death was selected for each cat and the diseases were classified (Togni et al. 2018, Withoeft et al. 2019). The causes of death were grouped as

Table 1. Molecular investigation by PCR (primers and probes) of infectious agents in tissues of domestic cats

Agents	Tissue	Primer (5´-3´)	Reference
Feline coronavirus (FCoV)	Lung and cerebral cortex	TAATGCCATACACGAACCAGCT GTGCTAGATTTGTCTTCGGACACC	Simons et al. (2005)
Leishmania sp.	Skin and subcutaneous	(C/G)(G/C)CC(C/A)CTA T(T/A)TTACACCAACCCC GGGGAGGGGCGTTCTGCGAA	Quaresma et al. (2009)
Feline parvovirus (FPV)	Intestine	GGATCACCATCTGCTGCTTG GCTGAGGTTGGTTATAGTGC	Yoon et al. (2009)
Feline immunodeficiency virus (FIV)	Lung and spleen	GCGCTAGCAGCTGCCTAACCGCAAAACCAC GTATCTGTGGGAGCCTCAAGGGAGAACTC	Szilasi et al. (2021)
Feline leukemia virus (FeLV)	Lung and lymph node	GCTCCCCAGTTGACCAGAGT TTTATAGCAGAAAGCGCGCG	Duda et al. (2020)
Feline calicivirus virus (FCV)	Lungs	GTAAAAGAAATTTGAGACAAT TACTGAAGWTCGCGYCT FAM-CAAACTCTGAGCTTCGTGCTTAAA-BHQ	Helps et al. (2005)
Felid alphaherpesvirus (FeHV)	Lungs	TGGTGCCTATGGAATAGGTAAGAGTT GTCGATTTTCATCCGCTCTGA HEX-AACGGCGAAGTACC-MGB	Helps et al. (2003)
Chlamydia sp.	Lungs	CCGCCAACACTGGGACT GGAGTTAGCCGGTGCTTCTTTAC FAM-CTACGGGAGGCTGCAGTCGAGAATC-BHQ1	Lienard et al. (2011)
Bordetella sp.	Lungs	ACTATACGTCGGGAAATCTGTTTG CGTTGTCGGCTTTCGTCTG FAM-CGGGCCGATAGTCAGGGCGTAG-BHQ1	Helps et al. (2005)
Mycoplasma felis	Lungs	GTG GGG ATG GAT TAC CTC GGA CTA TTA TCA AAA GCA CAT AAA C HEX-CTA CGG AGT ACA AGT TAC AAT TCA-BHQ1	Bennett (2015)
Pasteurella sp.	Lungs	ATCCGCTATTTACCCAGTGGGCTGTAAACGAACTCGCCAC	Townsend et al. (2001)

infectious and parasitic, neoplasms, degenerative, malformations, feline lower urinary tract disease (LUTD), traumas, iatrogenic, and "others" for diagnoses that do not fit into the previous groups. The gross and histological changes guided the ancillary examination.

Diagnostic tests. The investigation of infectious diseases was confirmed by microbiological tests performed in two cases of dermatitis one case of encephalitis. Two samples of fresh lung with macroscopic lesions were submitted to bacteriological culture (Markey et al. 2013). The molecular investigation in 38 cases fit the RNA extraction from archived formalin-fixed paraffin-embedded tissues (Shi et al. 2004). All cases of pneumonia were tested by PCR for a panel of infectious agents as *Pasteurella* sp., *Bordetella* sp., *Mycoplasma* sp., *Chlamydia* sp., feline calicivirus virus (FCV), felid alphaherpesvirus (FeHV), feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) (Di Martino et al. 2007, Schmal-Filius et al. 2020, Mello et al. 2023). The panleukopenia virus was PCR tested in cases of cats with enteritis. *Leishmania* sp. and *Sporothrix* sp. were tested in the dermatitis cases. The feline infectious peritonitis virus (FCoV) was tested in three cats with pyogranulomatous and fibrinous inflammation as meningitis and six cases of pneumonia. The primers and protocols are listed in Table 1.

Statistical methods. The results of the pathologic examinations, diagnostic tests, individual feline data, and location were recorded in a database using Microsoft Excel, and descriptive statistics were obtained.

RESULTS AND DISCUSSION

During the 2-year study, we examined 96 domestic cats presented for *post-mortem* examination. From these, 45% (44/96) were from Mato Grosso, and 55% (52/96) were from Rio de Janeiro. In total, 87 cases (90.6%) had a conclusive diagnosis, and nine (9.4%) were inconclusive due to no histological lesions.

In Mato Grosso, twelve of these cats were kittens (27.5%), sixteen young adults (36%), eight mature adults (18%) and one senior (2.5%); in seven cases (16%), age was not available. Twenty-three were males, and twenty were females; in one case, the sex was not identified.

In Rio de Janeiro, seven individuals were fetuses (13.5%), three cats were kittens (5.8%), twenty-one were young adults (40%), eleven mature adults (21.2%) and ten seniors (19.5%). Twenty-two were male, and twenty three were female. The seven fetuses were not sex identified. In total, 92 cats (96%) were Brazilian short hair.

The primary diagnosis groups were infectious and parasitic (36/96, 37.5%), neoplasm (15/96, 15.7%), other causes (8/96, 8.4%), malformation (7/96, 7.3%), degenerative (6/96, 6.2%), traumas (6/96, 6.2%), feline lower urinary tract disease (LUTD) (6/96, 6.2%), and iatrogenic (3/96, 3.1%).

The group infectious disease was composed by pneumonia (19/36, 52%) (Fig.1), sepsis (3/36), panleukopenia (3/36), sporotrichosis (2/36) (Fig.2), feline immunodeficiency virus (FIV) (3/36), platynosomiasis (1/36), feline infectious peritonitis (FCoV) (1/36), leishmaniasis (1/36), cryptococcosis (1/36) (Fig.3), bacterial endocarditis (1/36), and bacterial osteomyelitis (1/36).

The group neoplasms were lymphoma (5/14) (Fig.4-5), hemangiosarcoma (3/14), fibrosarcoma (2/14), squamous cell carcinoma (2/14), hepatic adenoma (1/14) (Fig.6), and meningioma (1/14).

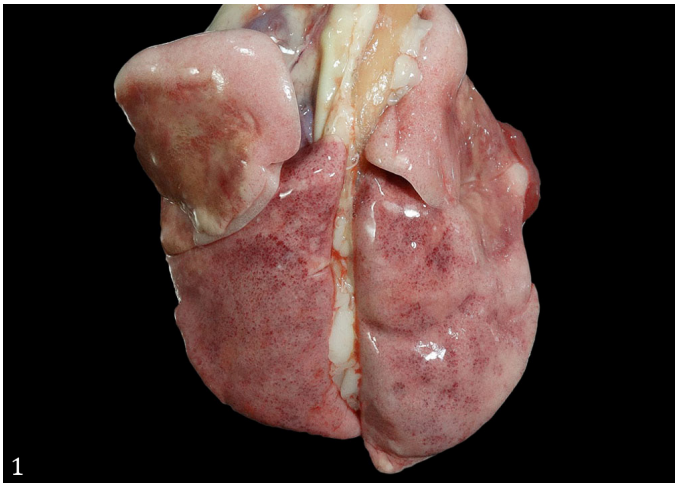

Fig.1. Causes of death in domestic cats. Consolidation of cranial lobes due to bacterial fibrinonecrotic bronchopneumonia in a feline from Rio de Janeiro.

Fig.2. Causes of death in domestic cats. Severe swelling in the nasal region due to sporotrichosis in a feline from Rio de Janeiro.

Fig.3. Causes of death in domestic cats. Red areas in the ectomarginal gyrus and ectosylvian sulcus and yellow regions of the cerebellar vermis due to fibrinous meningitis caused by cryptococcosis in a feline from Rio de Janeiro.

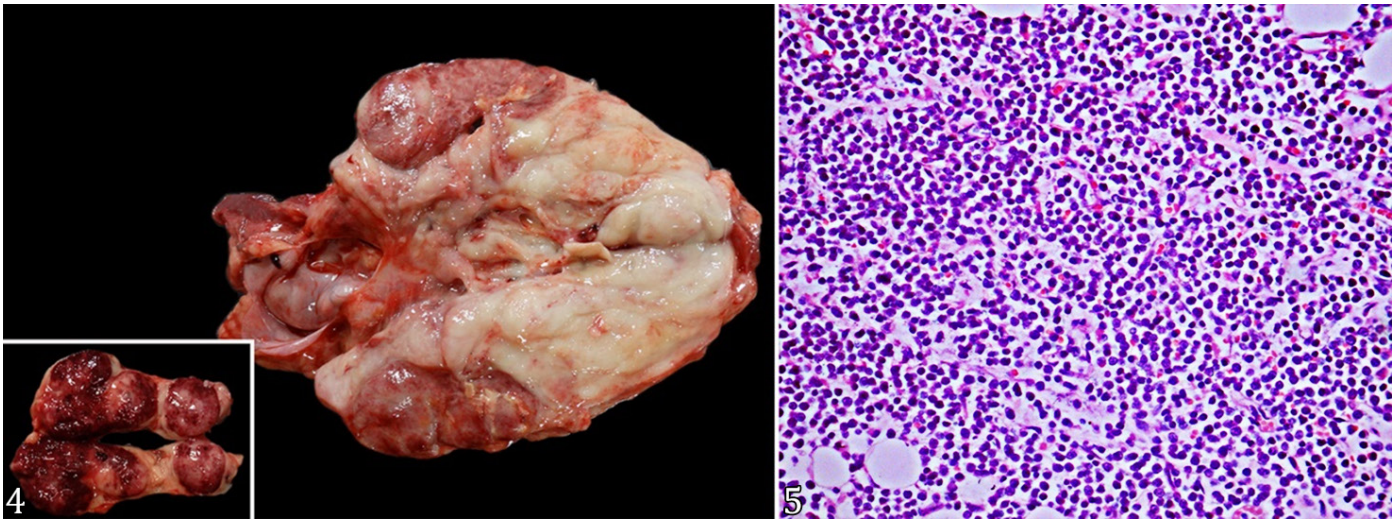

Fig.4-5. Causes of death in domestic cats. (**4**) The mediastinal lymph node is replaced by a friable white mass compatible with mediastinal lymphoma. Inset: The renal lymph node is markedly increased, and the cut surface is red and white mottled due to lymphoma. (**5**) Histologically, a monomorphic population of lymphocytes is replacing the lymph node parenchyma. HE, obj.40x.

The diagnosis of degenerative disease was chronic kidney disease (4/6), acute kidney disease (1/6) and malacia of the central nervous system (1/6).

The group trauma was composed of fractures of unknown cause (3/6), dog attack (1/6), motor vehicle collisions (1/6) and fall (1/6).

All cases of LUTD were characterized by urethral intraluminal obstruction by calculi and one urethral extraluminal obstruction by neoplasm. LUTD was diagnosed in four male and three female cats.

The "other" death causes were necrotic enteritis without an infectious agent detected, suppurative meningitis, lymphoplasmacytic dermatitis, chronic active pancreatitis, and one case of hypertrophic cardiomyopathy. Iatrogenic disturbances were drug gastritis, by high dosage of non-steroidal anti-inflammatory drugs, informed in the clinical history, fibrin necrotic glossitis caused by surgery procedure, and aspiration pneumonia (Table 2).

The cases with lesions compatible with infectious diseases were investigated by PCR. From these, all cases of bacterial pneumonia were tested by a panel of infectious agents. From the bacterial pneumonia cases, we have found two cats with *Mycoplasma* sp. infection and *Chlamydia* sp., and *Bordetella* sp. with one case of each agent. Two cases were cultivated by microbiology methods. In one case, only *Pseudomonas* sp. grew, and in the other, *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli* and *Staphylococcus* sp. were cultivated. Felid alphaherpesvirus (FeHV) was positive in four cases with concomitant bacterial pneumonia. Caliciviruses were not found in any case. FIV was found in eleven cases (11/19), FIV and feline leukemia virus (FeLV) together in (2/19), and FeLV was not found alone.

Three cats from Mato Grosso presented necrotic enteritis and lymphoid tissue necrosis and were infected with the panleukopenia virus. Feline immunodeficiency virus was positive in the cases compatible with lymphoid depletion in the spleen, lymph node, and occasionally in the bone marrow. Leishmaniasis was detected in a Rio de Janeiro cat presenting granulomatous dermatitis. Mycological techniques confirmed

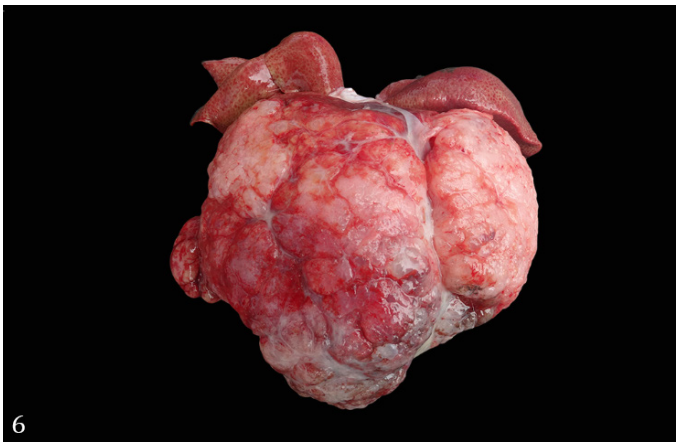

Fig.6. Causes of death in domestic cats. The hepatic parenchyma is diffusely replaced by multinodular white masses compatible with hepatic adenoma.

two cases of sporotrichosis and one case of cryptococcosis from Rio de Janeiro, which presented granulomatous dermatitis and mild granulomatous encephalitis, respectively.

The pathological, molecular and microbiological examinations were the base of this study to determine the causes of death and euthanasia during the 2020 and 2021 SARS-CoV-2 pandemic. We understand the challenge of retrospective studies and encourage research through pathological, molecular and microbiological approaches.

Infectious and parasitic diseases were the most frequent diagnostic, as well as other Brazilian research, in Santa Catarina (Withoeft et al. 2019) and Piauí (Batista et al. 2016), as well as in Milan, Italy (Grieco et al. 2021). Regardless of the lesions, the most frequent cause of feline death in our study was pneumonia. In our research, the main bacterial agents causing-pneumonia were *Pseudomonas* sp. and *Mycoplasma* sp. *Pseudomonas* sp. is most prevalent in mammals and presents the highest levels of antimicrobial resistance in dogs and cats (Li et al. 2021a). Herein, we evaluated the possibility of

immunosuppression of FIV and FeLV virus because, naturally, FIV-infected cats usually reflect secondary diseases, such as infections and neoplasia (Hartmann 2012). From cases of pneumonia, we have observed that more than half were positive for FIV, and some cases were concomitant with FeLV. This data was similar to other research (Slaviero et al. 2021, Mello et al. 2023). Although FIV does not cause a severe clinical syndrome, most naturally infected cats are predisposed to secondary infections and neoplasms (Hartmann 2012). Until now, no studies have compared the prevalence of lung diseases in cats naturally infected with FIV and those not infected to understand the relevance of FIV in respiratory diseases.

Another significant infectious disease was panleukopenia, which occurred only in Mato Grosso and affected kittens or cats from a shelter. This infectious disease demands attention mostly in shelters recognized as favorable environments (Li et al. 2021b). The surveys from South Brazil did not find cats with panleukopenia (Togni et al. 2018, Withoeft et al. 2019),

Table 2. Diagnoses of domestic cat diseases from Mato Grosso (MT) and Rio de Janeiro (RJ)

Type of lesions	State		Total	%
	MT	RJ		
Infectious and parasitic			36	37.5
Pneumonia	10	9		
Sepsis	2	1		
Panleukopenia	3	-		
Sporotrichosis	-	2		
Feline immunodeficiency (FIV)	3	-		
Platinosomiasis	-	1		
Feline infectious peritonitis (FCoV)	1	-		
Leishmaniosis	-	1		
Cryptococcosis	-	1		
Bacterial endocarditis	-	1		
Bacterial osteomyelitis	-	1		
Neoplasm			14	14.5
Lymphoma	1	4		
Hemangiosarcoma	-	3		
Fibrosarcoma	2	-		
Squamous cell carcinoma	1	1		
Hepatic adenoma	-	1		
Meningioma	-	1		
Other			8	8.4
Enteritis	2	1		
Meningitis	-	1		
Unspecific dermatitis	-	1		
Pancreatitis	-	1		
Hypertrophic cardiomyopathy	-	2		
LUTD	5	2	7	7.3
Malformation			7	7.3
Gastroschisis	-	7		
Degenerative			6	6.2
Chronic kidney disease	-	4		
Acute kidney disease	-	1		
Malacia*	1	-		
Trauma			6	6.2
Fractures of unknown cause	1	2		
Dog attack	-	1		
Motor vehicle collision	1	-		
Fall	1	-		
Iatrogenic			3	3.2
Drug gastritis	1	-		
Fibrinonecrotic glossitis	1	-		
Aspiration pneumonia	1	-		
Inconclusive	7	2	9	9.4
TOTAL			96	100.0

LUTD = lower urinary tract disease; * Malacia without associated cause.

and in Rio de Janeiro, we did not diagnose this disease. In our study, the most frequent neoplasm was lymphoma; this neoplasm is considered the most common in FeLV- and FIV-infected cats (Hartmann 2012). Current Brazilian research established that cats with FeLV infection have 3.9 more chances of lymphoma diagnosis (Mello et al. 2023). Our study did not establish this relationship because there were few FeLV cases.

The feline lower urinary tract disease (LUTD) is a diagnosis that fits a large group of lesions, including lower urinary tract infection, urolithiasis, traumas, neoplasms and lower urinary tract malformations (Osborne et al. 1996). We have found LUTD compatible with cystitis, urolithiasis and probably iatrogenic and traumatic lesions that fit histopathologically as necrohemorrhagic urethritis or cystitis. This disease is the most diagnosed in Mato Grosso, but we cannot correlate a specific cause. The most frequent cases of LUTD are male cats, mainly neutered (Foster 1967, Rich & Fabricant 1969). It was impossible to associate the neutered cats with LUTD due to a lack of information in the clinical histories. In our total domestic cats evaluated, we found that the males were most frequent (55/96), similar to other studies (Batista et al. 2016, Togni et al. 2018, Withoeft et al. 2019, Grieco et al. 2021). The species' habits always justify this finding to the search for in-estrus females and greater access to external environments, culminating in more significant contact with felines of unknown origin, facilitating the dissemination of infectious agents (Withoeft et al. 2019) and traumas. One cat from Mato Grosso was diagnosed with trauma due to a motor vehicle collision. However, RT-qPCR 43 days before the death demonstrated SARS-CoV-2 RNA, and immunohistochemistry demonstrated viral antigens in multiple organs six weeks after infection (Jarrah et al. 2022).

Of three cats with dermatitis in Rio de Janeiro, two were caused by *Sporothrix* spp. No cases of sporotrichosis were found in Mato Grosso. The mycological growth and histopathology fulfill the definition of a sporotrichosis diagnosis. One case of lymphohistiocytic dermatitis without yeast in histologic examination and negative to *Sporothrix* sp. and *Leishmania* sp. in PCR was defined as unspecific dermatitis. The sporotrichosis is endemic in Rio de Janeiro (Gremião et al. 2015) and was reported outside South America (Barnacle et al. 2023). This situation is a One Health challenge (Moreira et al. 2015). In Mato Grosso, dermatitis was not a cause of death or euthanasia reason. Few diagnoses of cat sporotrichosis from Mato Grosso were reported (Fernandes et al. 2004). All malformation cases were fetuses with gastroschisis from the same offspring. We have not investigated any infectious agents to the occurrence of these cases.

The molecular investigation using formalin-fixed paraffin-embedded samples (N=9) was efficient for extraction, but none of the cases allowed to complete the diagnoses of feline infectious peritonitis (FCoV). The limitation of using this kind of sample is RNA fragmentation due to formaldehyde exposure causing RNA-protein cross-linking. The literature indicates that body fluids and tissues from the omentum, mesenteric lymph nodes and spleen are the most valuable samples for analysis of FCoV infection by RT-PCR. In contrast, the kidneys, liver, lung, myocardium, and popliteal lymph nodes contain little or no viral RNA (Pedersen et al. 2015). We emphasize the need for studies on etiological diagnostic methods for FCoV infection. Although these cat cases series reporting reflects in part the Midwest and Southeast Brazil regional disease occurring pattern, more information in many regions of Brazil is needed.

CONCLUSIONS

The most frequent diagnoses in cats in Mato Grosso and Rio de Janeiro were infectious diseases during Brazil's first and second years of the SARS-CoV-2 pandemic. The most frequent lesion was pneumonia in both states, with observations of *Mycoplasma* sp., *Pseudomonas* sp., and Alphaherpesvirus (FeHV) infections.

We encourage studying domestic feline diseases in different Brazilian scenarios to compare infections and clarify the relevance of feline pathogens in a One Health approach.

Data availability.- The datasets used and analyzed during the current study are available from the corresponding author upon request.

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