

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
FACULDADE DE VETERINÁRIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

**MÔNICA SLAVIERO**

**ASPECTOS PATOLÓGICOS DE INFECÇÕES VIRAIS QUE AFETAM O TRATO  
RESPIRATÓRIO DE GATOS DOMÉSTICOS**

PORTO ALEGRE

2024

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**Tese apresentada como requisito para obtenção  
do grau de Doutor em Ciências Veterinárias na  
área de concentração em Medicina Veterinária  
Preventiva e Patologia: Patologia Animal e  
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**Orientadora:** Prof. Dra. Luciana Sonne

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Prof<sup>a</sup>. Dr<sup>a</sup>. Luciana Sonne

Orientadora e Presidente da Comissão

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Prof. Dr. Eduardo Conceição de Oliveira (UCS)

Membro da Comissão

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Prof. Dr. Djeison Lutier Raymundo (UFLA)

Membro da Comissão

---

Prof. Dr. David Driemeier (UFRGS)

Membro da Comissão

## RESUMO

Doenças infecciosas que envolvem o trato respiratório são frequentes em gatos e de grande importância na medicina felina devido à alta morbidade ou mortalidade, principalmente em filhotes ou adultos imunocomprometidos. O objetivo desse projeto foi investigar a ocorrência e lesões associadas de infecções virais no trato respiratório de gatos necropsiados no Setor de Patologia Veterinária (SPV-UFRGS). Visou-se elucidar a relevância das infecções virais nesses animais e determinar as principais características patológicas associadas a cada agente viral. Inicialmente relatou-se um caso de infecção natural por herpesvírus felino (FHV) em um gato adulto coinfectado pelo vírus da leucemia felina (FeLV) e *Escherichia coli* causando necrose hepática, traqueíte e pneumonia fibrinonecrótica. A imunodepressão em gatos FeLV-positivos provavelmente favorece uma situação de viremia por FHV, além do desenvolvimento de infecções bacterianas secundárias. Posteriormente, investigou-se a importância da infecção pelo vírus da peritonite infecciosa felina (PIF) no trato respiratório de gatos a partir de achados patológicos principalmente no pulmão e investigação da distribuição do antígeno viral por imuno-histoquímica (IHQ). Sessenta e seis gatos com PIF foram analisados. Observou-se frequente pleurite e envolvimento do parênquima pulmonar. Padrões de lesão macroscópica e histológica foram definidos e as lesões estavam comumente associadas à imunopositividade de coronavírus felino. Embora a PIF seja uma doença sistêmica, alguns gatos (21/66) desenvolveram lesões importantes na cavidade torácica, inclusive alguns com envolvimento do trato respiratório superior e com sinais respiratórios, sem outros sinais clássicos de PIF. Sugere-se que o trato respiratório possa desempenhar um papel importante na rota de infecção de PIF em alguns gatos. Por fim, investigou-se através da IHQ o envolvimento de FHV e calicivírus felino (FCV) em gatos que morreram com doença respiratória. Sessenta gatos foram incluídos no estudo e em 22 gatos identificou-se infecção por FHV, FCV ou ambos os vírus. Os principais achados patológicos e distribuição de antígeno viral nos tecidos afetados foram analisados. Ressalta-se a importância de coletar durante a necropsia porções de trato respiratório e digestório superior e a utilização da técnica de imuno-histoquímica para a confirmação do envolvimento de agentes infecciosos virais.

**Palavras-chaves:** Patologia felina; doenças infecciosas; trato respiratório; calicivírus; herpesvírus felino; PIF; pneumonia; imuno-histoquímica.

## ABSTRACT

Infectious diseases involving the respiratory tract are frequent in cats and of great importance in feline medicine due to high morbidity or mortality, especially in kittens or immunocompromised adults. This project aimed to investigate the occurrence of viral infections in the respiratory tract of cats that die with important local lesions, necropsied at the Setor de Patologia Veterinária (SPV-UFRGS). The aim was to elucidate the relevance of viral infections in these cats and to determine the pathological characteristics associated with each viral agent. Initially, we report a case of natural infection by FHV-1 in an adult FeLV-positive cat associated with hepatic necrosis, tracheitis, and fibrinonecrotic pneumonia, with secondary infection by *Escherichia coli*. Immunosuppression in FeLV-positive cats probably favor a situation of FHV viremia, in addition to the development of secondary bacterial infections. Subsequently, the importance of feline infectious peritonitis virus (FIP) infection in the respiratory tract of cats was investigated based on pathological findings mainly in the lung and investigation of the viral antigen distribution by immunohistochemistry (IHC). Sixty-six FIP-positive cats were analyzed. Pleuritis and lung parenchyma involvement were frequent. Gross and histological lesion patterns were defined and lesions were commonly associated with FIP antigen. Although FIP is a systemic disease, some cats (21/66) have developed major lesions in the thoracic cavity, including some with upper respiratory tract involvement and with respiratory signs, without other classic signs of FIP. It is suggested that the respiratory tract may play an important role in the route of FIP infection in some cats. Finally, the involvement of FHV and feline calicivirus (FCV) in cats that died of respiratory disease was investigated by IHC. Sixty cats were included in the study, and 22 cats were infected with FHV, FCV, or both viruses. The main pathological findings and distribution of viral antigen in affected tissues were studied. It is important to collect portions of the respiratory and upper digestive tract during necropsy and to use the immunohistochemistry technique to confirm the involvement of viral infectious agents.

**Keywords:** feline pathology; infectious diseases; respiratory tract; calicivirus; feline herpesvirus; FIP; pneumonia; immunohistochemistry.

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## 1 INTRODUÇÃO

O gato foi domesticado há mais de 9.000 anos quando o homem passou a utilizá-lo como forma de controle de ratos (Driscoll *et al.*, 2007). Desde então, a relação entre homens e gatos se tornou cada vez mais estreita, principalmente, nos últimos anos em que houve uma intensificação na criação de felinos. Estima-se que atualmente no Brasil a população de gatos domiciliados seja de mais de 33 milhões, com o maior crescimento acumulado entre os anos 2021-2022 em comparação às outras espécies de companhia (ABINPET, 2024). Conseqüentemente, o aumento crescente da população de felinos ocasionou naturalmente um avanço na procura por produtos e atendimentos clínicos veterinários. O mercado *Pet* fatura anualmente cerca de 47 bilhões, sendo o setor veterinário responsável por 15% deste valor, com crescimento de 16% do ano de 2023 (ABINPET, 2024).

A expectativa de vida em gatos tem aumentado nas últimas décadas, especialmente em gatos que recebem cuidados preventivos, médicos e nutricionais de seus tutores. O conhecimento das principais doenças que acometem esses animais e que acarretam o óbito é de extrema importância para o médico veterinário, e contribui para um diagnóstico e tratamento mais acurados, permitindo medidas profiláticas para redução de índices de mortalidade e melhora na qualidade de vida (Trapp *et al.*, 2010). Embora esse aumento da expectativa de vida associado ao aumento de cuidados preventivos ocasione um aumento na observação de doenças neoplásicas e crônicas em gatos, no Brasil as doenças infecciosas ainda desempenham um papel importante como causa de óbito, especialmente, considerando que os índices de retrovíroses ainda são elevados (Batista *et al.*, 2016; Togni *et al.*, 2018; Biezus *et al.*, 2019; De Mello *et al.*, 2023).

Dentre as doenças infecciosas, as que acometem o trato respiratório são algumas das principais causas de morbidade e mortalidade em gatos e um dos principais motivos para o atendimento na clínica médica de felinos (Gaskell; Dawson; Radford, 2012; O'Neill *et al.*, 2014; Little, 2016). Dentre as causas, o complexo respiratório felino (CRF) é uma doença multifatorial com alta prevalência em todo o mundo, principalmente em gatos que vivem em abrigos ou em qualquer situação em que haja contato próximo com outros gatos (Cannon, 2023; Litster, 2021; Cohn, 2011). Diferentes vírus, patógenos bacterianos e fatores ambientais e do hospedeiro podem causar, agravar e manter o CRF (Cannon, 2023).



Dentre os agentes infecciosos primários relatados que causam sinais clínicos, os vírus desempenham um papel importante. Calicivírus felino (FCV) e herpesvírus felino (FHV) são os principais agentes envolvidos nas doenças do trato respiratório superior, e menos frequentemente, do trato respiratório inferior. FCV e FHV possuem ampla distribuição mundial e respondem por aproximadamente 80 a 90% de todas as doenças respiratórias felinas (López; Martinson, 2017). *Chlamydomphila felis*, *Bordetella bronchiseptica* e *Mycoplasma felis* são agentes bacterianos identificados nesta síndrome, no entanto, são geralmente secundários à infecção por FCV e FHV, embora possam atuar como agentes primários em gatos filhotes e adultos imunocomprometidos (Gaskell; Dawson; Radford, 2012; Sykes, 2013). Ainda, coinfeções com outros patógenos bacterianos como *Pasteurella multocida*, *Streptococcus* spp. são relatados (Gaskell; Dawson; Radford, 2012).

Além da infecção por FCV e FHV, outros agentes virais podem estar envolvidos em manifestação de sinais clínicos respiratórios em gatos. Embora não seja uma condição primária do trato respiratório, a peritonite infecciosa felina (PIF) causada por um biotipo mutante virulento do coronavírus felino (FCoV) leva a efusões e inflamação piogranulomatosa em múltiplos tecidos, podendo acometer o tórax, e, conseqüentemente, levando à manifestação de sinais clínicos respiratórios (Pedersen, 2009). Outros patógenos virais primários de outras espécies podem menos frequentemente afetar o trato respiratório de gatos, como infecções pelo vírus da influenza de origem suína H1N1 e aviária H5N1 e H7N7 (Reperant *et al.*, 2012; Caswell; Williams, 2016), síndrome respiratória severa aguda (SARS-CoV), e recentemente, SARS-CoV-2 (Van Den Brand *et al.*, 2008; Gaudreault *et al.*, 2021; Hosie *et al.*, 2021). Adicionalmente, infecções respiratórias também podem ser associadas aos efeitos imunossupressores dos retrovírus felinos, o vírus da leucemia felina (FeLV) e o vírus da imunodeficiência felina (FIV) (López; Martinson, 2017).

Apesar de serem doenças rotineiramente vistas na clínica médica de felinos, há informações limitadas na literatura e lacunas no conhecimento sobre a frequência e patologia de infecções virais em gatos. Pesquisas científicas envolvendo as doenças do trato respiratório em gatos compreendem em sua maioria estudos clínicos e sorológicos (Binns *et al.*, 2000; Holst *et al.*, 2010; Henzel *et al.*, 2012; Henzel; Lovato; Weiblen, 2015; Nguyen *et al.*, 2019). Ainda, investigações epidemiológicas, de aspectos patológicos, causas envolvidas e até mesmo de características clínicas dos gatos que vem a óbito com lesões importantes nos tratos respiratório são pouco exploradas na literatura, na qual encontram-se em sua maioria uma grande amplitude de estudos *ante-mortem* (Binns *et al.*,

2000; Holst *et al.*, 2010; Henzel *et al.*, 2012; Henzel; Lovato; Weiblen, 2015; Nguyen *et al.*, 2019; Falcão *et al.*, 2020).

O conhecimento do perfil dos gatos que morrem em consequência de doença respiratória, causas, lesões locais e sistêmicas envolvidas, distribuição de antígenos e identificação de outras doenças associadas são de fundamental importância para um maior entendimento sobre essas enfermidades e assim saber direcionar melhor a conduta clínica, reduzindo futuros óbitos. Ainda, o levantamento de dados epidemiológicos, de frequência de doenças, bem como de descrições macroscópicas e histopatológicas constituem ferramentas importantes para auxiliar médicos veterinários no diagnóstico de enfermidades. Conhecer as doenças que ocorrem na espécie e no local estudado permite traçar estratégias de prevenção e, desta forma, aumentar a qualidade e a expectativa de vida dos animais.

Desta maneira, esta tese tem por objetivo principal compilar e documentar os aspectos epidemiológicos e patológicos de infecções virais no trato respiratório de gatos que morrem com doença respiratória, descrever as lesões macroscópicas e histopatológicas observadas nos principais agentes envolvidos, bem como investigar o envolvimento de FIV e FeLV nestes animais.

## 2 REVISÃO BIBLIOGRÁFICA

### 2.1 Herpesvírus felino

A infecção pelo herpesvírus em gatos é causada pelo herpesvírus felino tipo 1 (FHV-1, FHV), um dos principais agentes envolvidos no complexo respiratório felino, comumente encontrado em populações de felinos domésticos. É um importante causador de doença aguda e crônica do trato respiratório superior causando inflamação das mucosas nasal, ocular e traqueal, e, por isso, é também conhecido como vírus da rinotraqueíte felina. Entretanto, FHV pode causar também doença pulmonar, principalmente em gatos filhotes ou animais imunocomprometidos (Caswell; Williams, 2016).

FHV pertence ao gênero *Varicellovirus*, que pertence à subfamília Alphaherpesvirinae da família Herpesviridae. É um alphaherpesvirus constituído por uma fita dupla de DNA, com envelope lipídico e com glicoproteínas (Gaskell *et al.*, 2007). Assim como outros, FHV é relativamente frágil fora do hospedeiro e é altamente suscetível aos efeitos dos desinfetantes comuns (Eleraky; Potgieter; Kennedy, 2002; Gaskell *et al.*, 2007). O vírus pode sobreviver por apenas 18 horas em ambiente externo úmido, menos em condições secas. Também é relativamente instável como aerossol. Apenas um sorotipo de FHV-1 é reconhecido, embora alguns isolados possam apresentar virulências variáveis (Gaskell *et al.*, 2007).

FHV está distribuído mundialmente e é endêmico na maioria das populações felinas (Gaskell *et al.*, 2007). No Brasil, são relatadas soroprevalências em populações de gatos entre 30,6% e 38,1% (Henzel *et al.*, 2012; Henzel; Lovato; Weiblen, 2015). A morbidade pode chegar a 100% em gatos jovens e em grandes populações, incluindo aqueles encontrados em gatis, feiras e exposições, e a mortalidade é elevada entre recém-nascidos, gatos jovens e debilitados. Animais que se recuperam de doença clínica, geralmente em 7-10 dias, o vírus torna-se latente no gânglio trigeminal, reativado em situações de imunodepressão (Gaskell; Dawson; Radford, 2012). Porém, a mortalidade pode chegar a até 50%, associada à infecção generalizada entre gatos predispostos (Gaskell *et al.*, 2007). O vírus FHV persiste na população principalmente pelo contato entre gatos com infecção aguda e gatos suscetíveis, assim como pela infecção latente (Gaskell *et al.*, 2007).

Infecção por FHV é relacionada, principalmente, a rinites, sinusites, rinotraqueítes, conjuntivites e ceratites ulcerativas (Thiry *et al.*, 2009), embora também possa causar lesões

ulcerativas em pele e mucosa oral (Lommer, 2013). Menos frequentemente, infecções por FHV podem causar pneumonia broncointersticial. Apresentações mais raras incluem abortos e natimortos, meningoencefalite não supurativa, esofagite e gastrite necróticas (Hora *et al.*, 2013; Mcgregor; Sheehan; Simko, 2016; López; Martinson, 2017). Sinais clínicos frequentes incluem espirros e descargas oculonasais, que podem estar acompanhados de hipersalivação, depressão, inapetência e pirexia (Gaskell; Dawson; Radford, 2012).

A infecção pelo FHV ocorre pelas vias nasal, conjuntival e oral. O vírus é epiteliotrópico e se caracteriza por replicação lítica principalmente no epitélio nasal, faríngeo, traqueal e bronquial, trazendo prejuízos aos mecanismos naturais de defesa e favorecendo infecções bacterianas secundárias (Gaskell *et al.*, 2007). As lesões causadas por FHV tendem a ser reversíveis, porém o desenvolvimento de infecções bacterianas secundárias pode causar rinite e conjuntivite supurativas, além de favorecer o desenvolvimento de pneumonias e outras afecções sistêmicas importantes (López; Martinson, 2017). Em infecções por FHV, a ocorrência de viremia é rara porque a replicação do vírus é normalmente restrita a áreas de baixa temperatura corporal, como o trato respiratório superior. No entanto, há raros relatos de viremia e doença generalizada em animais debilitados ou em filhotes neonatais (Gaskell *et al.*, 2007).

Quando os pulmões são afetados, FHV causa pneumonia broncointersticial com necrose do epitélio bronquiolar e alveolar, espessamento dos septos alveolares e formação de edema (Bart *et al.*, 2000; López; Martinson, 2017). O vírus causa um importante comprometimento dos sistemas de defesa pulmonares e predispõe à pneumonia bacteriana secundária e coinfeção por calicivirus (López; Martinson, 2017).

Atualmente os métodos moleculares parecem mais sensíveis que o isolamento de vírus ou a imunofluorescência indireta para o diagnóstico de FHV. A utilização de PCR convencional, nested-PCR e PCR em tempo real é empregada rotineiramente por laboratórios de diagnóstico para detectar DNA de FHV em esfregaços conjuntivais, corneanos ou orofaríngeos, raspados corneanos, humor aquoso, sequestro corneano, sangue ou biópsias (Weigler *et al.*, 1997; Vogtlin *et al.*, 2002; Marsilio *et al.*, 2004). Todavia, resultados positivos dos testes devem ser interpretados com cautela uma vez que pequenas quantidades de ácidos nucleicos virais são detectáveis por PCR, que podem ou não estar associadas a doenças (Thiry *et al.*, 2009).

De maneira similar, anticorpos contra o FHV podem ser detectados no soro, no humor aquoso e no líquido cefalorraquidiano por ensaio de soro-neutralização ou ELISA (Dawson *et al.*,

1998, Maggs *et al.*, 1999). Porém, devido à infecção natural e à vacinação, a soroprevalência é alta em gatos, e a presença de anticorpos não se correlaciona com doença e infecção ativa (Maggs *et al.*, 1999). Além disso, a sorologia não distingue entre animais infectados e vacinados (Thiry *et al.*, 2009).

Quando amostras de biópsias estão disponíveis, a observação de corpúsculos de inclusão característicos na histopatologia é suficiente para o diagnóstico, porém estes são visualizados apenas na fase de replicação viral aproximadamente 2-7 dias após infecção (López; Martinson, 2017). Assim, técnicas diagnósticas auxiliares de detecção de antígeno diretamente no tecido podem ser utilizadas, como imuno-histoquímica, hibridização *in situ* e imunofluorescência indireta (Caswell; Williams, 2016).

## 2.2 Calicivirus felino

Calicivirus felino (FCV) é o segundo principal agente viral envolvido em doenças do trato respiratório em felinos. É um vírus de RNA fita simples, não envelopado, pertencente ao gênero *Vesivirus* da família *Caliciviridae* (Radford *et al.*, 2009). FCV é ligeiramente mais resistente que FHV, sobrevivendo no ambiente externo por vários dias a várias semanas em superfícies secas à temperatura ambiente e por mais tempo em condições mais frias e úmidas, além de ser mais resistente ao uso de desinfetantes domésticos (Povey, 1978).

Por ser um vírus de RNA, é comum a ocorrência de mutações genéticas durante a replicação do FCV (Radford *et al.*, 2009). Esta diversidade genética compromete a eficácia das vacinas. Complica a indução da imunidade vacinal de proteção cruzada e o controle da infecção e contribui para a persistência da infecção (Radford *et al.*, 2009). O surgimento de isolados de FCV altamente virulentos (doença sistêmica virulenta associada ao calicivirus felino – FCV-VSD) é outra consequência importante desta diversidade genética (Pedersen *et al.*, 2000). Contudo, todos os isolados de FCV pertencem ao mesmo sorotipo, apesar desta diversidade (Radford *et al.*, 2009).

Assim como o FHV, FCV possui distribuição mundial e é amplamente identificado na população de felinos, com uma prevalência geralmente mais elevada em lares com vários gatos, em abrigos e também em animais mais jovens (Bannasch; Foley, 2005; Binns *et al.*, 2000; Schulz *et al.*, 2015, Fernandez *et al.*, 2017). Embora considera-se que FHV possa causar doença mais grave, FCV tende a ser mais comum (Gaskell; Dawson; Radford, 2012). No Brasil, de maneira

similar, são relatadas soroprevalências em populações de gatos maiores que de FHV, entre 39,2% a 56,7% para FCV (Henzel *et al.*, 2012; Henzel; Lovato; Weiblen, 2015). O vírus é eliminado principalmente nas secreções oculares, nasais e orais, e a disseminação ocorre principalmente por contato direto com um gato infectado. Animais gravemente infectados são claramente uma das fontes mais importantes do vírus, mas a infecção também ocorre comumente em gatos infectados clinicamente recuperados. Em algumas situações, particularmente dentro de um gatil, também pode ocorrer transmissão indireta. Secreções contaminadas podem estar presentes nas gaiolas, nos utensílios de alimentação e limpeza e no pessoal (Gaskell; Dawson; Radford, 2012).

Embora não haja uma situação de latência como no FHV, o estado de portador (carreadores subclínicos) parece ser generalizado na população de gatos, com aproximadamente 10% dos animais domésticos e 25% a 75% dos gatos de abrigo ou colônia sendo positivos para FCV (Povey, 1978; Pedersen *et al.*, 2000; Helps *et al.*, 2002; Coyne *et al.*, 2006; Hofmann-Lehmann, 2022). O vírus persiste em portadores em tecidos tonsilares e outros tecidos orofaríngeos. Embora o mecanismo preciso de persistência não seja claro, é provável que inclua pressão de seleção imunológica que conduz à variação antigênica na proteína do capsídeo viral, o que permite que o vírus evite a resposta imune do hospedeiro (Radford *et al.*, 1997; Coyne *et al.*, 2007; Kang; Park, 2008). No entanto, outros fatores do hospedeiro, virais e ambientais também desempenham, sem dúvida, um papel na resposta imune e estado de portador (Gaskell; Dawson; Radford, 2012).

Infecções por FCV causam doença importante do trato respiratório superior com rinites, geralmente acompanhadas de lesões orais incluindo gengivites, estomatites e glossites ulcerativas (Radford *et al.*, 2009; Lommer, 2013). O vírus replica em tecidos orais e respiratórios e, após três a quatro dias de infecção, ocorre viremia, e assim o vírus pode ser detectado em tecidos viscerais, fezes e ocasionalmente na urina (Gaskell; Dawson; Radford, 2012). Pneumonias por FCV são incomuns; são mais prováveis de ocorrerem após exposição por aerossol ao invés de oronasal e cepas específicas podem estar relacionadas a um maior tropismo pelo pulmão (Caswell; Williams, 2016). Todavia, quando as pneumonias ocorrem, são relatadas principalmente em filhotes e animais imunocomprometidos (Monné-Rodríguez *et al.*, 2014; Slaviero *et al.*, 2021). Além disso, uma infecção virulenta sistêmica por FCV (*virulent systemic feline calicivirus* - VS-FCV) tem sido descrita nos Estados Unidos e na Europa (Pedersen *et al.*, 2000; Schorr-Evans *et al.*, 2003; Pesavento *et al.*, 2004; Reynolds *et al.*, 2009; Battilani *et al.*, 2013), relacionado a altos índices de mortalidade em gatos adultos, mesmo nos vacinados previamente. Nesses casos, é observado severa ulceração

em plano nasal, mucosa oral, lábios, orelhas e coxins, além de acentuado edema subcutâneo (principalmente em face e membros) e icterícia. Envolvimento sistêmico é relatado, com a ocorrência de pneumonia broncointersticial, necrose hepática, esplênica, pancreática e de tecidos linfoides comumente associados (Pesavento *et al.*, 2004, Battilani *et al.*, 2013). No Brasil, esta apresentação ainda não foi descrita. Porém, a ampla diversidade genética de cepas de FCV e relatos emergentes no mundo a cerca destas infecções virulentas por calicivirus sistêmicos em diferentes populações de gatos, associadas ao fato da vacina utilizada no Brasil ser a mesma há anos, levanta o alerta da possibilidade destas apresentações também ocorrerem no Brasil e apenas serem desconhecidas até o presente momento (Henzel; Lovato; Weiblen, 2015).

Como as lesões muitas vezes são inespecíficas e muito similares às causadas pelo FHV, é necessário auxílio laboratorial para o diagnóstico definitivo. PCR e isolamento viral podem ser utilizados para diagnóstico, porém, de maneira similar ao que ocorre com o FHV, é necessário cautela entre um resultado positivo e a associação com doença clínica. Assim, a imunohistoquímica possui grande importância na detecção do antígeno viral diretamente em lesões em secções teciduais (Caswell; Williams, 2016).

### **2.3 Peritonite infecciosa felina**

A peritonite infecciosa felina (PIF) é uma das doenças infecciosas mais importantes na medicina felina e causa de morte em gatos, com distribuição mundial, afetando principalmente gatos jovens de abrigos e gatis (Thayer *et al.*, 2022). É causada por um biotipo mutante virulento do coronavírus felino (FCoV), conhecido como vírus da peritonite infecciosa felina (FIPV), onde há uma mudança da replicação viral primária nos enterócitos para a replicação e ativação nos monócitos circulantes, transformando uma infecção local em sistêmica (Poland *et al.*, 1996; Vennema *et al.*, 1998; Pedersen *et al.*, 2008).

A patologia da PIF está relacionada à inflamação piogranulomatosa generalizada em tecidos associada à vasculite, principalmente devido à vasculite, principalmente, flebite (Kipar *et al.*, 2005). A diferenciação entre as formas “úmida” e “seca” tem sido usada para diferenciar casos com efusão daqueles com apenas piogranulomas, mas ambos são a mesma entidade patológica (Thayer *et al.*, 2022). Os sinais clínicos diferem de acordo com os tecidos afetados, mas comumente

incluem distensão abdominal (Thayer *et al.*, 2022). Os sinais respiratórios são menos observados, sendo a dispneia mais comumente relacionada ao derrame pleural (Pedersen, 2009).

Os achados patológicos em gatos positivos para PIF estão bem estabelecidos na literatura; no entanto, há pouca informação sobre doenças respiratórias associadas à PIF. Sabe-se que a PIF pode causar pneumonia piogranulomatosa (Macdonald *et al.*, 2003; Sherding, 2006; Pedersen, 2009), mas a importância da PIF para o sistema respiratório ainda é pouco compreendida. Os pulmões não são considerados um alvo primário da PIF e o seu envolvimento é frequentemente considerado uma consequência de doença sistêmica, geralmente com lesões localizadas no parênquima subjacente à pleura (Caswell; Williams, 2016; Haake *et al.*, 2020). No entanto, alguns gatos parecem apresentar sinais respiratórios como espirros e secreção nasal em uma fase inicial da PIF (Pedersen, 1987; Hok, 1993; Adie, 2012; André; Miller; Whittaker, 2020). Além disso, um estudo recente relata um caso de rinite piogranulomatosa associada à detecção imuno-histoquímica local de PIF, sugerindo que a infecção respiratória faz parte do espectro da doença associada à PIF (André; Miller; Whittaker, 2020).

Apesar das diversas pesquisas ao longo dos anos sobre a PIF, ela ainda é uma das doenças com maior risco de vida em gatos, e o diagnóstico *ante mortem* da PIF ainda representa um desafio para clínicos, patologistas e virologistas. O grande desafio no diagnóstico da doença é diferenciar a infecção por FCoV da PIF, uma vez que atualmente se sabe que gatos saudáveis infectados por FCoV também podem apresentar infecção sistêmica (Kipar *et al.*, 2006). Além disso, gatos clinicamente saudáveis podem ser positivos para anticorpos anti-FCoV sem desenvolver PIF (Thayer *et al.*, 2009).

As técnicas de imunocitoquímica e imunofluorescência direta demonstraram ser ferramentas úteis no diagnóstico de PIF efusiva; no entanto, não são aplicáveis para o diagnóstico *ante mortem* de PIF não efusiva (Hartmann *et al.*, 2003; Felten *et al.*, 2016). A imuno-histoquímica (IHQ) é uma técnica diagnóstica importante e a principal ferramenta para o diagnóstico definitivo da PIF, uma vez que apenas gatos com PIF apresentam carga viral alta o suficiente para a detecção do antígeno viral dentro dos macrófagos. Apesar da necessidade de métodos mais invasivos necessários para obter amostras de tecido, o diagnóstico pela IHQ pode ser realizado tanto *ante mortem* quanto *post-mortem* (Tammer *et al.*, 1995; Thayer *et al.*, 2009).



## 2.4 Desafio no diagnóstico de infecções virais respiratórias

Por ser comumente uma consequência de uma interação multifatorial complexa de patógenos respiratórios, estresse e susceptibilidade animal, as enfermidades do trato respiratório possuem um diagnóstico causal muitas vezes desafiador. O isolamento viral e sorologias positivas de FHV e FCV, por exemplo, em gatos saudáveis, enfatizam a dificuldade de controle e prevenção desses vírus (Henzel; Lovato; Weiblen, 2015). Além disso, exames de PCR rotineiros não são capazes de diferenciar cepas vacinais de infecções naturais (Burns *et al.*, 2011). De maneira semelhante, a grande maioria de bactérias envolvidas nessas afecções são oportunistas, e assim fazem parte da microbiota natural, sendo encontradas tanto em gatos saudáveis como doentes (Gaskell; Dawson; Radford, 2012).

Portanto, exames virológicos e bacteriológicos associados à realização de exame histopatológico e identificação de antígenos virais em tecidos por meio da técnica de imunohistoquímica são uma importante ferramenta diagnóstica. Os exames auxiliam na determinação da causa e diferenciação do agente causal de enfermidades do trato respiratório e digestório superior em gatos, e conseqüentemente auxilia na escolha do tratamento mais apropriado (Burns *et al.*, 2011).

## 2.5 Importância da associação entre retrovíroses felinas e outras infecções virais

Vírus da leucemia felina (FeLV) e vírus da imunodeficiência felina (FIV) são retrovírus com distribuição mundial e um potencial distinto para causar doenças em gatos (Hartmann, 2012).

Atualmente observa-se grande variação entre diferentes países nas frequências relatadas de retrovíroses felinas. Enquanto baixa soroprevalência de FIV e FeLV é observada em países da América do Norte e Europa (FIV 3,6% e FeLV 3,1% nos EUA e Canadá [Burling *et al.*, 2017]; FIV 3,2% e FeLV 3,6% na Alemanha [Gleich *et al.*, 2009]; FIV 6% e FeLV 5% no Reino Unido [Hosie *et al.*, 2009]), prevalências maiores são relatadas em países como Austrália (FIV 6-14%; FeLV 1-4% [Westman *et al.*, 2016]), Malásia (FIV 10% e FeLV 12% [Sivagurunathan *et al.*, 2018]) e Costa Rica (FIV 8,8% e FeLV 16,7% [Blanco *et al.*, 2008]). No Brasil, quando avaliadas populações de felinos recebidos em Hospitais Universitários, frequências maiores são relatadas, variando de 7,6% a 31% para FIV e 10,1% a 28,4% para FeLV (Costa *et al.*, 2017; Biezus *et al.*,

2019; De Mello *et al.*, 2023). Em estudos de linfomas, esses índices são ainda maiores, com mais de 50% dos gatos FeLV positivos, e 21,6% a 37,5% FIV positivos (Cristo *et al.*, 2019; Mello *et al.*, 2019; Leite-Filho *et al.*, 2020). Os principais fatores relacionados a maior ocorrência observada incluem baixas taxas de vacinação e práticas de manejo de felinos, como superpopulações de felinos e acesso à rua (Biezus *et al.*, 2019).

Tanto o vírus da FIV quanto da FeLV é capaz, por diferentes mecanismos, de imunossupressão com infecções oportunistas, entretanto, imunossupressão pelo vírus da FeLV é mais severa (Tenorio *et al.*, 1991; Hartmann, 2012). Consequentemente, diferentes infecções secundárias e coinfeções são frequentes em gatos FeLV positivos. São relatadas coinfeções importantes com FCoV e FHV; além de estar relacionado a doenças como: micoplasmoses, criptosporidiose, toxoplasmose, aspergilose e dermatofitoses (Hartmann, 2012; Najafi *et al.*, 2014; Zandonà *et al.*, 2018).

Em um estudo de pneumonias fatais em gatos, 75% dos gatos com pneumonia viral por FHV, FCV, ou ambos, apresentaram coinfeção com algum retrovírus felino (FIV/FeLV; Slaviero *et al.*, 2021). Em outro estudo, gatos FeLV positivos tiveram 1,6 a 2,2 vezes mais chances de morrer por doença infecciosa e por PIF que gatos não infectados (De Mello *et al.*, 2023). PIF é descrita como uma das doenças infecciosas mais prevalentes em gatos necropsiados infectados com FeLV (Essex *et al.*, 1975; Essex *et al.*, 1977; Reinacher 1989; Suntz *et al.*, 2010). Gatos com infecção por FeLV são mais suscetíveis ao desenvolvimento de PIF, pois condições imunossupressoras podem levar a altos níveis de replicação do FCoV, aumentando mutações (Hardy, 1982; Poland *et al.*, 1996; Pedersen; Allen; Lyons, 2008). Além disso, a infecção por FeLV pode reativar a infecção em gatos portadores do vírus PIF na forma latente ou sequestrada (Pedersen, 1987). No entanto, em países com baixa prevalência de FeLV, menos de 10% dos casos de PIF estão associados a este vírus (Pedersen, 2009).

Similarmente, gatos em estágio terminal da infecção pelo FIV também parecem estar predispostos ao desenvolvimento da PIF (Poland *et al.*, 1996). Embora não haja comprovação estatística entre maior risco de óbito por doenças virais em gatos FIV, óbitos em gatos FIV positivos e com pneumonia associada a FCV e/ou FHV são relatados (De Mello *et al.*, 2023). Embora estes vírus possam ser isolados de gatos saudáveis, estudos anteriores relataram que coinfeções entre FIV, FCV e FHV eram mais prevalentes em gatos com doença do trato respiratório superior (Binns; Dawson, *et al.*, 1995; Najafi *et al.*, 2014). Em outro estudo, o FIV foi relatado em 24% das

pneumonias fatais em gatos infectados concomitantemente com outros patógenos (Slaviero *et al.*, 2021).

A imunossupressão do FIV no trato respiratório é principalmente pelo declínio de linfócitos T CD4, uma vez que essas células, uma das principais envolvidas na defesa imunológica adaptativas do pulmão na formação de resposta tanto humoral quanto celular, são o principal alvo do vírus (Cohn; Reiner, 2007; Hosie *et al.*, 2009). A imunossupressão do FeLV é relacionada à linfopenia com perda tanto de células CD4 quanto CD8, além de neutropenia (Ogilvie *et al.*, 1988). Ainda, ocorre o desenvolvimento de neutrófilos e macrófagos defeituosos, os quais atuam normalmente como os principais mecanismos fagocíticos de defesa de alvéolos, contribuindo com a morte por infecções secundárias/opportunistas em gatos FeLV-positivos (Ogilvie *et al.*, 1988; Cohn; Reiner, 2007). Além disso, o vírus da FeLV diminui a resposta IgG, a qual junto da IgM possui um papel importante na imunidade local nas porções respiratórias de condução do ar (narinas a brônquios extrapulmonares e intrapulmonares), e transicional (bronquíolos), especialmente na prevenção da aderência de patógenos ao sistema ciliar (López; Martinson, 2017). Adicionalmente, FIV/FeLV não só predis põem à ocorrência de pneumonias na espécie, como possuem um papel na severidade da enfermidade (Dear, 2020). Ainda, uma maior prevalência e aumento da gravidade da inflamação oral tem sido relatada em gatos coinfectados com FIV e FCV, ou com FeLV e FCV (Tenorio *et al.*, 1991).

### **3 RESULTADOS**

Os resultados da presente tese serão apresentados na forma de artigos, conforme os sub-  
itens abaixo por ordem cronológica de realização:

#### **3.1 Artigo 1**

“Generalized and fatal felid alphaherpesvirus-1 natural infection with liver involvement in a feline leukaemia virus-positive adult cat: a case report”

#### **3.2 Artigo 2**

“Pathological patterns, occurrence, and distribution of viral antigen in the lower respiratory tract of cats with feline infectious peritonitis”

#### **3.3 Artigo 3**

“Occurrence and pathological findings of feline herpesvirus and feline calicivirus in the respiratory tract and upper digestive tract of cats”

### 3.1 Artigo 1

Neste item é apresentado o artigo intitulado “**Generalized and fatal felid alphaherpesvirus-1 natural infection with liver involvement in a feline leukaemia virus-positive adult cat: a case report**”. Este artigo encontra-se publicado no periódico científico “*Veterinary Research Communications*”, , v. 46, n. 4, p. 1319-1324, 2022, com endereço de DOI: <https://doi.org/10.1007/s11259-022-09977-6> .

**“Generalized and fatal felid alphaherpesvirus-1 natural infection with liver involvement in a feline leukaemia virus-positive adult cat: a case report”**

Mônica Slaviero<sup>1\*</sup>, Luiza Presser Ehlers<sup>1</sup>, Bruno Albuquerque de Almeida<sup>1</sup>, Paula Reis Pereira<sup>1</sup>, Welden Panziera<sup>1</sup>, Fernanda Vieira Amorim da Costa<sup>2</sup>, Saulo Petinatti Pavarini<sup>1</sup>, Luciana Sonne<sup>1</sup>

1 Department of Veterinary Pathology, College of Veterinary Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.

2 Department of Animal Medicine, College of Veterinary Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

\* Corresponding author:

Mônica Slaviero

Department of Veterinary Pathology, College of Veterinary Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Postal Address: Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil.

E-mail: monicaslav.vet@gmail.com

**Abstract**

Generalized and fatal felid alphaherpesvirus-1 (FeHV-1) natural infection with liver involvement is rarely reported in cats, and the occurrence of herpesvirus viraemia with internal organ histologic lesions in adult cats is unknown. A 1.5-year-old cat, female, mixed breed, positive for feline leukaemia virus (FeLV) presented in a veterinary teaching hospital with sneezing, nasal discharge, anorexia, and diarrhoea after two weeks, evolving to inspiratory dyspnoea. Complete blood count and serum biochemistry analysis showed marked leukopenia and thrombocytopenia. After clinical worsening and lack of treatment response, the cat was euthanized. Pathological findings included hepatic necrosis, fibrinonecrotic tracheitis, and bronchointerstitial pneumonia. Marked amounts of coccobacillary bacteria were observed covering the necrotic tracheal and bronchial mucosa, at the cytoplasm of alveolar macrophages, and free in alveoli lumen, mimetizing a primary bacterial tracheitis and pneumonia. Both lung and tracheal bacteria exhibited marked immunolabeling in anti-*Escherichia coli* immunohistochemistry. In addition, rare epithelial cells of bronchi contained

round, eosinophilic, intranuclear viral inclusion bodies (4-7  $\mu\text{m}$ ) that marginate the chromatin, characteristic of FeHV-1 infection. Strong multifocal anti-FeHV-1 immunolabeling was observed in necrotic epithelial cells of the liver, trachea, and lungs. Generalized herpesviruses infection with the occurrence of acute hepatic necrosis and severe respiratory illness is a potential differential diagnosis in FeLV-positive cats with respiratory signs. The immunodepression in these cats probably favours a FeHV-1 viraemia in addition to the development of opportunistic bacterial infections, such as *Escherichia coli*, and it is associated with a poor outcome.

Keywords: Cats, Herpesviridae infections, Viral hepatic necrosis, Herpesvirus viraemia, Retroviruses, Immunohistochemistry, Case report.

### **Resumo**

Infecção generalizada natural e fatal por herpesvírus felino tipo-1 (FeHV-1) com envolvimento hepático é raramente reportada em gatos, e a ocorrência de viremia por herpesvírus com lesões histológicas em órgãos internos em gatos adultos é desconhecida. Um gato fêmea, 1,5 anos, sem raça definida, positiva para vírus da leucemia felina (FeLV), foi atendida em um hospital veterinário de ensino com espirros, descarga nasal, anorexia e diarreia há duas semanas, evoluindo para dispneia inspiratória. Análise hematológica e bioquímica demonstraram acentuada leucopenia e trombocitopenia. Após piora clínica e falta de resposta ao tratamento, o gato foi eutanasiado. Achados patológicos incluíram necrose hepática, traqueíte fibrinonecrótica e pneumonia broncointersticial. Acentuada quantidade de agregados bacterianos cocobacilares foram observados recobrando a mucosa necrótica da traqueia e brônquio, no citoplasma de macrófagos alveolares e livres no lúmen alveolar, mimetizando uma pneumonia e traqueíte primárias bacterianas. Tanto o pulmão quanto a traqueia exibiram acentuada imunomarcção anti-*Escherichia coli*. Adicionalmente, raras células epiteliais de brônquios continham corpúsculos de inclusão arredondados, eosinofílicos e intranucleares (4–7  $\mu\text{m}$ ) que marginavam a cromatina periféricamente, característico de infecção por FeHV-1. Acentuada e multifocal imunomarcção anti-FeHV-1 foi observada em células epiteliais necróticas do fígado, traqueia e pulmão. Infecção generalizada por herpesvírus com ocorrência de necrose hepática aguda e doença respiratória grave é um potencial diferencial diagnóstico diferencial em gatos FeLV com sinais respiratórios. A imunodepressão nesses gatos provavelmente favorece a viremia de FeHV-1 e o desenvolvimento

de infecções bacterianas oportunistas, como *Escherichia coli*, o que pode estar associado a um pior prognóstico.

Palavras-chave: gatos, infecções por herpesvírus, necrose hepática viral, viremia por herpesvírus, retrovíroses, imuno-histoquímica, relato de caso.



## Introduction

Felid alphaherpesvirus 1 (FeHV-1) is a double-stranded DNA virus belonging to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. This virus is highly contagious with worldwide distribution (Gaskell et al. 2007). FeHV-1 is considered one of the main causes of acute upper respiratory tract disease in cats, as well as lifelong infection via latency with possible reactivation (Gaskell et al. 2007). Despite widespread vaccination, FeHV-1 infections remain a permanent risk, especially in multi-cat situations due to high challenges or in immunocompromised animals (Thiry et al. 2009).

FeHV-1 is an epitheliotropic and cytopathic virus mainly related to ulcerative and necrotic rhinitis, sinusitis, rhinotracheitis, conjunctivitis, and keratitis in cats, although it can also cause ulcerative lesions in the skin and oral mucosa (Thiry et al. 2009; Argenta et al. 2017). In more severe cases, FeHV-1 infections can also cause bronchointerstitial pneumonia, especially in kittens and immunocompromised adult cats, with high mortality (Chvala-Mannsberger et al. 2009; Monné Rodriguez et al. 2017; Slaviero et al. 2021).

Generalized infections by other alphaherpesviruses with liver involvement are commonly reported in different species, especially in puppies or equine and bovine foetuses (Van Maanen 2002; Crook et al. 2012; Greene 2012); however, in cats, this presentation is poorly described. Scarce cases of generalized FeHV-1 infections with liver and lung involvement in feline foetuses and kittens are reported in experimental studies in the 70s, following intravenous or genital FeHV-1 inoculation of the pregnant queen (Bittle and Peckham 1971; Hoover and Griesemer 1971). Generalized infections after FeHV-1 replication in natural sites still have contradictions. Although the absence of viraemia has been reported in cats inoculated intranasally (Hoover and Griesemer 1971), a brief period of viraemia without systemic lesions has occurred in cats undergoing primary disease (Westermeyer et al. 2009). Additionally, natural generalized FeHV-1 infection with liver necrosis in kittens is reported in some studies also in the 70s (Spradbrow et al. 1971; Shields and Gaskin 1977), and in one kitten from a study of FeHV-1 pneumonia (Chvala-Mannsberger et al. 2009); however, the occurrence of this presentation in adult cats is uncertain.

This report describes a case of hepatic necrosis, tracheitis, and pneumonia associated with a fatal FeHV-1 natural infection in a feline leukaemia virus (FeLV)-positive adult cat, associated with opportunistic *Escherichia coli* infection, using histopathology and immunohistochemistry as diagnostic techniques.

### Case presentation

A 1.5-year-old female mixed-breed cat, 2 kg, with outdoor access, was presented to a veterinary teaching hospital with a history of sneezing, nasal discharge, anorexia, and diarrhoea for two weeks. On physical examination, the cat had a poor body condition score, fever (40°C), prostration, mucopurulent nasal and ocular discharge, and inspiratory dyspnoea. The animal was hospitalized for treatment (day 0). Data on vaccinal status was not available.

Complete blood count and serum biochemistry analysis showed marked leukopenia (1500/mm<sup>3</sup>; reference interval: 5500-19500/mm<sup>3</sup>) and thrombocytopenia (90.000/mm<sup>3</sup>; reference interval: 200.000-377.000/mm<sup>3</sup>). Differential analysis was not performed due to intense leukopenia. Thorax radiography and abdominal ultrasound were performed; however, without significant alterations. A second complete blood count was performed on day +4, see **Online Resource 1**. An even greater decrease in white blood cell count was observed (750/mm<sup>3</sup>). Snap test for feline immunodeficiency virus (FIV) and FeLV were performed, and the cat was positive for FeLV infection and negative for FIV (SNAP FIV Antibody/ FeLV Antigen Combo, IDEXX Laboratories, Westbrook, ME, USA).

Treatment consisted of fluid therapy with sodium lactate Ringer's solution, amoxicillin with potassium clavulanate (20 mg/kg SC q12h), acetylcysteine (70 mg/kg IV q8h), dipyrone (25 mg/kg IV q12h), cobamamide with cyproheptadine (1000 µg/animal PO q12h and 2 mg/animal PO q12h, respectively), ranitidine (1 mg/kg SC q12h), vitamin B complex (Bionew® 0,2 mg/kg IV q24h), oxygen therapy and nebulization with saline solution three times daily. After day +4, an antiemetic drug was added due to constant vomiting (metoclopramide 0.5 mg/kg IV q8h), and antibiotic therapy was extended to ampicillin (20 mg/kg IV q8h), metronidazole (15 mg/kg IV q12h), and enrofloxacin (5 mg/kg IV q12h). After clinical worsening and no response to the treatment, euthanasia was performed on day +6 and the cat was subsequently submitted to a complete post-mortem examination.

At necropsy, the cat exhibited poor body condition and marked pale mucous membranes. Grossly, the lungs were uncollapsed, with focal cranioventral consolidation. The tracheal mucosa presented marked deposition of

friable yellow material, and the liver had pale random areas. Samples of the trachea, lung, liver, heart, bone marrow, thyroid glands, pancreas, brain, cerebellum, spleen, stomach, small intestine, large intestine, adrenal glands, kidneys, and urinary bladder were collected, fixed in 10% neutral buffered formalin, routinely processed for histology, and stained with haematoxylin and eosin (HE). Lung, tracheal, and liver sections were submitted to immunohistochemistry (IHC) anti-FeHV-1, anti-feline calicivirus (FCV), anti-*E. coli*, and anti-caspase 3 (apoptosis marker). IHC anti-FeLV was performed in a bone marrow section to confirm FeLV infection. IHC antibodies and protocols used were based on previous published literatures (**Online Resource 2**) and are reported in **Table 1**. Universal HRP-Polymer (MACH 4, Biocare Medical®) was used as a detection system in all cases. Reactions were revealed with 3-amino-9-ethylcarbazol chromogen (AEC, Dako®). Positive controls for IHC comprised tissues previously confirmed as positive for the respective antigens. As a negative control, the primary antibody was replaced by Universal Negative Control Serum (Biocare Medical CA, USA).

Histological examination of the liver showed multifocal, random, foci of coagulative necrosis of hepatocytes, which cover 40% of the parenchyma in the analysed sections (**Fig.1A**). These foci presented strong and multifocal anti-FeHV-1 immunolabeling (**Fig.1B**). In the respiratory tract, the tracheal mucosa was diffuse and markedly necrotic, associated with fibrin deposition (**Fig.2A**), and intense amounts of coccobacillary bacteria covering mucosa epithelial cells. The submucosa was thickened by fibrin deposition, and an inflammatory infiltrate of lymphocytes, plasma cells, macrophages, and neutrophils. Thrombosis was also observed in the blood vessels of the submucosa. Microscopic evaluation of the lung exhibited multifocal and marked necrosis of bronchial and bronchiolar epithelial cells associated with fibrin deposition. Pneumocytes and airway epithelial cells sometimes presented with shrunken cytoplasm, pyknotic or fragmented nuclei. Alveolar, bronchial, and bronchiolar lumen contained varying amounts of fibrin, oedema, degenerate neutrophils, sloughed epithelial cells, and macrophages (**Fig.2B**). Rare epithelial cells of bronchi contained round, eosinophilic, intranuclear viral inclusion bodies (4-7  $\mu\text{m}$ ) that marginate the chromatin, characteristic of FeHV-1 infection. Strong multifocal anti-FeHV-1 immunolabeling was observed in the necrotic epithelium of the trachea, bronchi, and bronchioles, in the intranuclear inclusion bodies, in the cytoplasm of peribronchial glands, pneumocytes, and macrophages (**Fig. 2C and D**). Intense amounts of coccobacillary bacteria were observed covering the necrotic bronchial mucosa, at the cytoplasm of alveolar macrophages, and free in the

alveoli lumen. Both lung and tracheal bacteria exhibited marked anti-*E. coli* immunolabeling (see **Online Resource 3, Supplementary Figure 1**). No immunolabeling for *E. coli* was observed in the liver parenchyma. No anti-FCV immunolabeling was observed in the trachea and lung. Expression of caspase-3 was observed in epithelial cells in the lung, and no immunolabeling was present in the liver (**Online Resource 3, Supplementary Figure 2**). Bone marrow cells showed strong multifocal and cytoplasmatic immunolabeling anti-FeLV, see **Online Resource 3, Supplementary Figure 3**.

## Discussion

Diagnosis of FeHV-1 and concomitant FeLV and *E. coli* infection in an adult cat in the present study was based on the clinical, pathological, and immunohistochemical findings. Ante-mortem diagnosis of herpesvirus as a causative agent of disease can be challenging. FeHV-1 seroprevalence in cats is high due to wide distribution among feline populations, by natural infections and vaccination, and the presence of antibodies does not correlate with disease and active infection (Maggs et al. 1999). Furthermore, routine PCR tests are not able to differentiate vaccine strains from natural infections (Burns et al. 2011). Post-mortem diagnosis only by histopathological analysis can also be difficult if characteristic inclusion bodies cannot be identified or if a marked amount of bacteria is present in the tissue resembling a primary bacterial infection, similarly to the present case. Therefore, the IHC technique is particularly useful to detect viral or bacterial antigens in characteristic lesions when formalin-fixed and paraffin-embedded tissues are available. Similarly, although *E. coli* may be involved in pneumonia in immunocompromised cats (Slaviero et al. 2021), it may also be an agent of the natural microbiota or a bacteria contaminating the tissue sample (Greene 2012). Thus, to confirm the association of disease with bacteria, the IHC technique was an important diagnostic tool.

The detection of FeHV-1 antigen by IHC technique in the liver with necrotic lesions in the FeLV-positive cat of the present study is an important indication of the possibility of herpesvirus viraemia following natural infection in immunocompromised cats. Alphaherpesviruses are known to have tropism for epithelial cells and cytolytic effect, and FeHV-1 primary replication is recognized to occur in tissues with low body temperature, mainly in the upper respiratory tract, and eyes (Gaskell et al. 2007). High morbidity and low mortality are usually expected (Thiry et al.

2009). However, in some cases, pneumonia may occur, particularly in kittens, young cats, or immunocompromised adults (Chvala-Mannsberger et al. 2009; Monné Rodriguez et al. 2017; Slaviero et al. 2021), and a poor outcome is expected. In the present case, the alterations observed in the respiratory tract are similar to those previously described (Chvala-Mannsberger et al. 2009; Monné Rodriguez et al. 2017; Slaviero et al. 2021). In contrast, the hepatic necrosis presentation is an unexpected finding.

Generalized herpesvirus infections with hepatic necrosis are commonly described in puppies and aborted bovine and equine foetuses with congenital infections (Van Maanen 2002; Crook et al. 2012; Greene 2012). In cats, scarce studies report hepatic necrosis in foetuses and kittens after inoculation of FeHV-1 in pregnant queens (Bittle and Peckham 1971; Hoover and Griesemer 1971). Although no viraemia was observed in pregnant cats inoculated intranasally (Hoover and Griesemer 1971), hepatic necrosis associated with respiratory disease after natural infection has been reported in kittens from catteries or colonies of experimental cats (Spradbrow et al. 1971; Shields and Gaskin 1977), similar to the present case. Emerging evidence suggests that cats infected with FeHV-1 may experience a brief viraemic phase beyond the primary infected tissues (Westermeyer et al. 2009), spreading the virus to distant connective tissues (Swenson et al. 2012) or abdominal organs (Hora et al. 2013); however, in contrast to our study, no histological lesions are reported in these cases.

FeLV-positive cats tend to have a higher frequency or severity of infections than cats with no retroviruses (Greene 2012; Slaviero et al. 2021). The severity of the respiratory condition and fatal outcome in the present case are probably related to primary FeHV-1 damage associated with the immunocompromised state of the host and secondary *E. coli* infection. Although the differential analysis was not possible in the present case, neutropenia and lymphopenia are commonly observed in FeLV-positive cats. While the lymphopenia occurs by the destruction of lymphocytes through direct viral replication, neutropenia is most likely due to virus-induced immune-mediated mechanism (Greene 2012). Neutrophils in FeLV-positive cats may also have decreased chemotactic and phagocytic function. In addition to low neutrophil counts, this favours bacterial infections (Greene 2012), and probably favours the *E. coli* infection in the present case. Thus, it is important for practicing veterinarians to perform complete blood counts and to consider FeLV coinfection in adult cats with respiratory signs and cytopenias. Although qPCR was not performed, positive immunochromatographic assay and detection of viral envelope protein (gp70) in bone marrow

cells in association with leukopenia are strong indicators of progressive FeLV, which favours the development of coinfections (Hofmann-Lehmann and Hartmann 2020), as observed in the present study.

The pathological and immunohistochemical findings observed in the present case demonstrate that there are two patterns of cell death caused by FeHV-1. Tracheitis and pneumonia are probably a consequence of continuous cell-to-cell viral spread from the upper airways, and the expression of caspase-3 in these tissues confirm cell death by apoptosis, as described by Monné Rodriguez et al. (2017), although necrosis also appears as result from virus-induced neutrophil influx (Monné Rodriguez et al. 2017). On the other hand, liver damage appears to be related to coagulative necrosis, as a result of viral replication, with no evidence of apoptosis. The moderate coagulative necrosis without cellular response and no increase in hepatic enzymes indicate acute cell death, possibly secondary due to viraemia. In these cases, the virus probably spreads from the respiratory tract to other tissues by circulating leukocytes, similar to that suggested in cattle (Uzal 2016) and FeHV-1-infected young cats (Tham and Studdert 1987). However, studies with more cats are needed for a better understanding of the pathogenesis of systemic feline herpesvirus infections. In the differential diagnosis of liver injury, it is important to consider other infectious causes. A novel hepadnavirus has been described as a cause of liver injury in domestic cats; however, these cases are often associated with chronic inflammation or hepatocellular carcinoma (Pesavento et al. 2019).

Unfortunately, the source of FeHV-1 infection, in this case, is unknown. Recrudescence of FeHV-1 usually causes mild disease, although concurrent infection with immunosuppressive viruses may lead to more severe disease (Greene 2012). However, viraemia appears to be important in the pathogeny of primary herpetic disease and is less likely to contribute to the pathogeny of recrudescence herpetic disease (Westermeyer et al. 2009), suggesting a primary infection in the present case.

The FeLV immunosuppression in the present case probably favoured the development of severe respiratory infection and FeHV-1 viraemia, leading to hepatic necrosis. The marked leukopenia and *E. coli* secondary infection possibly contributed to the poor outcome. Thus, in addition to cat foetuses, neonates, and kittens, the possibility of viraemia and generalized disease by FeHV-1 in adult cats should be considered in FeLV-positive cats with respiratory signs, even as isolated cases.

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**Data availability statements: 5.** All data generated or analysed during this study are included in this published article and its supplementary information files.

**Ethics approval:** We authors of the article entitled “Generalized and fatal felid alphaherpesvirus-1 natural infection with liver involvement in a feline leukaemia virus-positive adult cat: a case report” declared, for all due purposes, the project was approved by the Research Committee (COMPESQ) of the Universidade Federal do Rio Grande do Sul under number 41935. Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

**Consent to participate:** Not applicable.

**Consent to publish:** Not applicable.

### Figure captions

**Fig 1** Felid herpesvirus-1 (FeHV-1) hepatic necrosis in an adult feline leukaemia virus-positive cat. (A) Liver. A focus of coagulative necrosis in the liver parenchyma. Hepatocytes are often anucleated with preserved cell outlines, and opaque to intense cytoplasmic eosinophilia is observed in necrotic cells. Haematoxylin and eosin stain (HE), 200x magnification. (B) Liver. Strong anti-FeHV-1 immunolabelling is observed in necrotic hepatocytes. Immunohistochemistry (IHC), AEC chromogen, 200x magnification

**Fig 2** Respiratory findings in an adult feline leukaemia virus-positive cat with felid herpesvirus-1 (FeHV-1) infection and secondary bacterial infection. (A) Trachea. Marked necrosis of the mucosa (asterisk) is associated with cellular debris and fibrin deposition. The submucosa is diffusely thickened by inflammatory infiltrate that surrounds the submucosal glands and by fibrin deposition (arrow). Multifocal thrombosis is also noted (arrowhead). Haematoxylin and eosin stain (HE), 100x magnification. (B) Lung. Bronchioles and adjacent alveolar spaces occluded by a marked inflammatory infiltrate. HE, 100x magnification. (C) Trachea. Strong and multifocal anti-FeHV-1 immunostaining in necrotic mucosa and epithelial cells of submucosal glands. Immunohistochemistry (IHC), AEC chromogen, 100x magnification. (D) Lung. Strong and multifocal immunolabelling of FeHV-1 in necrotic bronchial epithelial cells (asterisk) and peribronchial glands (arrowhead). IHC, AEC chromogen, 100x magnification

## 5.2 Artigo 2

Neste item é apresentado o artigo intitulado “**Pathological findings and patterns of feline infectious peritonitis in the respiratory tract of cats**”. Este artigo encontra-se publicado no periódico científico “*Journal of Comparative Pathology*, v. 210, p. 15-24, 2024, com endereço de DOI: <https://doi.org/10.1016/j.jcpa.2024.02.001>.”

**“Pathological findings and patterns of feline infectious peritonitis in the respiratory tract of cats”**

Mônica Slaviero, Fernanda G Cony, Rodrygo C da Silva, Cíntia De Lorenzo, Bruno A de Almeida, Marianna Bertolini, David Driemeier, Saulo P Pavarini, Luciana Sonne

Veterinary Pathology Laboratory, Department of Veterinary Clinical Pathology, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Corresponding author: M Slaviero, Veterinary Pathology Laboratory, Department of Veterinary Clinical Pathology, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9090 - Agronomia, Porto Alegre - RS, 91540-000, Brazil. E-mail address: monicaslav.vet@gmail.com

**Abstract**

Feline infectious peritonitis (FIP) is an important cause of death in cats. Thoracic manifestations are less common than abdominal, and FIP-associated respiratory disease is poorly documented. This study aimed to investigate pathological findings in the respiratory tract of FIP-positive cats, and the occurrence and distribution of feline coronavirus-antigen in the respiratory tract using immunohistochemistry (IHC). Clinical and epidemiological information on affected cats was also analysed. Data concerning FeLV and FIV infection were collected. A retrospective study was carried out on 112 cats with FIP, of which 66 presented inflammatory histological lesions in the respiratory tract (58.9%) and were included in this study. Three major gross patterns were defined: marked fibrin deposition in the thoracic cavity with lung atelectasis; marked fibrin deposition in the thoracic cavity with lung pyogranulomas; and lung pyogranulomas without thoracic effusion. Histological analysis showed primary lesions in the visceral pleura and lung parenchyma at a similar frequency, with multifocal to diffuse presentations. Marked lesions were commonly observed. Five major histological patterns were defined: pleuritis; pleuritis and vasculitis/perivascular injury in the lung parenchyma; pleuritis and pneumonia; perivascular injury in the parenchyma without pleuritis; and pneumonia without pleuritis. FIP-antigen was detected in macrophages of the pleura and lung parenchyma (perivascular, peribronchial, foci of necrosis and inflammation, BALT). Co-infections with retroviruses were detected in 47 cats (71.2%), mainly FeLV (62.2%). Although FIP is a systemic disease, some cats developed significant lesions in the

thoracic cavity, including involvement of the upper respiratory tract and presenting respiratory signs, without other classic signs of FIP. This work contributes to furthering knowledge of FIP in the respiratory system, helping veterinarians to recognize the different presentations of this disease.

Keywords: feline coronavirus, pneumonia, pleuritis, retrospective studies, immunohistochemistry.

## **Resumo**

A peritonite infecciosa felina (PIF) é uma importante causa de morte em gatos. As manifestações torácicas são menos comuns que as abdominais, e a doença respiratória associada à PIF é pouco documentada. Este estudo teve como objetivo investigar achados patológicos no trato respiratório de gatos positivos para PIF e a ocorrência e distribuição do antígeno do coronavírus felino no trato respiratório por meio de imuno-histoquímica (IHQ). Também foram analisadas informações clínicas e epidemiológicas dos gatos afetados. Foram coletados dados referentes à infecção por FeLV e FIV. Foi realizado um estudo retrospectivo em 112 gatos com PIF, dos quais 66 apresentavam lesões histológicas inflamatórias no trato respiratório (58,9%) e foram incluídos neste estudo. Foram definidos três padrões macroscópicos principais: deposição acentuada de fibrina na cavidade torácica com atelectasia pulmonar; deposição acentuada de fibrina na cavidade torácica com piogranulomas pulmonares; e piogranulomas pulmonares sem efusão torácica. A análise histológica mostrou lesões primárias na pleura visceral e no parênquima pulmonar em frequência semelhante, com apresentações multifocais a difusas. Lesões acentuadas foram comumente observadas. Foram definidos cinco padrões histológicos principais: pleurite; pleurite e vasculite/lesão perivascular no parênquima pulmonar; pleurite e pneumonia; lesão perivascular no parênquima sem pleurite; e pneumonia sem pleurite. O antígeno PIF foi detectado em macrófagos da pleura e do parênquima pulmonar (perivascular, peribrônquica, focos de necrose e inflamação, BALT). Coinfecções com retrovírus foram detectadas em 47 gatos (71,2%), principalmente FeLV (62,2%). Embora a PIF seja uma doença sistêmica, alguns gatos desenvolveram lesões significativas na cavidade torácica, incluindo envolvimento do trato respiratório superior e apresentando sinais respiratórios, sem outros sinais clássicos de PIF. Este trabalho contribui para aprofundar o conhecimento da PIF no sistema respiratório, ajudando os veterinários a reconhecer as diferentes apresentações desta doença.

Palavras-chave: coronavírus felino, pneumonia, pleurite, estudos retrospectivos, imunohistoquímica

## 1. Introduction

Feline infectious peritonitis (FIP) is one of the most important infectious diseases in feline medicine and a cause of death in cats, with a worldwide distribution, mainly affecting young cats from shelters and catteries [1]. It is caused by a mutant virulent biotype of feline coronavirus (FCoV) known as feline infectious peritonitis virus (FIPV), where there is a switch from primary viral replication in enterocytes to replication and activation in circulating monocytes, transforming a local infection into a systemic one [2,3,4].

FIP pathology is related to generalised pyogranulomatous inflammation in tissues associated with vasculitis, mainly due to inflammation of veins [5]. Differentiation between the “wet” and “dry” forms has been used to differentiate cases with effusion from those with only pyogranulomas, but both are the same disease entity [1]. Clinical signs differ according to the affected tissues but commonly include abdominal distention [1]. Respiratory signs are less observed, with dyspnoea being more commonly related to pleural effusion [6].

Pathological findings in FIP-positive cats are well-established in the literature; however, there is little information about FIP-associated respiratory disease. It is known that FIP can cause pyogranulomatous pneumonia [6,7,8], but the importance of FIP for the respiratory system is still poorly understood. The lungs are not considered a primary target of FIP, and its involvement is often considered to be a consequence of systemic disease, generally with localized lesions in the parenchyma underlying the pleura [9,10]. However, some cats seem to show upper respiratory signs

such as sneezing and nasal discharge at an early stage of FIP [11–14]. Also, a recent study reports a case of pyogranulomatous rhinitis associated with local immunohistochemical detection of feline infection peritonitis virus (FIPV), suggesting that respiratory infection is part of the FIP-associated disease spectrum [14].

Thus, this study aimed to investigate pathological findings in the respiratory tract of cats with FIP infection, the occurrence and distribution of FIPV antigen in the lung identified by immunohistochemistry, and to define lung injury patterns in these cats.

## **2. Material and Methods**

### *2.1 Selection of cases and clinical-epidemiological information*

A retrospective study of respiratory lesions associated with FIP infection in domestic cats was carried out by analysing necropsy reports filed at the Department of Veterinary Pathology, Universidade Federal do Rio Grande do Sul, Porto Alegre, in Southern Brazil. The database was searched for cases diagnosed as FIP in the veterinary pathology laboratory between January 2010 and December 2022. The necropsy reports were reviewed, and the description of the lung was analysed in each case. Cases that presented histological inflammatory lesions in the pleura and lung were included in the study. Cases with only circulatory alterations in these tissues, such as oedema and congestion, were excluded.

Information about the breed, sex, and age were collected. Age was further divided into: kitten (0-1 year-old), young adult (1-6 year-old), mature adult (7-10 year-old), and senior (>10 year-old) [15]. Additionally, information about feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infection detected by IHC were collected from the clinical forms and data

from De Mello et al., 2023 [16]. When available, clinical history and the reason for clinical care were assessed.

## *2.2 Gross analysis*

Necropsy photographs and reports were reviewed for the standardization of gross findings in the lungs, and evaluation of the occurrence of effusions (pleural, pericardial, abdominal, or none). It was analysed whether the thoracic cavity had the most significant gross lesions in relation to the other cavities; whether thoracic injuries were concomitant with abdominal/central nervous system injuries; and if there were no gross lesions in the thorax. The cases were divided into three gross patterns: 1- thoracic cavity filled with marked deposition of liquid, fibrillar, gelatinous or pasty material in the visceral and parietal pleura. Pulmonary parenchyma was atelectatic, without other parenchymal alterations. 2 - thoracic cavity filled with marked deposition of liquid, fibrillar, gelatinous or pasty material in the visceral and parietal pleura. Uncollapsed pulmonary parenchyma, with multiple punctate white nodules randomly distributed throughout the lung lobes. 3 - little or non-existent fluid in thoracic cavity content. The lung lobes were not collapsed. The lung parenchyma often had marked pallor colour, with multiple white nodules randomly distributed.

## *2.3 Histological analysis*

Histological descriptions of the necropsy reports were reviewed for assessment of tissues affected in the selected cases. Histological slides of lung tissue were examined when available, or paraffin-embedded tissues were retrieved, cut at 3  $\mu\text{m}$ , and stained with haematoxylin and eosin (HE) for light microscopic examination. Lesions in the pleura, blood vessels, airways, and alveolar



spaces were evaluated. According to the histological features observed in the pleura and lung parenchyma, histopathological patterns were observed as: 1A – diffuse pleuritis; 1B – multifocal pleuritis; 2 – pleuritis and perivascular injury; 3 – pleuritis and pneumonia; 4 – perivascular injury; 5 – pneumonia. When available, portions of the upper respiratory system with concomitant pulmonary lesions were also analysed. The intensity of pulmonary lesions in the available histological sections was analysed by two veterinary pathologists and categorized into mild (<10% involvement of the lung parenchyma in the analysed sections), moderate (10-50%), and severe (>50%).

### *2.3 Immunohistochemical analysis*

The occurrence, distribution, and intensity of FIPV antigen in the lung sections were evaluated with immunohistochemistry using an anti-feline monoclonal coronavirus antibody (FIPV3-70, Santa Cruz Biotechnology, Dallas, Texas, USA; 1:200 dilution). Endogenous peroxidase activity was blocked by incubating tissue sections in a 10% solution of hydrogen peroxide in methanol for 15 minutes. The viral antigen was retrieved by boiling the sections for 40 min at 96°C in a digital pressure cooker in Tris-EDTA buffer (pH 9.0). To block nonspecific reactions, the cuts were treated with 5% skimmed milk diluted in distilled water for 15 minutes at room temperature. Novolink™ Max Polymer Detection System (Leica Biosystems Newcastle upon Tyne, UK) was used as a detection system in all cases. Reactions were revealed with 3-amino-9-ethylcarbazol chromogen (AEC, Dako®), and the slide was counterstained with Mayer's haematoxylin. Positive IHC control comprised of previously confirmed as positive tissues for the respective antigens. As a negative control, the primary antibody was replaced by Universal Negative Control Serum (Biocare Medical CA, USA).

The distribution was evaluated according to the regions of the lung in which immunostaining was observed (pleura, perivascular, lung parenchyma, peribronchial and bronchial-associated lymphoid tissue). The intensity of the immunolabeling in the lung sections was analysed by three veterinary pathologists and categorized into mild (up to 20 immunolabeled cells in ten highest magnification fields (HPF), 2,37mm<sup>2</sup>); moderate (21-50 cells); marked (>50 macrophages). Additionally, data about previous immunohistochemistry in tissues other than the lung was also collected when available.

#### *2.4 Statistical analysis*

An association of nominal categorical variables of histological pattern and retroviral status, lesion intensity and retroviral status, histological pattern and lesion intensity, and histological patterns and gross pattern was tested using the Chi-Square Test or Fisher's Exact Test. The result of Fisher's Exact Test was used when the number of cells with expected results less than 5 was greater than 25%. In cases where the result was statistically significant, a comparison between columns was performed using the Z Test with Bonferroni correction. Statistical significance was assigned when P values were less than 0.05. Analysis were performed using the commercial software IBM SPSS Statistics for Windows (Version 22.0, IBM Corp., Armonk, NY).

### **3. Results**

#### *3.1 Cases and clinical-epidemiological information*

From January 2010 to December 2022, 2,089 post-mortem examinations were carried out on cats. FIP was identified as the cause of death in 112 (5.4%) cats. Sixty-six of these cats (58.9%) had histological inflammatory lesions in the lung and pleura and were included in the study.

Most of the cats were mixed-breed (59; 89.4%), four (6.1%) were Persian, one (1.5%) Siamese, one Maine Coon (1.5%), and one Scottish Fold (1.5%). Twenty-five of these cats were kittens (37.9%), 35 young adults (53.1%), three mature adults (4.5%), and three seniors (4.5%) (Rate three months to 16 years; median 1 year; mean 4.5 years). Forty-two (63.6%) cats were male and 24 (36.4%) were female. There were 41 FeLV-positive cats (62.2%), three FIV-positive cats (4.5%), three FIV and FeLV-positive cats (4.5%), and 19 cats (28.8%) with no retroviruses.

In 23 cats (23/66; 34.8%) it was reported that the reason for clinical care involved respiratory signs. These signs involved sneezing and nasal discharge in eight cats, of which six had no effusion. Other 12 cats had dyspnoea secondary to pleural effusion and one cat had dyspnoea without effusion. In the other cats (43/66), the reasons were mainly abdominal distension, neurological signs, apathy, and anorexia.

### *3.2 Gross findings*

Forty-nine of the 66 cats (74.2%) with respiratory lesions had effusions distributed in the thoracic, pericardial, and/or abdominal cavity. The other 17 cats did not have effusion in any of their body cavities. Thoracic effusion occurred in 30 cats (45.5%) and often affected both the left and right hemithorax. The complete distribution of effusions can be seen in the **Fig. 1** and **Supplementary Table 1**.

Three main gross patterns of injury could be observed in the lungs. Of the 66 cats, 18 of them presented the thoracic cavity filled with marked deposition of yellow to reddish liquid, fibrillar, gelatinous or pasty material in the visceral and parietal pleura. The pulmonary parenchyma was diffusely and markedly atelectatic with pleural irregularity, without inflammatory parenchymal alterations (Pattern 1 – **Fig. 2A-B**). Similarly, 13 cats showed marked deposition of

yellow liquid, fibrillar, gelatinous or pasty material in the visceral and parietal pleura. However, in these cases, the lung parenchyma was often not collapsed, with multiple white nodules of a few millimetres randomly distributed throughout the lung lobes (Pattern 2 - **Fig. 2C-D**). In another 23 cats, the deposition of yellowish fibrillar material in the thoracic cavity was little or non-existent. The lung lobes were not collapsed, sometimes with a rib impression. The lung parenchyma often had marked pallor colour, with multiple white nodules of a few millimetres randomly distributed (Pattern 3 - **Fig. 2E-F**). Of these, four cats presented, in addition to the characteristics already mentioned, areas of cranioventral consolidation, or dark red multifocal areas in the lung parenchyma, which corresponded to secondary bacterial infection. One cat had marked thickening and irregularity of the visceral pleura, forming multiple nodules in the lung parenchyma, which was markedly pale, corresponding to parasitic infection. Finally, 12 cats did not present gross lesions in the lungs, of which five had abdominal effusions.

In 21 cats, the thoracic lesions were the main site of gross injury (in comparison with the abdominal cavity or the nervous system), 14 corresponding to the cats that presented previous respiratory signs. In six of these cats (6/21), the thoracic cavity was the only site of lesion. Additionally, four of these 21 cats also had alterations reported in the upper respiratory tract: three had thickening of the nasal turbinates associated with the deposition of yellowish material, and one had thickening of the larynx. Another 33 cats had thoracic injuries of the same intensity or lesser intensity than abdominal or CNS tissues.

### *3.3 Histological findings*

Of the 66 cats, the visceral pleura was involved in 48 cases (histological patterns 1, 2, and 3; **Fig. 3**) (72.7%) of which 40 were associated with effusion. The lung parenchyma was commonly

affected, with 50 cases presenting some degree of parenchymal injury (histological patterns 2, 3, 4, and 5; **Fig. 3**) (75.7%), 35 cases of these with pneumonia, and 15 only with vascular lesions.

Sixteen cats (24.2%) had only pleural involvement. Seven of these cats (10.6%) cases presented a diffuse, markedly thickening of the pleura (Pattern 1A, **Fig. 3A**), all associated with the occurrence of effusion. Nine cats (13.6%) had multifocal thickening in the pleura (Pattern 1B, **Fig. 3B**), three without any effusion. Twelve cats (18.2%) had pleuritis and pulmonary vasculitis/perivasculitis with transmural infiltrates of macrophages, neutrophils, and few lymphocytes (Pattern 2, **Fig. 3C**). Peribronchiolar blood vessels were frequently affected. The alveoli and airways in these cases showed no alterations. In addition to the pleura, the lung parenchyma of 19 cats (28.8%) showed marked pyogranulomatous or suppurative inflammation in alveolar spaces (Pattern 3, **Fig. 3D**) associated with vasculitis and/or airway inflammation (bronchopneumonia).

Nineteen cats (28.8%) did not present any lesions in the pleura (histological patterns 4 and 5). Of these, three cats (4.6%) had only pyogranulomatous vasculitis/perivasculitis in the parenchyma (Pattern 4, **Fig. 3E**). In the other 16 cases (24.2%), pneumonia was observed, with involvement of alveoli and airways (Pattern 5, **Fig. 3F**).

Histological findings of pneumonia can be observed in **Fig. 4**. Of the 35 cases of pneumonia (histological patterns 3 and 5), multifocal aggregates of coccoid bacterial concomitant with pyogranulomatous inflammation and vascular lesions were noted in four cases. Three of these cats with bacterial pneumonia were FeLV positive (one kitten and two 3-year-old adults) and one negative for retroviruses (kitten) (Cases 4, 20, 52 and 54 in Supplementary Table 1. In one case, a 3-year-old FeLV-positive cat, a marked amount of parasitic structures morphologically compatible

with *Aelurostongylus abstrusus* was also observed. In five cases, only bronchopneumonia with suppurative inflammation was observed.

Additionally, in the cases in which the upper respiratory tract was collected, three cats showed marked desquamation of the respiratory epithelium and thickening of the nasal stroma due to inflammatory infiltrate of neutrophils, lymphocytes, and macrophages. In the case where the larynx was collected, multifocal areas of mucosal necrosis and pyogranulomatous inflammation were observed.

Regarding the intensity of these lesions, 38 cats (57.6%) had these lesions markedly, with significant impairment of the lung parenchyma. It is important to point out that all 21 cases that had the main lesion in the thoracic cavity were included here. Four of the six cats with a gross lesion only in the thoracic cavity also presented pulmonary lesions only on histology. The other two cats also had inflammatory lesions of varying degrees in the abdominal viscera and central nervous system. Eleven cats (16.7%) had moderate lesions and 17 cases (25.7%) had mild lesions, the latter being mainly in the abdomen or central nervous system.

Illustrated and graphical comparison between histological and gross patterns can be seen in **Fig. 5 and Fig. 6**. Graphical comparison between histological patterns, lesion intensity, and retroviral status can be seen in **Fig. 6**.

### *3.5 Immunohistochemical findings*

Of the 66 cases, 59 cases had paraffin-embedded sections of the lung available and IHC was performed. In the other seven cases, anti-FIP IHC was previously performed in other tissues (nervous system and lymph node), but lung sections were not available. Of the 59 cases, 45 showed immunostaining in the lung for FIPV antigen. Of the four cases with upper respiratory tracts, two

were positive in the nasal cavity and one in the larynx. Of the 14 negative cases in the lung, seven corresponded to cases in which the histology showed bacterial pneumonia or bronchopneumonia lesions only (**Supplementary Table 1**). These cases had positive immunolabeling in other tissues, confirming FIP infection.

Multifocal, strong immunolabeling was observed in the cytoplasm of macrophages both in the pleura and in the lung parenchyma (**Fig. 7**). Immunolabeling only in the pleura was observed in 11/45 cases, 14/45 only in the lung parenchyma, and 20/45 in both. In the pleura, immunolabeling was observed in macrophages associated with the pyogranulomatous inflammatory infiltrate; in areas with connective tissue proliferation, the IHC failed to show viral antigen. In the lung parenchyma, this immunolabeling was observed mainly in macrophages adjacent to blood vessels of medium to large calibre (17/45), to the epithelium of bronchi and bronchioles (10/45), and in foci of necrosis and pyogranulomatous inflammation in alveolar septa and alveolar spaces (21/45). Less frequently, FIP antigen was observed in macrophages of the bronchial lumen (2/45) and bronchial-associated lymphoid tissue (BALT) hyperplasia (2/45). Marked immunolabeling was observed in 20/45 of these cases, 10/45 moderate, and 15/45 mild (**Supplementary Table 1**). Positive immunolabeling in macrophages was observed in the larynx and stroma nasal in 2/3 cases.

### *3.6 Statistical analyses results*

The results indicated that there is an association between gross and histological patterns (p-value <0.001). Gross pattern 1 was more associated with histological patterns 1 and 2, while gross pattern 2 was more related to histological pattern 3 (**Fig. 6 and Supplementary Table 2**). No association between the histological pattern and the retroviral status (p-value:0.974), between the

intensity of the histological lesion and the retroviral status (p-value:0.112), or between the intensity of the histological lesion and histological pattern (p-value:0.222) were observed.

#### **4. Discussion**

In this study, lesions in the lower respiratory tract in FIP-positive cats were frequent, and distinct pathological patterns could be identified, with some cats showing severe thoracic lesions. Susceptibility to the development of FIP in cats involves host characteristics including age at the time of FCoV infection, susceptibility to monocyte infection, concurrent diseases, and/or other stressors when FCoV infection is occurring [1,6,17,18]. FIP disease predominantly affects young intact male cats under two years of age, very old, or immunosuppressed individuals [10,19], similar to what was observed in the study. Affected cats were mainly male in this study, and more than ninety percent were kittens and young adults. Although some purebred cats appear to be more susceptible to FCoV infection [19,20], most cats in this study were mixed-breed; however, this is related to the epidemiology of the study's geographic site [16,21].

Coinfections with retroviruses were commonly found in this study, with more than seventy percent of cats affected, especially with FeLV. FIP is one of the most frequent infectious diseases in necropsied FeLV-positive cats [22,23]. In a recent study, the odds of viral diseases were higher in FeLV-cats, with 2.2 times more diagnoses of feline infectious peritonitis [16]. In addition, although it was not possible to establish a relationship between lesion intensity and retroviral status in this study, serious viral infections that lead to death are more common in FeLV-positive cats [16]. FeLV coinfections interfere with the immunity of cats infected with FCoV and favour high levels of FCoV replication increasing mutations [2,4]. Cats with advanced FIV infection are also more susceptible to FIP when exposed to FECV [2]. Coinfections with bacterial and parasitic



agents were also observed in this study, mainly in FeLV-positive cats. This finding reflects the immunosuppression caused by FeLV, since these cats have a greater chance of developing bacterial infectious diseases [16], but FIP too. Also, FIP is frequently associated with lymphopenia, which is a non-specific find indicative of immunosuppression [6,24], and an increase in bacterial infections can be seen in these cats [6].

Clinical respiratory signs were reported in some cats in the present study, which later presented important lesions in the lower respiratory tract at necropsy and histology. Previous studies have suggested that upper respiratory infections characterized by conjunctivitis or rhinitis preceded the development of FIP, although FCoV is not routinely considered a common respiratory pathogen in cats [14,25]. Coronaviruses primarily infect the upper respiratory and gastrointestinal tract of mammals and birds [26]. In contrast, FCoV infects primarily the enterocytes; however, after mutations, macrophages are infected, and the disease acquires a systemic presentation [18]. The lungs in FIP infection are not considered a primary target and their involvement is considered more like a consequence of systemic disease [10]. In contrast, cats with respiratory signs in the present study had significant pneumonias, including those associated with upper respiratory infection in the few cases that these tissues were collected.

Recently, the occurrence of pyogranulomatous rhinitis with FIP-antigen detection after respiratory signs has been reported, suggesting that the respiratory tract is a potential transmission route for FCoV [14]. Previous experimental studies using aerosol challenge detected FIPV antigen in macrophages and multinucleated giant cells in tracheobronchial lymph nodes, lungs, and trachea two days after challenge, and in liver, spleen, kidneys, and omentum only three and four days after [7]. Additionally, other experiments showed that the most specific pathogen-free cats that died with FIP had shown previous or concurrent signs of upper respiratory tract disease [18]. Respiratory

signs may be an early indicator of FIP; the nasal cavity seems to be involved in the pathogenesis of FIP [14], and perhaps it may be the main entry point for pulmonary lesions. However, FCoV present in the nasal cavity may also suggest a role of significant hematogenous spread of the virus as the virus produces vasculitis and the nasal cavity is extremely vascular [14], and the lower respiratory tract (lungs and pleura) can be severely affected by anatomical proximity.

Pathological findings in the respiratory tract were observed in more than half of the cats that died from FIP in our diagnostic laboratory. In contrast, pleural effusions are widely described in other studies, but pulmonary lesions have been less reported. In a previous study with experimentally infected animals, the lungs, heart, and other thoracic tissues appeared normal in 15/19 necropsied cats [24]. This may represent a difference between natural and experimental infection, or even a geographic difference, including contrasts in coinfections with retroviruses. FeLV and FIV-positive cats have systemic immunologic compromise which leads to reduced pulmonary defences and the respiratory tract being more exposed to other pathogens [28].

The gross findings observed in the present study are characteristic of FIP infections; however, they have some characteristics that contrast with the literature data. First, fibrin deposition in the thoracic cavity in cats with effusive FIP is often cited as less evident compared to the abdominal cavity [6]. Thoracic effusions are usually described in conjunction with abdominal effusions, and the single occurrence of thoracic effusion is little commented [18]. In these cases, pyogranulomas are particularly prevalent in the abdominal organs, and they tend to follow the course of the cranial mesenteric artery [6], with lesions less common in the thorax and centred on the pleura and pericardium, with dark and rubbery lungs [6,9]. Similarly, in non-effusive FIP, lesions are considered to be prominent mainly in abdominal viscera, and thoracic lesions are generally localized, mainly in the pleura and underlying parenchyma. In contrast to these data,

marked fibrin deposition in the thoracic cavity was an important finding in cats of this study, even being in some cases the only cavity affected grossly. White nodular lesions in the lung surface and parenchyma were a common finding, which histologically corresponded to cases not only of pleuritis, but also perivascular involvement in the parenchyma and pneumonia, both multifocal and diffuse forms. When the gross presentation of FIP was primarily in the thorax, it was likely to accompany pneumonia. On the other hand, almost thirty percent of the cats in the present study showed marked deposition of fibrin without multifocal granulomas, and in some cats, this was the only gross finding. It is important in these cases to differentiate FIP infection from infection caused due to *Pasteurella multocida*, the main cause of pyothorax in cats [29].

Histological analysis showed that, when there are lesions in the respiratory tract in FIP-cats, they are often significant. Also, although uncommon, pyogranulomas can be unique in thoracic viscera, even in histology, with no other site affected; in these cases, immunohistochemistry is essential to confirm the diagnosis of FIP. Generalized vasculitis and perivasculitis, especially of small to medium-sized venules, are the key to microscopic FIP diagnosis [5]. Macrophages replicate the mutated coronavirus and carry it to target tissues such as the peritoneum, pleura, kidney, uvea, and nervous system, resulting in widespread immune-mediated vasculitis, disseminated perivascular pyogranulomatous inflammation, and exudative fibrinous polyserositis [8].

The lung parenchyma was affected at a similar frequency to the visceral pleura in this study. When free fibrin deposition was present in the thoracic cavity, diffuse or multifocal pleuritis was noted. Pleural effusion is the most common thoracic manifestation in FIP-positive cats [6]. Multifocal pleuritis without effusion was also observed, demonstrating that parenchymal lesions in the pleura occur before effusion. These cases likely represent an initial form of lung inflammation,

with the involvement of hematogenous spread in localized peripleural venules. Medium and large-calibre pulmonary veins, often close to the bronchi, were commonly affected in the present study, resulting in a multifocal and perivascular pattern. Some cats with non-effusive FIP presented localized granulomas in the lungs (6). Similarly, pyogranulomatous inflammation in the pulmonary parenchyma was observed, not only as localized lesions but in a more diffuse pattern. Small venules of alveolar septa may probably be involved in these cases; however, their delicate stroma associated with necrosis prevented the identification of small venules. Also, inflammation extending to airways was commonly seen; although infectious agents most often reach the pleura from blood, they also can reach from bronchopneumonia lesions [9]. Data on pneumonia in cats with FIP are still scarce. It is known that pyogranulomatous pneumonia can be found in cats with FIP on thoracic radiographs or at necropsy, but in most cases, it has little relevance [8]. In addition to focal lung lesions, there may be diffuse interstitial pneumonia, sometimes most severe close to the visceral pleura [9]. When the gross presentation of FIP was primarily in the thoracic cavity, pneumonia was a common find, with or without pleuritis. Interestingly, when no gross lesions are seen, if there is histological lung involvement, it is more likely to be pneumonia.

Identification of FIP-antigen associated with pleural and lung lesions was a frequent finding in the present study. The virus is strongly associated with macrophages in lesional tissues and effusions [24], and the IHC technique is considered the gold standard for the diagnosis of FIP [30]. Nonetheless, in some cases of bronchopneumonia and pleuritis, it was not possible to identify the viral antigen in the lung. Secondary respiratory infection in these cases cannot be completely ruled out; however, the presence of FIP antigen could be minimal and undetectable on IHC. Pleural defence mechanisms against infectious agents are limited, and even a few organisms reaching the pleural surfaces can cause severe damage [9]. The immunostaining cannot differentiate between

non-mutated and mutated viruses, but the presence of a high amount of antigen is necessary for positive staining. Thus, negative IHC results do not exclude FIP because FCoV antigens can be variably distributed within lesions [1]. On the other hand, the immunostaining observed in the airways and subjacent parenchyma, larynx, and nasal turbinates showed that these tissues had high viral load and provided evidence that respiratory infection by feline coronavirus is part of the spectrum of FIP-associated disease.

As this was a retrospective study, it has limitations. First, the lack of routine upper respiratory tract collection in feline necropsies prevented a more complete analysis of respiratory tract involvement. Second, information regarding environmental conditions, contact with other cats, and radiographic images were not available. Third, information on bacteriological isolation was not available, preventing the identification of which bacteria were involved in secondary infections.

The occurrence of FIP-positive cats with significant lesions in the thoracic cavity associated with previous respiratory signs, lesions in the upper respiratory tract, and detection of FIP antigen in respiratory tissues suggests that the respiratory tract may play an important role in the route of FIPV infection in some cats. These data emphasize the importance of collecting the complete upper respiratory tract, in addition to the lungs, at the necropsy from these cats and carrying out future studies to better clarify the pathogenesis of FIP-associated respiratory disease.

## **5. Conclusion**

Pathological findings in the respiratory tract were frequent in cats with FIP. Gross findings included marked fibrin deposition in the thoracic cavity, with or without pyogranulomas in the lung parenchyma. Pyogranulomas without pleural effusion were also observed, and pneumonia was

commonly associated with these cases. Primary lesions in the lung parenchyma occur at a similar frequency to lesions in the visceral pleura, not only with vascular lesions but also in the airways. Although most cases were effusive, a significant percentage were non-effusive, making the diagnosis more challenging. Although FIP is a systemic disease, some cats develop pathologically significant lesions in the thoracic cavity, including the involvement of the upper respiratory tract and presenting respiratory signs, without other classic signs of FIP. More studies are needed to better understand the pathogenesis of FIP in these cats.

### **Statement of author contributions**

All authors contributed to the revision of the paper approving the submitted version and agreed to the copyright conditions. Mônica Slaviero: Conceptualization, Investigation, Methodology, Writing-original draft preparation. Fernanda G Cony: Investigation, Methodology, Writing-review and editing. Rodrygo C da Silva: Investigation, Methodology, Writing-review and editing. Cíntia De Lorenzo: Methodology, Writing-review and editing. Bruno A de Almeida: Statistical analysis, Writing-review and editing. Marianna Bertolini: Writing-review and editing. David Driemeier: Investigation, Writing-review and editing. Saulo Petinatti Pavarini: Investigation, Writing-review and editing, Luciana Sonne: Conceptualization, Investigation, Methodology, Project administration, Writing-review and editing.

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The authors declared no conflicts of interest concerning the research, authorship or publication of this article.

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## **Figure legends**

**Fig. 1.** Occurrence and distribution of effusion in the 66 FIP-positive cats with histological lung involvement. Yellow circle: pleural effusion; Orange circle: abdominal effusion; Blue circle: pericardial effusion.

**Fig. 2.** Gross patterns of lung lesions in cats with FIP. **(A)** Pattern 1. Thoracic cavity filled with marked deposition of yellow fibrillar material in the visceral and parietal pleura. **(B)** Pattern 1. The lung parenchyma shows atelectasis and marked irregularity of the pleura. **(C)** Pattern 2. Thoracic cavity filled with marked deposition of yellow gelatinous material. **(D)** Pattern 2. The lung lobes show areas of pleural thickening, are not collapsed, and have dozens of randomly distributed punctate nodules. **(E)** Pattern 3. The thoracic cavity does not show the same deposition of yellow material as patterns 1 and 2. **(F)** Pattern 3. The lung lobes are uncollapsed, pale in colour, and with dozens of randomly distributed white punctiform nodules.

**Fig. 3.** Lung, histological patterns of lung lesions in cats with FIP. **(A)** Pattern 1A: In these cases, the pleura was markedly thickened by fibrin deposition and pyogranulomatous inflammation (asterisk). Haematoxylin and eosin stain (HE). **(B)** Pattern 1B. Multifocal thickening of the pleura (asterisk). Granulomatous inflammation was the main finding, with fibrin deposition much less evident compared to pattern 1A. HE. **(C)** Pattern 2. The pleura (asterisk) and blood vessels (arrow) of the pulmonary parenchyma showed pyogranulomatous inflammation. Adjacent alveolar spaces (arrowhead) showed no alterations. HE. **(D)** Pattern 3. The pleura is covered with fibrin (arrow). A marked inflammatory infiltrate is observed both in the pleura (asterisk) and alveolar spaces (arrowhead) and, sometimes, in the airways (double asterisk). HE. **(E)** Pattern 4. Inflammation is noted only perivascularly in the parenchyma (asterisk). HE. **(F)**. Pattern 5. A marked inflammatory

infiltrate is observed obliterating alveolar spaces (asterisk), in association with perivascular injury (arrow). HE.

**Fig. 4.** Lung, histological findings of pneumonia in FIP-positive cats. Cats with histological pattern 5. (A) Multifocal pattern with fibrin deposition in alveoli associated with pyogranulomatous inflammation was a frequent finding. Haematoxylin and eosin stain (HE). (B) Some cats had a more diffuse and interstitial pattern, often associated with vasculitis (asterisk). HE. (C) A diffuse pattern associated with intense inflammation in the alveoli and airways was also observed. Macrophage infiltration in the airways was commonly seen (asterisk). HE. (D) Peribronchiolar inflammation was observed, mainly in cats with significant lesions in the thorax. HE.

**Fig. 5.** Comparison between gross and histological patterns of lung lesions in cats with FIP. Gross pattern 1: in these cases, 94.4% (17/18) of the cats had pleuritis on histology, with or without perivascular involvement (histological pattern 1A and 2). Multifocal pleuritis (1B) and perivascular involvement (4) were also observed. Gross pattern 2: the pleura was involved in all cases, with 84.6% (11/13) of the cats showing pleuritis and pneumonia (3). Multifocal pleuritis was noted in two cats (1B). Gross pattern 3: pneumonia was a frequent finding in this pattern, with 69.6% (16/23) presenting pneumonia without or with pleuritis (5 and 3). Pleuritis with perivascular involvement was the third more common histological pattern (2). Multifocal pleuritis (1B) and perivascular injury (4) were also noted. Lung with no gross alteration: in the histology, pneumonia without pleuritis was the major finding (5). Multifocal pleuritis (1B), pleuritis with perivascular injury (2), pleuritis with pneumonia (3) and perivascular injury was also observed (4).

**Fig. 6.** Distribution of histological patterns and lesion intensity compared to the frequency of retroviral infection, as well as the histological pattern and lesion intensity. Note also the distribution of histological and gross patterns, which included a positive statistical association (p-value<0.001).

**Fig. 7.** Distribution of FIP-antigen in the lung parenchyma, IHC anti-FIP, AEC chromogen. **(A)** Multifocal immunolabeling can be observed in the cytoplasm of macrophages in the pleura, associated with inflammatory infiltrate of lymphocytes and plasma cells. **(B)** Multifocal cytoplasmatic immunolabeling in macrophages adjacent to a pulmonary venule. **(C)** Necrotic bronchiole associated with multifocal macrophages showing marked immunolabeling of FIP-antigen. **(D)** Respiratory epithelium of a bronchus with focal necrosis associated with an inflammatory infiltrate of lymphocytes and macrophages. Multifocal macrophages exhibit strong cytoplasmic immunostaining.

### 5.3 Artigo 3

Neste item é apresentado o artigo intitulado “**Occurrence and pathological findings of feline herpesvirus and feline calicivirus in the respiratory tract and upper digestive tract of cats**”.

Este artigo será submetido no periódico científico “*Topics in Companion Animal Medicine*”.

## CONSIDERAÇÕES FINAIS

- A infecção sistêmica por herpesvirus é pouco frequente; quando ocorre, trato respiratório e fígado são tecidos importantes de ação viral;
- Infecções generalizadas por FHV podem ser observadas em gatos imunodeprimidos, e infecção por FeLV bem como coinfeções por outros agentes, como *Escherichia coli* devem ser consideradas e investigadas;
- A PIF resulta em lesões significativas na cavidade torácica e pulmão ocorrem com frequência, inclusive com envolvimento do trato respiratório superior e com sinais respiratórios;
- Três padrões principais macroscópicos associados a PIF foram definidos: deposição acentuada de fibrina na cavidade torácica com atelectasia pulmonar; deposição acentuada de fibrina na cavidade torácica com piogranulomas pulmonares; e piogranulomas pulmonares sem efusão torácica;
- Cinco padrões histológicos principais de PIF foram definidos: pleurite; pleurite e vasculite/lesão perivascular no parênquima pulmonar; pleurite e pneumonia; lesão perivascular no parênquima sem pleurite; e pneumonia sem pleurite;
- Considera-se importante considerar PIF em gatos com sinais respiratórios, mesmo sem outros sinais clássicos de PIF;
- Aproximadamente 36% dos gatos na população estudada que morrem com doença respiratória apresentaram envolvimento viral por FCV e/ou FHV, o que demonstra a importância em investigar agentes virais em gatos com doença respiratória;
- Coinfeções entre FCV e FHV e retrovíroses FIV e FeLV são frequentes na população estudada. Alguns gatos também apresentaram infecções bacterianas secundárias;
- Tanto macroscopicamente como histologicamente lesões em gatos com FCV e FHV acontecem principalmente em trato respiratório superior, trato respiratório inferior e trato digestório superior (esôfago e cavidade oral);
- Pneumonias são frequentes e provavelmente estão relacionadas à causa do óbito em todos os gatos infectados por FCV, e na maioria dos gatos infectados com FHV;

- Pneumonias em gatos FHV e FCV-positivos ocorrem concomitante a lesões em porções mais craniais do trato respiratório e digestório, o que demonstra a importância em se analisar esses tecidos;
- Lesões histológicas em casos de FCV e FHV foram observadas principalmente em cavidade nasal, oral, laringe, traqueia, esôfago e pulmão, associadas à detecção de antígeno viral pela IHQ;
- Infecção por FCV está mais frequentemente associada à pneumonia intersticial fibrinossuprativa e lesões em cavidade oral e esôfago;
- Gatos com FHV apresentam rinites significativas, lesões ao longo de laringe e traqueia, com ou sem pneumonia broncointersticial;
- É importante considerar a possibilidade de envolvimento viral em gatos com lesões ulcerativas e necróticas em cavidade nasal, oral, esôfago, traqueia, laringe e/ou pulmão, e a imuno-histoquímica pode ser uma ferramenta diagnóstica importante;
- É importante sempre considerar a coleta sistemática de todos os tecidos do trato respiratório superior, trato digestório superior e trato respiratório inferior os durante a necropsia de gatos com sinais respiratórios para melhor elucidar a causa da morte.



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