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LANA ISABELLA GILA

**Avaliação molecular da circulação de hepadnavírus em gatos domésticos do
triângulo mineiro**

Uberlândia
2024

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triângulo mineiro**

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, da Faculdade de Medicina Veterinária, da Universidade Federal de Uberlândia, como requisito parcial para obtenção do título de Mestre em Ciências Veterinárias

Área de concentração: Investigação Etiológica

Orientadora: Prof^a. Dra. Aline Santana da Hora

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Dedicatória

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RESUMO

O vírus da hepatite B (HBV) é uma das principais causas de hepatopatias em humanos, incluindo hepatite crônica e carcinoma hepatocelular. O hepadnavírus de gatos domésticos (*Domestic cat hepadnavirus* - DCH), um recém descrito hepadnavírus, pertencente a mesma família viral do HBV, foi identificado em um felino em 2018, e, posteriormente, relatado em vários outros países. No Brasil, a presença do vírus não foi investigada. Portanto, constitui-se como objetivo avaliar a ocorrência de hepadnavírus em gatos domésticos na região do triângulo mineiro. Foram utilizadas 334 amostras de DNA extraído de sangue total, que foram submetidas à Nested-PCR Pan-*Hepadnaviridae* e à PCR em tempo real para DNA proviral do vírus da imunodeficiência felina [FIV (3,0%; 10/334)] e do vírus da leucemia felina [FeLV (27,2%; 91/334)]. Informações epidemiológicas como idade, sexo, além de dados sobre a atividade sérica da alanina aminotransferase [ALT (43,4%; 23/53)] foram obtidos dos prontuários dos gatos. Apesar da população amostral apresentar potenciais fatores de risco para a infecção pelo hepadnavírus de gatos domésticos (DCH), como a infecção por FeLV e o aumento da atividade sérica da enzima hepática ALT, não foi observada a presença deste vírus na região estudada.

Palavras-chave: *Hepadnaviridae*; diagnóstico molecular; doenças virais; hepatite; felinos

ABSTRACT

The hepatitis B virus (HBV) is one of the main causes of liver diseases in humans, including chronic hepatitis and hepatocellular carcinoma. Domestic cat hepadnavirus (DCH), a recently described hepadnavirus belonging to the same viral family as HBV, was identified in a feline in 2018 and subsequently reported in various other countries. In Brazil, the occurrence of the virus has not been investigated. Therefore, the objective was to assess the occurrence of hepadnavirus in domestic cats in the Triângulo Mineiro region. A total of 334 DNA samples extracted from whole blood were used, subjected to Nested-PCR Pan-Hepadnaviridae, and real-time PCR for proviral DNA of feline immunodeficiency virus [FIV (3,0%; 10/334)] and feline leukemia virus [FeLV (27,2%; 91/334)]. Epidemiological information such as age, sex, and data on serum alanine aminotransferase [ALT (43,4%; 23/53)] activity was obtained from the cats' records. Despite the sample population presenting potential risk factors for domestic cat hepadnavirus (DCH) infection, such as FeLV infection and increased serum ALT enzyme activity, the presence of this virus was not observed in the studied region.

Key words: *Hepadnaviridae*; molecular diagnosis; viral diseases; hepatitis; feline

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CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

1 Introdução e revisão de literatura

Domestic cat hepadnavirus (hepadnavírus de gatos domésticos - DCH) é um vírus envelopado, pertencente ao gênero *Orthohepadnavirus*, família *Hepadnaviridae*, ordem *Blubervirales* e reino *Riboviria*. Constitui-se por DNA de dupla fita e se destaca por executar uma etapa de transcrição reversa durante seu processo replicativo, portanto, com a presença de um intermediário de RNA (Magnius et al., 2020). O genoma do hepadnavírus de gatos domésticos possui 3.187 nucleotídeos e difere em mais de 33% de todas as outras espécies virais do gênero *Orthohepadnavirus*, representando, assim, uma nova espécie (Aghazadeh et al., 2018).

A família *Hepadnaviridae* é composta por cinco gêneros *Parahepadnavirus*, *Metahepadnavirus*, *Herpetohepadnavirus*, *Avihepadnavirus*, e *Orthohepadnavirus*. Membros das espécies de *Orthohepadnavirus* foram identificados em seis ordens de mamíferos: *Carnivora*, *Eulipotyphla*, *Rodentia*, *Artiodactyla*, *Primates* e *Chiroptera* (Koonin et al., 2020).

As espécies virais pertencentes ao *Orthohepadnavirus* causam manifestações hepáticas nos hospedeiros, como o vírus da hepatite B (*Hepatitis B virus* - HBV), responsável por infectar cronicamente 400 milhões de pessoas e por, aproximadamente, 80% dos casos de carcinomas hepatocelulares (Sinn et al., 2014). O hepadnavírus dos gatos domésticos, assim como HBV, realiza a replicação viral no núcleo dos hepatócitos e apresenta associação com hepatites crônicas e carcinomas hepatocelulares (Pasavento, 2019).

A primeira descrição de DCH ocorreu em 2018 na Austrália, em um gato eutanasiado em 2016, devido às manifestações clínicas apresentadas pelo animal, associadas a um quadro de linfoma de células grandes e coinfeção com vírus da imunodeficiência felina (FIV). O diagnóstico foi obtido por detecção molecular em material proveniente da neoplasia. Além desse animal, outros oito gatos (6,5%; 8/123) também foram positivos para DCH em amostras extraídas de sangue total disponíveis em um biobanco (Aghazadeh et al., 2018).

No que diz respeito à epidemiologia de DCH, na pesquisa de Lanave et al., 2019, na Itália, o DNA de DCH foi observado em 10,8% (42/390) das amostras de soro sanguíneo de gatos. A presença deste vírus foi observada em 12,4 % (26/209) das amostras de soro e em 20% (3/15) das amostras hepáticas, na Tailândia

(Piewbang et al., 2020). Anpuanandam et al., 2021, observaram positividade em um total de 12,3% (31/253) das amostras de sangue total e de 14,9% (13/87) das amostras de fígado, na Malásia.

No Japão, o DNA de DCH foi observado em apenas 0,78% (1/128) das amostras de sangue total (Takahashi et al., 2022). Já na América do Norte, Stone et al., 2022, testaram amostras de sangue total (97,8%; 485/496) e amostras hepáticas (2,2%; 11/496) e apenas uma amostra do grupo testado de sangue total (0,2%; 1/485) foi positiva para DCH. Piewbang et al., 2022 também testaram amostras de sangue total e amostras hepáticas, obtendo 18,5% (103/557) e 9,2% (11/119) de positividade para hepadnavírus de gatos domésticos.

Em Hong Kong, amostras de sangue total foram avaliadas e 11,1% (57/513) foram positivas para DCH (Capozza et al., 2023a). Adıgüzel, et al., 2023, na Turquia, observaram positividade em 3,1% (17/550) das amostras de sangue total, 0,4% (2/550) de soro, 0,4% (2/550) de órgãos e 0,2% (1/550) de efusão abdominal. Já em Taiwan, em 11, 3% (8/71) das amostras de sangue total foi observado o material genético de DCH (Silva et al., 2023).

O DNA do DCH também foi detectado por Whardani et al., 2023 em 4,7% (25/532) das amostras de sangue total e em 12,5% (1/8), 1,14% (1/88), 2,54% (10/394) e 1,65% (3/182) das amostras de swab auricular, nasal, oral e retal, respectivamente. No Chile, o DNA de DCH foi detectado no soro em 1,67% (2/12) dos gatos (Choi et al., 2023).

Alguns estudos relataram analogia de DCH com a disseminação do vírus humano (HBV), por meio de fluidos corporais como sangue, soro, saliva e sêmen, além da transmissão transplacentária (Capozza et al., 2023b; Lanave et al., 2019; Wardhani et al., 2023). Porém, em um estudo observacional, dois gatos naturalmente co-infectados com DCH e retrovírus felinos (FIV e/ou FeLV, em teste imunocromatográfico), apresentaram esfregaços orais, conjuntivais, prepuciais e retais repetidamente negativos para DCH por PCR (Capozza et al., 2021).

Além dos sinais clínicos associados ao fígado, como as hepatites crônicas, carcinomas hepatocelulares (Pasavento, 2019) e, mais recentemente, a hepatite linfoplasmocítica crônica em gatos (Piewbang et al., 2022), um estudo descreveu a infecção sistêmica por DCH em três gatos, associada a lesões inflamatórias em vários órgãos, incluindo fígado, rim, intestino, coração e pulmões (Piewbang et al., 2020).

A presença do vírus também já foi descrita em 2/72 (2,8%) amostras de efusão peritoneal de gatos. Um deles apresentou efusões pericárdica, pleural e peritoneal, juntamente com hepatomegalia e com a presença de múltiplas lesões hiperecogênicas difusas por todo o parênquima hepático, um leve aumento de AST e ALT também foram observados. O segundo gato possuía histórico de enzimas hepáticas elevadas. Na data da consulta, a ultrassonografia abdominal revelou a presença de peritonite difusa e aumento dos linfonodos, juntamente com a presença de efusão peritoneal, porém, com mudanças nos exames sanguíneos, indicando provável insuficiência hepática, como hipoalbuminemia, diminuição da atividade enzimática da ALT e hiperbilirrubinemia (Ratti et al., 2023).

Existe uma divergência de opiniões sobre a correlação entre o hepadnavírus de gatos domésticos e a presença de linfoma. Beatty et al., 2023 enfatizam a necessidade de cautela na interpretação dos resultados obtidos por Piewbang et al., 2023, acerca da associação de DCH com a formação neoplásica de linfoma. Beatty et al., 2023 sugere que, outros fatores, não considerados por Piewbang et al., 2023, como (1) a não identificação de gatos persistentemente infectados, (2) a utilização de linfoma apenas de gatos positivos para o vírus no soro e (3) a não utilização de tecidos não linfomatosos como controle negativo para os testes, podem influenciar nos resultados encontrados pelos autores.

Apesar da detecção molecular de DCH ter ocorrido em poucos animais (321/3931), algumas características foram relacionadas a positividade para este vírus. Apesar dos autores as apresentarem como fatores de riscos, neste estudo tais aspectos serão citados como possíveis fatores de risco, uma vez que o número de animais positivos para DCH é baixo, ainda não é possível afirmar que sejam realmente fatores de riscos.

Após o primeiro caso de DCH descrito ter sido associado ao FIV (Aghazadeh et al., 2018), estudos subsequentes corroboraram a associação entre a infecção por hepadnavírus de gatos domésticos e os retrovírus (Lanave et al., 2019; Piewbang et al., 2020; Anpuanandam et al., 2021). Níveis aumentados de marcadores indicativos de danos estruturais ou funcionais ao fígado, principalmente o aumento da atividade sérica da enzima alanina aminotransferase (ALT), também já foram descritos como consequências da infecção por DCH (Lanave et al., 2019, Anpuanandam et al., 2021, Capozza et al., 2023b).

Além dos testes moleculares para DCH que incluem PCRs Pan-*Hepadnaviridae* (Wang et al., 2017) e específicas para DCH (Aghazadeh et al., 2018), outros autores avaliaram a presença de hepadnavírus dos gatos domésticos em determinadas localidades por meio de testes sorológicos com anticorpo específico, baseado no antígeno recombinante do núcleo de hepadnavírus de gatos domésticos (Capozza et al., 2023b; Fruci et al., 2022).

Nos últimos anos, houve um aumento notável no desenvolvimento de novos medicamentos para o HBV, com múltiplos alvos apresentando propriedades antivirais promissoras. No entanto, ensaios clínicos maiores e mais prolongados são necessários para avaliar a eficácia desses medicamentos na indução de uma cura completa, sem a presença de circulação viral nos hepatócitos dos humanos (Phillips; Jagatia; Chokshi, 2022). Até o momento nenhuma pesquisa foi realizada para avaliar tratamentos de gatos infectados por DCH.

A abordagem preventiva do vírus da hepatite B inclui a implementação de programas de vacinação de alcance global, que já estão disponíveis há mais de 30 anos (Nguyen et al., 2020). Como a descoberta de DCH é recente, são requeridas pesquisas adicionais para aprofundar a compreensão do vírus, particularmente no que diz respeito aos aspectos relacionados à sua transmissão. Novos estudos acerca da frequência, patogenia e transmissão permitirão a implementação de estratégias de manejo eficazes para prevenir a infecção por DCH em felinos.

2 Objetivo

Investigar a ocorrência de hepadnavírus de gatos domésticos em gatos da região do triângulo mineiro.

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CAPÍTULO 2 – ARTIGO

Preventive Veterinary Medicine

Avaliação molecular da circulação de hepadnavírus em gatos domésticos do triângulo mineiro, Brasil

Resumo

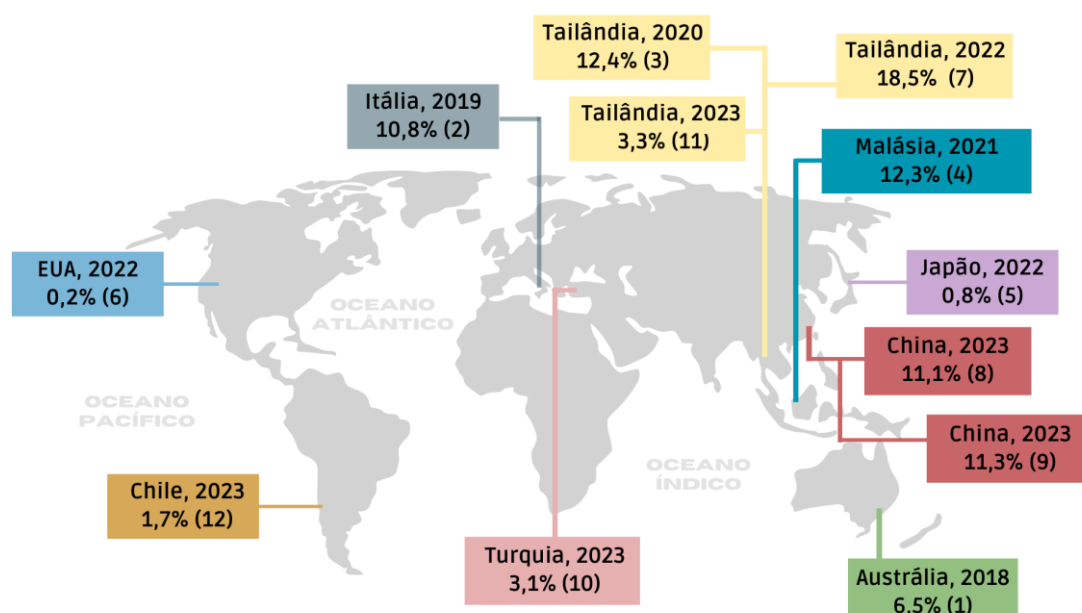
Com o crescente aumento do número de gatos em todo o mundo, faz-se necessário conhecer a circulação de patógenos emergentes e reemergentes que podem infectar a espécie. Neste contexto, destaca-se um vírus recém-descoberto e com potencial patogênico a ser mais bem elucidado, o hepadnavírus de gatos domésticos (*Domestic cat hepadnavirus* - DCH), um vírus DNA, pertencente ao gênero *Orthohepadnavirus* e à família *Hepadnaviridae*. Como sua ocorrência no Brasil ainda é desconhecida, objetivou-se avaliar a presença de DCH em gatos. Foram utilizadas 334 amostras de DNA extraído de sangue total de felinos domésticos, provenientes do biobanco de um laboratório de investigação etiológica, situado na região do triângulo mineiro, no estado de Minas Gerais, Brasil. A detecção molecular de DCH foi realizada por meio de Nested-PCR Pan-*Hepadnaviridae* e a identificação de DNA proviral de FIV e de FeLV foi conduzida por PCR em tempo real. A população estudada foi composta por gatos de idade entre 2 a 166 meses e positivos para DNA proviral de FeLV (25,7%; 86/334), FIV (1,5%; 5/334) e coinfectados por FIV e FeLV (1,5%; 5/334). Além disso, 43,4% (23/53) dos gatos apresentavam aumento da atividade sérica da enzima hepática alanina aminotransferase (ALT). Apesar da população estudada apresentar possíveis fatores de risco para a infecção por DCH, como infecção por FeLV e aumento da atividade sérica da ALT, a presença deste vírus não foi observada na região.

Palavras chave: Doenças virais emergentes; hepatopatia viral felina; *Hepadnaviridae*

1 Introdução

Domestic cat hepadnavirus (hepadnavírus de gatos domésticos - DCH) é um vírus DNA de dupla fita, pertencente ao gênero *Orthohepadnavirus* e à família *Hepadnaviridae* (Magnius et al., 2020). O vírus da hepatite B (HBV) é protótipo deste gênero e responsável por infectar cronicamente 400 milhões de pessoas (Sinn et al., 2014). Após o primeiro registro de DCH na Austrália, em 2018, a presença do vírus foi relatada em diversos países, com ocorrência variando entre 0,2% (Stone et al., 2022) a 18,5% (Piewbang et al., 2022).

Figura 1. Distribuição mundial da ocorrência de hepadnavírus de gatos domésticos em amostras de sangue total ou soro.



Fontes: (1) Aghazadeh et al., 2018 (2) Lanave et al., 2019; (3) Piewbang et al., 2020; (4) Anpuanandam et al., 2021; (5) Takahashi et al., 2022; (6) Stone et al., 2022; (7) Piewbang et al., 2020; (8) Capozza et al., 2023a; (9) Adigüzel et al., 2023; (10) Silva et al., 2023; (11) Wardhani et al., 2023; (12) Choi et al., 2023.

Na Oceania e na Europa, as frequências moleculares são relativamente mais altas quando comparadas às encontradas mais recentemente nas Américas. Na Oceania a frequência variou de 6,5% (8/123) a 18,5% (103/557) (Aghazadeh et al., 2018; Piewbang et al., 2020; Anpuanandam et al., 2021; Takahashi et al., 2022; Piewbang et al., 2022; Capozza et al., 2023a; Silva et al., 2023; Wardhani et al.,

2023). Na Itália, a ocorrência foi de 10,8% (42/390) (Lanave et al., 2019). Nas Américas, apenas dois países relataram a presença de DCH até o momento. Os Estados Unidos da América do Norte com a menor frequência descrita (0,2%; 1/496) (Stone et al., 2022) e o Chile com 1,67% (2/120) (Choi et al., 2023).

Com relação às condições associadas ao DCH, Lanave et al., 2019; Piewbang et al., 2020; Anpuanandam et al., 2021 observaram associação entre a infecção por DCH e a infecção pelos retrovírus felinos. A presença de elevados níveis de indicadores de lesões estruturais ou funcionais no fígado, particularmente observados pelo aumento da atividade sérica da enzima alanina aminotransferase (ALT), também podem estar relacionados à infecção por DCH (Lanave et al., 2019, Anpuanandam et al., 2021, Capozza et al., 2023b).

Neste contexto, avaliar a presença de DCH, possibilita a obtenção de novas informações acerca da ocorrência, principalmente em países onde a circulação deste vírus ainda é desconhecida, como no Brasil. Além disso, estimar a ocorrência do patógeno em determinada região, propicia oportunidades para relacionar a presença de DCH com seus fatores de risco, como idade, sexo, *status* para as retroviroses felinas e alterações em exames, como a atividade de enzimas hepáticas. Portanto, o objetivo do presente estudo foi de avaliar a presença de material genético de hepadnavírus de gatos domésticos no triângulo mineiro, Brasil.

2 Material e Métodos

2.1. Amostras e dados dos animais

Neste estudo, foram utilizadas 334 amostras de DNA genômico extraído de sangue total de gatos domésticos que estavam armazenadas em ultrafreezer -20°C , no biobanco do Laboratório de Investigação Etiológica Veterinária da Universidade Federal de Uberlândia (LIVe Vet-UFU) - Minas Gerais, Brasil. Para o estudo, não foi necessária aprovação do comitê de ética animal por se tratar de amostras já coletadas (Anexo 1). As amostras foram provenientes de atendimentos veterinários do Hospital Veterinário (HV) da UFU e de clínicas e hospitais veterinários da região, compreendendo o período de janeiro de 2021 a junho de 2023.

Dados epidemiológicos, como idade (classificados segundo Quimby et al., 2021), sexo, *status* para retroviroses (diagnóstico por qPCR na mesma amostra testada para DCH) e dados sobre a atividade sérica da ALT foram obtidos dos

prontuários dos gatos. Somente foram registrados os dados referentes ao exame bioquímico realizado na mesma semana da coleta da amostra utilizada para este estudo.

2.2. Extração do material genético

A extração do material genético foi realizada com o kit Wizard® Genomic DNA Purification (Promega Biotecnologia), de acordo com o protocolo do fabricante, a partir de 200 µL de sangue total.

2.3. Controle endógeno

A presença de inibidores de PCR nas amostras e alguma possível falha no processo de extração de DNA foram excluídas pela amplificação de um fragmento do gene GAPDH (gliceraldeído-3-fosfato desidrogenase) com 282pb (Birkenheuer et al., 2003). Para a qPCR, utilizou-se 1X da enzima GoTaq® qPCR Master Mix (Promega Corporation), 200 nM de cada primer (Tabela 1), 2 µL de DNA e água livre de nucleases q.s.p. 10 µL. Amostras de gatos positivas, previamente estabelecidas no laboratório, foram instituídas como controle positivo para o controle endógeno e água livre de nucleases como controle negativo. As condições de qPCR estabelecidas envolveram uma denaturação inicial a 95°C por 30 segundos, seguida por 30 ciclos a 55°C por 30 segundos e 72°C por 30 segundos, seguido por ciclo de melting em termociclador em tempo real (Rotor-Gene Q, Qiagen).

2.4. PCR em tempo real (qPCR) para detecção de FIV e FeLV

Para PCR em tempo real para detecção de um fragmento do gene da proteína gag de FIV, utilizou-se 1X de GoTaq® qPCR Master Mix (Promega Corporation), 200 nM de cada primer (Tabela 1), 5 µL de DNA e água livre de nucleases q.s.p. 20 µL. Foram utilizados primers previamente descritos (Padovani, 2017), com modificações do primer antisenso de FIV para este estudo.

Já para reação de FeLV, para detecção da região U3 da LTR, utilizou-se primers descritos em literatura (Tabela 1). A reação constituiu-se de 1X de GoTaq® qPCR Master Mix (Promega Corporation), 500 nM de cada primer (Tabela 1), 4 µL de DNA e água livre de nucleases q.s.p. 20 µL.

A ciclagem térmica de ambas as reações consistiu na ativação da DNA polimerase a 95°C por 15 segundos e 45 ciclos de denaturação a 60°C por 1 minuto

e extensão a 72°C por 30 segundos, seguido por um ciclo de melting em termociclador em tempo real (Rotor-Gene Q, Qiagen).

Os controles positivos foram instituídos com uso de amostras de gatos que testaram positivo para ambos os vírus, previamente estabelecidas no laboratório, e água livre de nucleases, como controle negativo.

2.5. Nested-PCR convencional para detecção do gene da polimerase

A Nested-PCR convencional para detecção do gene da polimerase das espécies virais pertencentes à família *Hepadnaviridae* foi realizada com oligonucleotídeos previamente descritos (Wang et al., 2017). Para a PCR, utilizou-se a enzima DreamTaq® Green PCR Master Mix (ThermoFisher Scientific), 500 nM de cada primer (Tabela 1), 2,5 µL de DNA e água livre de nucleases q.s.p. 25 µL. Foi utilizado como controle positivo o vírus da hepatite B (HBV), cordialmente cedido pelo Dr. João Renato Rebello Pinho (Hospital Israelita Albert Einstein) e água livre de nucleases como controle negativo. Para Nested-PCR utilizou-se a mesma enzima, 500 nM de cada primer (Tabela 1), 2 µL de produto amplificado da PCR e água livre de nucleases q.s.p. 25 µL.

As condições de ciclagem, da PCR e Nested-PCR incluíram uma denaturação inicial a 95°C por 2 minutos, seguida por 45 ciclos a 48°C (PCR) ou 50°C (Nested-PCR) por 30 segundos e 72°C por 40 segundos, com uma extensão final de 72°C por 4 minutos.

Os produtos amplificados, juntamente com um marcador de peso molecular [BH 100pb DNA ladder H3 RTU, Bio-Helix], o controle positivo e o controle negativo, foram dispostos em um gel de agarose 1,5% com corante SYBR® Safe DNA Gel Stain (ThermoFisher Scientific) diluído a 1:10.000. Em seguida, os produtos foram submetidos à eletroforese em cuba horizontal com solução tampão TBE, pH 8,0 (Tris-borato 0,045M: EDTA 0,001M). O gel de agarose foi visualizado sob luz ultravioleta e a imagem obtida foi fotodocumentada (L-PIX Touch, Locus).

Tabela 1 – Descrição dos primers utilizados para detecção de gliceraldeído-3-fosfato desidrogenase (GAPDH), hepadnavírus de gatos domésticos (DCH - pertencente à família *Hepadnaviridae*), vírus da imunodeficiência felina e vírus da leucemia felina

PCR	Identificação	Sequência (5' – 3')	Referência
Pan- <i>Hepadnaviridae</i> (PCR)	Primer HBV-pol-F1 (forward)	TGACTSGTGGTGGACTTCTC	Wang et al., 2017)
Pan- <i>Hepadnaviridae</i> (PCR)	Primer HBV-pol-R2 (reverse)	CATATAASTRAAAGCCAYACAG	
Pan- <i>Hepadnaviridae</i> (Nested-PCR)	Primer HBV-pol-F2 (forward)	AGTRAAYTGAGCCAGGAGAAAC	
Pan- <i>Hepadnaviridae</i> (Nested-PCR)	Primer HBV-pol-R1 (reverse)	TGCCATCTTCTTGTTGGTTC	
FeLV (qPCR)	Primer FeLV-U3-exo-F (forward)	AACAGCAGAAGTTTCAAGGCC	Tandon et al., 2005
FeLV (qPCR)	Primer FeLV-U3-exo-R (reverse)	TTATAGCAGAAAGCGCGCG	
FIV (qPCR)	qFIV-F (forward)	RTGGTTYACAGCCTTYTCDGC	Padovani, 2017
FIV (qPCR)	qFIV-R (reverse)	GGTACGATCATAYTCWGCTGTCA	Modificado de Padovani, 2017 para este estudo
GAPDH (qPCR)	GAPDH-F	CCTTCATTGACCTCAACTACAT	Birkenheuer et al., 2003
GAPDH (qPCR)	GAPDH-R	CCAAAGTTGTCATGGATGACC	

Fonte: A autora.

PCR: reação em cadeia pela polimerase; qPCR: reação em cadeia pela polimerase em tempo real.

2 Resultados

Dentre os animais estudados, 50% (167/334) eram machos e 50% (167/334) eram fêmeas. Com relação à idade, 26,6% (89/334) eram filhotes (menos de 1 ano), 60,7% (203/334) eram adultos jovens (entre 1 e 6 anos), 8,9% (30/334) eram adultos maduros (entre 7 e 10 anos) e 3,5% (12/334) eram idosos (com mais de 10 anos).

Apenas 15,9% (53/334) dos prontuários dos animais apresentavam os resultados dos exames bioquímicos com informações sobre ALT. O aumento da atividade sérica de ALT foi observado em 43,4% (23/53) (variação de 84 U/L a 430 U/L) dos gatos em que este parâmetro estava disponível. O valor maior que 83 U/L

(Weiss et al., 2010) foi utilizado como parâmetro para determinar um aumento da atividade sérica de ALT.

Com relação às reações de controle endógeno, todas as amostras que apresentaram aumento exponencial da curva de amplificação e valor de 87°C na curva de melting foram consideradas positivas.

Para as reações de FIV e FeLV, ao final dos 45 ciclos, as amostras foram consideradas positivas quando apresentaram aumento exponencial da curva de amplificação e valor específico para temperatura de melting de 77°C e 85°C, para FIV e FeLV, respectivamente. Para qPCR de FeLV, 25,7% (86/334) amostras foram positivas, já FIV, 1,5% (5/334) de positividade e 1,5% (5/334) estavam coinfectados por FIV e FeLV.

Para interpretação da Nested-PCR para detecção do gene da polimerase das espécies virais pertencentes à família *Hepadnaviridae*, a banda observada no gel de agarose foi comparada com o marcador de peso molecular e com o controle positivo (258pb). Nenhuma amostra foi positiva.

3 Discussão e Conclusão

Até o momento, a distribuição por sexo e por idade não foi identificada como um fator de risco para a infecção por DCH. No que se refere à idade, na Malásia, 64,5% (20/31) (Anpuanandam et al., 2021) dos gatos positivos possuíam mais de dois anos de idade. Em outros países, a associação entre a infecção e a faixa etária não foi estatisticamente relevante (Lanave et al., 2019; Piewbang et al., 2020; Stone et al., 2022; Takahashi et al., 2022). Nesta pesquisa, mesmo com 50% (167/334) de machos e 50% (167/334) de fêmeas e com 60,7% (203/334) dos gatos sendo adultos jovens a presença de DCH não foi observada.

O primeiro caso de DCH publicado foi em um indivíduo imunocomprometido com *status* positivo para FIV e, no mesmo trabalho, 75% (6/8) dos animais que testaram positivo para DCH, estavam coinfectados com FIV (Aghazadeh et al., 2018). Estudos posteriores também relacionaram a infecção por DCH aos retrovírus (Lanave et al., 2019; Piewbang et al., 2020; Anpuanandam et al., 2021).

No estudo realizado por Lanave et al., 2019, quase metade (45,2%; 14/31) dos gatos positivos para DCH na PCR apresentava co-infecção pelos retrovírus (FIV e/ou FeLV), porém sem informação do método diagnóstico. Na Tailândia, 73,0%

(19/26) das amostras positivas para DCH também apresentavam provírus de FIV e/ou FeLV, sendo um gato (1/26) coinfected por FIV, FeLV e DCH (Piewbang et al., 2020).

Anpuanandam et al., 2021 observaram coinfeção entre DCH e FIV e/ou FeLV em 19,35% (6/31) das amostras, com o diagnóstico das retroviroses por *point-of-care* (POC) de antígeno de FeLV e anticorpos de FIV (GenBody, Korea). Nos Estados Unidos da América do Norte, gatos de abrigos com uma ocorrência alta de FIV (12,0%; 58/485) e gatos de uma residência com infecções progressivas por FeLV (11,7%; 57/485) foram testados, sem informação do método diagnóstico para os retrovírus, mas com nenhuma (0/55) amostra positiva para DCH (Stone et al., 2022).

O gato positivo para DCH no estudo conduzido por Takahashi et al., 2022 (0,78%; 1/128), foi negativo para FIV e FeLV. As outras amostras testadas negativas para DCH não receberam *status* para as retroviroses. Capozza et al., 2023a, não avaliaram o *status* para FIV e FeLV dos animais.

No Chile, o *status* de FIV e FeLV foi estabelecido através de PCR e RT-PCR para FeLV e POC (SNAP FIV/FeLV combo IDEXX). Com relação ao provírus de FeLV, 14,1% (17/120) dos gatos foram positivos e com relação aos anticorpos de FIV, 2,5% (3/120) demonstraram positividade. Nenhum dos gatos positivos para DCH (2/120) eram coinfectedos com FIV ou FeLV (Choi et al., 2023).

Neste estudo, todos os animais receberam *status* para provírus de FIV e FeLV. A frequência de FIV foi baixa (3%; 10/334) quando comparada a frequência de FeLV, que ultrapassou 25% da população estudada (27,2%; 91/334). Apesar das frequências de retrovírus terem sido maiores do que as encontradas no outro estudo realizado na América do Sul, no Chile (Choi et al., 2023), nesta população, não foi observado à presença de hepadnavírus de gatos domésticos.

Em um estudo conduzido na Itália, 6,8% (3/44) dos gatos positivos para DCH possuíam anormalidades bioquímicas potencialmente condizentes com doença hepática (como aumento de ALT). Já um gato (1,9%; 1/52) positivo para DCH pertencia ao grupo sem anormalidades bioquímicas condizentes com lesão hepática (Scavone et al., 2022).

No estudo realizado por Lanave et al., 2019, também observaram que gatos com viremia por DCH provavelmente irão apresentar valores elevados de ALT.

Foram analisados 20 (47,6%; 20/42) resultados de exames dos pacientes, incluindo hemograma e bioquímicos, e, níveis aumentados de marcadores indicativos de danos estruturais ou funcionais no fígado [aspartato aminotransferase (AST), ALT, fosfatase alcalina (FA), gamaglutamiltranspeptidase (GGT) e bilirrubina total] estavam presentes em 10/20 (50%) dos gatos.

Para Anpuanandam et al., 2021, 52,2% (12/23) dos gatos positivos para DCH, apresentaram ALT aumentada, demonstrando que gatos com ALT alterada têm 21% de chances a mais de ter uma amostra de sangue positiva para DCH. Apenas um aumento da ALT maior que 95 U/L, foi considerado (Anpuanandam et al., 2021), se a referência máxima da enzima fosse considerada até 83 U/L (Weiss et al., 2010), como neste estudo, as chances de amostras positivas para DCH poderiam ser ainda maiores, já que um pequeno aumento da atividade de ALT nos gatos pode sugerir lesão hepática (Scott, 2008).

Na pesquisa observacional realizada por Capozza et al., 2021, um dos gatos, que permaneceu infectado por DCH, manteve os níveis de ALT sérica aumentada, durante os 11 meses de pesquisa, com cinco testes positivos para DCH.

Neste estudo, embora uma parcela da população (43,4%; 23/53) apresentou elevação na atividade sérica de ALT, aumentando as chances de observação de animais infectados com DCH, a presença do vírus não foi detectada.

Outros estudos utilizaram amostras hepáticas para testes moleculares (Pesavento et al., 2019; Piewbang et al., 2020, 2022) e sorologia para anticorpos específicos para DCH como método diagnóstico (Capozza et al., 2023b; Fruci et al., 2022). Neste trabalho, não foram avaliadas amostras de fígado ou a prevalência de anticorpos na população de gatos.

A presença de DNA *Pan-Hepadnaviridae* não foi detectada na região do triângulo mineiro, Brasil, por meio da análise de amostras sanguíneas de gatos domésticos, utilizando a técnica de Nested-PCR. Em estudos subsequentes, a ampliação da cobertura geográfica para abranger diferentes regiões do país, assim como o aumento do tamanho amostral, pode fortalecer os resultados deste estudo ou potencialmente revelar a circulação do vírus em território nacional. Adicionalmente, a inclusão de amostras de tecido hepático e a utilização de

abordagens além da PCR, como a análise da frequência de anticorpos-DCH na população felina, podem fornecer entendimentos adicionais sobre a exposição dos gatos ao vírus e suas possíveis repercussões na saúde da espécie.

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ANEXOS

ANEXO 1 – Comitê de ética UFU



UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Reitoria
Pró-Reitoria de Pesquisa e Pós-Graduação
Comissão de Ética na Utilização de Animais
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PARECER Nº 91/2023/CEUA/PROPP/REITO
PROCESSO Nº 23117.089368/2023-00
INTERESSADO(S): ALINE SANTANA DA HORA

ANÁLISE FINAL Nº 23117.089368/2023-00 DA COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS

Projeto Pesquisa: "Avaliação molecular da circulação de hepadnavírus em gatos domésticos do triângulo mineiro".

Pesquisador Responsável: Aline Santana da Hora

Declaro para os devidos fins, que o projeto intitulado "Avaliação molecular da circulação de hepadnavírus em gatos domésticos do triângulo mineiro" **não manipula diretamente animais vivos para colheita de amostras biológicas**. Desse modo, por não ferir a ética relacionada à experimentação animal e, estando em conformidade com a legislação federal, Lei Nº 11.794, de 8 de outubro de 2008, não há necessidade de apreciação e aprovação pela Comissão de Ética na Utilização de Animais-CEUA.

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ANEXO 2 – Normas Preventive Veterinary Medicine



PREVENTIVE VETERINARY MEDICINE

An International Journal reporting on Methodological and Applied Research in Veterinary Epidemiology, Animal Disease Prevention & Control and Animal Health Economics, and on the contributions of Veterinary Epidemiology to One Health, including Environmental Health

AUTHOR INFORMATION PACK

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ISSN: 0167-5877

DESCRIPTION

Preventive Veterinary Medicine is one of the leading international resources for scientific reports on animal health programs and preventive veterinary medicine. The journal follows the guidelines for standardizing and strengthening the reporting of biomedical research which are available from the CONSORT, MOOSE, PRISMA, REFLECT, STARD, and STROBE statements. The journal focuses on: Epidemiology of health events relevant to domestic and wild animals; Economic impacts of epidemic and endemic animal and zoonotic diseases; Latest methods and approaches in veterinary epidemiology; Disease and infection control or eradication measures; The "One Health" concept and the relationships between veterinary medicine, human health, animal-production systems, and the environment; Development of new techniques in surveillance systems and diagnosis; Evaluation and control of diseases in animal populations. The journal encourages the submission of clinical and field-trial studies, particularly those related to new vaccines and other preventive measures. These studies, however, should follow the Consort Statement (<http://www.consort-statement.org>) or Reflect Statement (<http://reflect-statement.org>).

Prevalence studies may be considered for publication, but only if the results are likely to be of international interest (i.e. it must be possible to generalize the findings using scientifically based approaches). For these studies, key considerations in the review process will include (but are not limited to): consideration of both animal-level and herd-level demographics in the sampling design; the study population's relevance to the authors' described target population; the potential for confounding; and how well the sample-size justification assures high precision. The sensitivity and specificity of non-perfect tests used must be declared; the true rather than the apparent prevalence must be presented.

Submissions of reviews of relevant topics are also encouraged, but these should follow the systematic-review process addressed by the guidelines in the following two websites: <http://jama.ama-assn.org/cgi/reprint/283/15/2008>; <http://prisma-statement.org>.

Preventive Veterinary Medicine does not publish studies on experimental development of diagnostic assays without the appropriate field evaluation. Guidelines for the evaluation of diagnostic assays are followed in the review process (<http://www.stard-statement.org>).

AUDIENCE

Research Workers in veterinary epidemiology and animal health.

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Review articles should cover subjects falling within the scope of the journal which are of active current interest.

A *Short Communication* is a concise but complete description of a limited investigation, which will not be included in a later paper. Short Communications should be as completely documented, both by reference to the literature and description of the experimental procedures employed, as a regular paper. They should not occupy more than 6 printed pages (about 12 manuscript pages, including figures, tables and references).

Letters to the Editor offering comment, or useful critique on material published in the journal are welcomed. The decision to publish submitted letters rests purely with the Editor-in-Chief. Any letter received, and approved for publication, will be sent to the Corresponding Author of the paper to which it refers for a response. Both letter and response (if received) will then be published together. It is hoped that the publication of such letters will permit an exchange of views which will be of benefit to both the journal and its readers.

A *Commentary paper* may include research, application, demonstration, and/or teaching components. It should not exceed 1,000 words (not counting references, tables, and figures). It should be supported with scientifically logical evidence, publications, and/or professional observations. It is the responsibility of the contributor(s) to ensure that the language of the submission is at a high-quality, professional level of English (or peer review may not be possible).

BEFORE YOU BEGIN

Ethics in publishing

Please see our information on [Ethics in publishing](#).

Ethics in Animal Experimentation

Circumstances relating to animal experimentation must meet the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences. They are obtainable from: Executive Secretary C.I.O.M.S., c/o WHO, Via Appia, CH-1211 Geneva 27, Switzerland, or at the following URL: http://www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm. Unnecessary cruelty in animal experimentation is not acceptable to the Editors of *Preventive Veterinary Medicine*.

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Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the [Sex and Gender Equity in Research \(SAGER\) guidelines](#) and the [SAGER guidelines checklist](#). These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the [resources on this page](#) offer further insight around sex and gender in research studies.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason

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It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the [Guide to Publishing with Elsevier](#)). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

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Manuscripts should have **numbered lines**, with wide margins and **double spacing** throughout, i.e. also for abstracts, footnotes and references. **Every page of the manuscript, including the title page, references, tables, etc., should be numbered.** However, in the text no reference should be made to page numbers; if necessary one may refer to sections. Avoid excessive usage of italics to emphasize part of the text.

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Subdivision - unnumbered sections

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results

Results should be clear and concise.

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This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: [example Highlights](#).

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Your abstract should not be longer than 400 words.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

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If no funding has been provided for the research, it is recommended to include the following sentence:

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Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

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1. Authors and editors are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, and the International Code of Zoological Nomenclature.
2. All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names when the English term is first used, with the exception of common domestic animals.
3. All biocides and other organic compounds must be identified by their Geneva names when first used in the text. Active ingredients of all formulations should be likewise identified.
4. For chemical nomenclature, the conventions of the International Union of Pure and Applied Chemistry and the official recommendations of the IUPAC-IUB Combined Commission on Biochemical Nomenclature should be followed.

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1. Give the meaning of all symbols immediately after the equation in which they are first used.
2. For simple fractions use the solidus (/) instead of a horizontal line.
3. Equations should be numbered serially at the right-hand side in parentheses. In general only equations explicitly referred to in the text need be numbered.
4. The use of fractional powers instead of root signs is recommended. Powers of e are often more conveniently denoted by exp.
5. In chemical formulae, valence of ions should be given as, e.g. Ca²⁺, not as Ca⁺⁺.
6. Isotope numbers should precede the symbols, e.g. ¹⁸O.
7. The repeated writing of chemical formulae in the text is to be avoided where reasonably possible; instead, the name of the compound should be given in full. Exceptions may be made in the case of a very long name occurring very frequently or in the case of a compound being described as the end product of a gravimetric determination (e.g. phosphate as P₂O₅).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

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Electronic artwork

General points

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- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the published version.

- Submit each illustration as a separate file.
- Ensure that color images are accessible to all, including those with impaired color vision.

A detailed [guide on electronic artwork](#) is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

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- h. Descriptions of the **formal random mechanism** (e.g., lottery or table of random numbers) and the list frame (enumerating every eligible subject and/or cluster) used at any step claimed to be "random"
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- d. Declaration of the **stakeholders** for any risk assessment
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