UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA

Vanessa Campos Paizan

Detecção molecular de vírus respiratórios de importância em saúde pública em amostras biológicas de mamíferos silvestres atropelados em diferentes estados brasileiros

Dissertação apresentada à Faculdade de Medicina, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de Botucatu, para obtenção do título de Mestra em Doenças Tropicais

Orientadora: Profa. Dra. Virgínia Bodelão Richini Pereira

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UNIVERSIDADE ESTADUAL PAULISTA

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ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE VANESSA CAMPOS PAIZAN, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM DOENÇAS TROPICAIS, DA FACULDADE DE MEDICINA - CÂMPUS DE BOTUCATU.

Aos 26 de fevereiro de 2026, às 9h, por meio de Videoconferência, realizou-se a defesa de DISSERTAÇÃO DE MESTRADO de VANESSA CAMPOS PAIZAN, intitulada "Detecção molecular de microrganismos causadores de doenças respiratórias de importância em Saúde Pública em amostras de mamíferos silvestres". A Comissão Examinadora foi constituída pelos seguintes membros: Profa. Dra. VIRGINIA BODELÃO RICHINI PEREIRA (Orientador(a) - Participação Presencial) do(a) Instituto Adolfo Lutz / Bauru/SP, Profa.Dra. SIMONE BALDINI LUCHEIS (Participação Presencial) do(a) Dep. de Produção Animal e Medicina Veterinária Preventiva / FMVZ/Botucatu - Unesp, Prof. Dr. THIAGO LUÍS MAGNANI GRASSI (Participação Virtual) do(a) Depto. de Medicina Veterinária / FZEA/Pirassununga - USP, Após a exposição pela mestranda e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, a discente recebeu o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Virgi B R. Pereira

Profa. Dra. VIRGINIA BODELÃO RICHINI PEREIRA

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RESUMO

O conceito de Saúde Única é uma abordagem fundamental para compreender a interdependência entre a saúde humana, animal e ambiental, no cenário de emergência e reemergência de patógenos respiratórios, como os vírus influenza e o SARS-CoV-2, responsáveis por epidemias e pandemias. Nesse contexto, a vigilância ecoepidemiológica de patógenos em populações silvestres torna-se estratégica, e o presente estudo teve como objetivo investigar, por meio de técnicas moleculares, a ocorrência de vírus respiratórios em mamíferos silvestres mortos por atropelamento, provenientes de diferentes regiões do Brasil, contribuindo para o mapeamento de áreas potenciais de risco de circulação viral. Foram analisadas 1.057 amostras coletadas de tecidos (pulmão, fígado, baço e coração) obtidas de 397 animais coletados em rodovias de 12 estados brasileiros, em dois períodos distintos (2002–2011 e 2021–2022). Os ácidos nucleicos foram extraídos e quantificados, e a integridade genética avaliada por reação em cadeia da polimerase convencional (cPCR) dos genes constitutivos. A triagem molecular de SARS-CoV-2, Influenza A e B e Vírus Sincicial Respiratório foi realizada pela PCR quantitativa (qPCR). Quando detectado Influenza A, realizou-se a subtipagem, incluindo H1N1 pdm09 e H3N2 por qPCR, e H5N1 por cPCR. Os resultados indicaram que 43,04% (455/1057) das amostras exibiram amplificação do gene constitutivo, confirmando integridade suficiente para as análises moleculares. Entre os tecidos, os pulmões apresentaram 54,9% (207/377) de amplificação, destacando este órgão como o mais indicado para triagem viral em mamíferos silvestres. A análise estatística demonstrou maior sucesso de amplificação em amostras criopreservadas a −80°C, com eficiência superior no período mais recente (2021–2022) em relação ao anterior (2002–2011). Foi detectada positividade para Influenza em um espécime de *Conepatus semistriatus* (jaritataca) procedente do estado de Minas Gerais, com subtipagem inconclusiva, direcionando a continuidade da investigação por sequenciamento de nova geração a ser investigada em projeto complementar. Conclui-se que o uso de animais silvestres atropelados como sentinelas, aliado à criopreservação e a metodologias moleculares sensíveis, constitui uma estratégia eficaz para vigilância passiva, viabilizando a detecção precoce de patógenos zoonóticos e o monitoramento da circulação viral na interface humano-animal, fortalecendo políticas de saúde pública sob a perspectiva da Saúde Única.

Palavras-chave: saúde única; ecoepidemiologia; vigilância em saúde pública; animais selvagens; reação em cadeia de polimerase.

ABSTRACT

The One Health concept provides an essential perspective for understanding the interconnectedness of human, animal, and environmental health, particularly in the context of emerging and re-emerging respiratory pathogens, such as influenza viruses and SARS-CoV-2, responsible for epidemics and pandemics. In this context, the eco-epidemiological surveillance of pathogens in wildlife represents a strategic approach, serving as a crucial component for the early identification of potential zoonotic threats. The present study aimed to investigate the occurrence of respiratory viruses in road-killed wild mammals from different regions of Brazil using molecular approaches, contributing to the identification of areas at potential risk for viral circulation. A total of 1,057 tissue samples (lung, liver, spleen, and heart) from 397 animals were analyzed. Specimens were collected along highways in 12 Brazilian states during two distinct periods (2002–2011 and 2021–2022). Nucleic acids were extracted, quantified, and evaluated for genetic integrity through conventional polymerase chain reaction (cPCR) targeting constitutive genes. Molecular screening for SARS-CoV-2, Influenza A and B, and Respiratory Syncytial Virus (RSV) was performed using quantitative PCR (qPCR). Influenza A–positive samples were further subjected to subtyping assays for H1N1 pdm09 and H3N2 by qPCR, and H5N1 by cPCR. Overall, 43.04% (455/1,057) of the samples exhibited successful amplification of constitutive genes, indicating adequate nucleic acid integrity for downstream molecular analyses. Among the evaluated tissues, lung samples exhibited the highest amplification rate (54.9%; 207/377), supporting their suitability for viral screening in wild mammals. Statistical analyses demonstrated significant amplification success in samples cryopreserved at −80 °C, as well as improved performance in specimens collected during the more recent period (2021–2022) compared to those from 2002–2011. Influenza virus was detected in a specimen of *Conepatus semistriatus* (striped hog-nosed skunk) from the state of Minas Gerais; however, subtyping was inconclusive, prompting further investigation using Next-Generation Sequencing (NGS) in a complementary study. These findings indicate that the use of road-killed wild mammals as sentinels, combined with appropriate cryopreservation and sensitive molecular methodologies, represents an effective strategy for passive surveillance. This approach supports the early detection of zoonotic pathogens and enhances the monitoring of viral circulation at the human–animal interface, thereby reinforcing public health actions within the One Health perspective.

Keywords: one Health; eco-epidemiology; public health surveillance; wildlife; polymerase chain reaction.

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1. INTRODUÇÃO

1.1. Saúde Única

O conceito de Saúde Única representa uma abordagem integrada e indispensável à compreensão da interdependência entre a saúde humana, animal e ambiental, reconhecendo que as alterações nos ecossistemas impactam, direta ou indiretamente, todos os seres que compartilham o mesmo ambiente. Em um mundo globalizado, essas alterações podem ultrapassar fronteiras e desencadear surtos epidêmicos ou pandemias, como evidenciado na história recente (Gebreyes *et al.,* 2014; Jones *et al*., 2025).

Nesse contexto, a abordagem de Saúde Única promove a cooperação entre diferentes áreas do conhecimento e setores da sociedade, fortalecendo ações interdisciplinares e interinstitucionais (Bidaisee; Macperson, 2014). Essa perspectiva reforça que a integração é essencial para prevenir e responder de forma eficaz às doenças emergentes, especialmente as zoonóticas, abrangendo também aspectos fundamentais como o fortalecimento dos serviços laboratoriais, o controle de doenças tropicais negligenciadas, o enfrentamento da resistência antimicrobiana, a segurança alimentar e a promoção da saúde ambiental (Cunningham; Daszak; Wood, 2017; Pitt; Gunn, 2024). A implementação dessa abordagem permite ações coordenadas e políticas integradas voltadas à proteção da saúde e ao bem-estar coletivo em escala global (Schneider; Oliveira, 2020; Zinsstag *et al.,* 2022).

As mudanças climáticas do planeta impactam a saúde de forma multimodal, ondas de calor, eventos climáticos exacerbados como furacões, inundações, secas, deficiências nutricionais, mudanças no uso da terra, sistemas de produção animal, uso irracional de antibióticos, crescimento e deslocamento populacional por diversos fatores como guerra, viagens, pobreza, comércio global e doenças emergentes afetam

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