**UNIVERSIDADE DE CAXIAS DO SUL**
**ÁREA DO CONHECIMENTO DE CIÊNCIAS DA VIDA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE ANIMAL**

**Pedro Augusto Freire de Sá Pontes**

# PARVOVÍRUS SUÍNO TIPO 1: DINÂMICA EVOLUTIVA E IMPLICAÇÕES PARA A EFICÁCIA VACINAL

Caxias do Sul
2025

**Pedro Augusto Freire de Sá Pontes**

# PARVOVÍRUS SUÍNO TIPO 1: DINÂMICA EVOLUTIVA E IMPLICAÇÕES PARA A EFICÁCIA VACINAL

Dissertação apresentada à Universidade de Caxias do Sul – Programa de Pós-Graduação em Saúde Animal, para obtenção do Título de Mestre em Saúde Animal.

Área de Concentração: Saúde Animal

**Orientador(a):**
Prof. Dr. André Felipe Streck

**Coorientador(a):**
Profa. Dra. Scheila de Avila e Silva

Caxias do Sul
2025

Dados Internacionais de Catalogação na Publicação (CIP)
Universidade de Caxias do Sul
Sistema de Bibliotecas UCS - Processamento Técnico

P814p Pontes, Pedro Augusto Freire de Sá
Parvovírus suíno tipo 1 [recurso eletrônico] : dinâmica evolutiva e implicações para a eficácia vacinal / Pedro Augusto Freire de Sá Pontes. – 2025.
Dados eletrônicos.

Dissertação (Mestrado) - Universidade de Caxias do Sul, Programa de Pós-Graduação em Saúde Animal, 2025.
Orientação: André Felipe Streck.
Coorientação: Scheila de Avila e Silva.
Modo de acesso: World Wide Web
Disponível em: https://repositorio.ucs.br

1. Parvovirus suíno. 2. Epidemiologia. 3. Vacinas. 4. Bioinformática. I. Streck, André Felipe, orient. II. Silva, Scheila de Avila e, coorient. III. Título.

CDU 2. ed.: 636.4:578.82/.83

Catalogação na fonte elaborada pela(o) bibliotecária(o)
Carolina Machado Quadros - CRB 10/2236

**Pedro Augusto Freire de Sá Pontes**

# PARVOVÍRUS SUÍNO TIPO 1: DINÂMICA EVOLUTIVA E IMPLICAÇÕES PARA A EFICÁCIA VACINAL

Dissertação apresentada à Universidade de Caxias do Sul – Programa de Pós-Graduação em Saúde Animal, para obtenção do Título de Mestre em Saúde Animal.

Área de Concentração: Saúde Animal
**Orientador(a):**
Prof. Dr. André Felipe Streck
**Coorientador(a):**
Profa. Dra. Scheila de Avila e Silva
**Aprovado em (02/07/2025)**

**Banca Examinadora**
Profa. Dra. Simone Silveira
Universidade do Oeste de Santa Catarina (UNOESC)

Profa. Dra. Danielle Gava
Universidade do Estado de Santa Catarina (UDESC)

Prof. Dr. Vagner Ricardo Lunge
Universidade de Caxias do Sul (UCS)

Ao meu avô, Joel de Sá, em memória. Seu trabalho, carinho e generosidade pavimentaram a estrada que me trouxe até aqui. Que este trabalho honre o seu legado e seja prova de que nenhum de seus esforços foi em vão.

# AGRADECIMENTOS

Expresso minha sincera gratidão ao meu orientador, Professor Dr. André Felipe Streck pela sua amizade, paciência, disponibilidade, apoio, dedicação e orientação ao longo desta jornada. À minha coorientadora, Professora Dra. Scheila de Avila e Silva, pela sua paciência, ensinamentos e doces na sala do grupo de bioinformática. Ao Professor Dr. Vagner Ricardo Lunge, pelas suas aulas sempre bem-humoradas e dúvidas sanadas. Agradeço os professores do Mestrado em Saúde Animal que contribuíram indiretamente para este trabalho, ampliando os meus conhecimentos.

Agradeço à minha família, o meu pilar. À minha mãe, Maria Amélia Freire de Sá, por todo amor e carinho. Aos meus padrinhos, Gilberto Freire de Azevedo Cabral e Valéria Cheik Cabral, pelo apoio, carinho e por estarem sempre presentes na minha vida. Ao meu primo, Felipe Cheik Cabral, pela amizade e companheirismo ao longo de todos estes anos.

Um agradecimento especial à minha tia, Professora Dra. Maria de Fátima Freire de Sá. Sua perseverança e crença incondicional em meu potencial, seu apoio e incentivo constante foram a força motriz que tornou a conclusão deste trabalho possível. Ao meu tio, José Alceu Lorandi, pelo apoio, carinho e pela torcida constante.

Minha gratidão aos meus colegas e amigos do Laboratório de Diagnóstico em Medicina Veterinária, cuja amizade e companheirismo tornaram os longos dias mais leves e os desafios, menores. Levo cada um de vocês em minha trajetória.

Por fim, agradeço ao patrocinador não-oficial deste projeto, a Monster Energy. A contribuição de sua cafeína para a conclusão deste trabalho foi estatisticamente significativa ($p < 0,05$)

**“No momento em que nos comprometemos, a providência divina também se põe em movimento.**
**(...) Qualquer coisa que você possa fazer ou sonhar, você pode começar.**
**A coragem contém em si mesma, o poder, o gênio e a magia.”.**
**— Johann Wolfgang von Goethe (atribuição popular).**

# RESUMO

O parvovírus suíno tipo 1 (PPV1) é um dos principais agentes etiológicos responsáveis por causar falhas reprodutivas em granjas suinícolas, frequentemente associado à síndrome SMEDI (natimortalidade, mumificação, morte embrionária e infertilidade), resultando em perdas econômicas significativas. Embora a vacinação seja a principal medida de controle, sua alta resistência e o surgimento de novas variantes levantam preocupações sobre a eficácia das vacinas comerciais atualmente utilizadas. O objetivo deste estudo foi investigar a dinâmica evolutiva do PPV1 por meio de uma abordagem integrada entre bioinformática e imunoinformática, avaliando a divergência antigênica e suas implicações para a proteção vacinal. Para isso, foram analisadas 200 sequências completas do gene VP2, abrangendo o período de 1963 a 2021. Os resultados revelaram a existência de cinco linhagens genéticas distintas, sendo que duas delas não possuem representação de cepas vacinais de referência, indicando possível falha na cobertura imunológica. Foi estimada uma alta taxa evolutiva, de $1{,}57 \times 10^{-5}$ substituições/sítio/ano, comparável à de vírus RNA. Os sítios 320 e 436 da proteína VP2 foram identificados sob forte pressão de seleção positiva, e mutações específicas em regiões antigênicas foram correlacionadas com a deriva antigênica e patogenicidade. Além disso, foram identificados epítopos de células T promissores com alta afinidade de ligação a alelos SLA. Conclui-se que o PPV1 apresenta linhagens geneticamente divergentes com potencial de escape imunológico. Tais achados ressaltam a necessidade de vigilância genômica contínua e da atualização de imunógenos.

**Palavras-chave:** bioinformática; SMEDI; vacinas; PPV1; epidemiologia.

## ABSTRACT

Porcine parvovirus type 1 (PPV1) is one of the main etiological agents responsible for causing reproductive failures in swine farms, frequently associated with SMEDI syndrome (stillbirth, mummification, embryonic death and infertility), resulting in significant economic losses. Although vaccination is the main control measure, its high resistance and the emergence of new variants raise concerns about the efficacy of currently used commercial vaccines. The objective of this study was to investigate the evolutionary dynamics of PPV1 through an integrated approach between bioinformatics and immunoinformatics, evaluating antigenic divergence and its implications for vaccine protection. For this purpose, 200 complete VP2 gene sequences were analyzed, spanning the period from 1963 to 2021. The results revealed the existence of five distinct genetic lineages, of which two lack representation of reference vaccine strains, indicating possible failure in immunological coverage. A high evolutionary rate of $1.57 \times 10^{-5}$ substitutions/site/year was estimated, comparable to that of RNA viruses. Sites 320 and 436 of the VP2 protein were identified under strong positive selection pressure, and specific mutations in antigenic regions were correlated with antigenic drift and pathogenicity. Additionally, promising T-cell epitopes were identified with high binding affinity to SLA alleles. In conclusion, PPV1 exhibits genetically divergent lineages with immune escape potential. These findings highlight the need for continuous genomic surveillance and the updating of immunogens.


**Keywords**: bioinformatics; SMEDI; variants; PPV1; epidemiology.

# SUMÁRIO

**1. Introdução ..... 13**

**2. Objetivos ..... 15**

2.1 Objetivo geral ..... 15

2.2 Objetivos específicos ..... 15

**3. Revisão da Literatura ..... 16**

3.1 Parvovírus Suíno Tipo 1 ..... 16

3.1.1 Histórico ..... 16

3.1.2 Etiologia ..... 16

3.1.3 Caracterização Estrutural ..... 17

3.1.4 Epidemiologia ..... 19

3.1.5 Patogenia ..... 21

3.1.6 Relação Sistema Imune e Hospedeiro ..... 24

3.1.7 Sinais Clínicos ..... 25

3.1.8 Diagnóstico ..... 25

3.1.9 Manejo Clínico ..... 27

3.1.10 Controle ..... 28

3.1.11 Profilaxia ..... 29

**4. Artigo Publicado em Periódico ..... 31**

**5. Discussão ..... 76**

**6. Conclusão ..... 80**

**7. Perspectivas ..... 81**

**8. Referências ..... 82**

# LISTA DE FIGURAS

Figura 1. Modelo em 3D da estrutura do capsídeo do PPV1 .....17

Figura 2. Representação esquemática do genoma do parvovírus suíno (PPV1) .....18

Figura 3. Exemplo de ninhada SMEDI, classificados por tamanho. .....23

# LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

| | |
|---|---|
| **PPV1** | Parvovírus suíno do tipo 1 (*Porcine parvovirus type 1*) |
| **PCV2** | Circovírus suíno tipo 2 (*Porcine circuvirus type 2*) |
| **AAC** | Composição de aminoácidos (*Amino Acid Composition*) |
| **ACC** | Autocovariância e covariância cruzada (*Auto and Cross-Covariance*) |
| **AIC** | Critério de Informação de Akaike (*Akaike Information Criterion*) |
| **ANNs** | Redes neurais artificiais (*Artificial Neural Networks*) |
| **BA** | Afinidade de ligação (*Binding Affinity*) |
| **BEAST** | *Bayesian Evolutionary Analysis by Sampling Trees* |
| **BIC** | Critério de Informação Bayesiano (*Bayesian Information Criterion*) |
| **BSP** | *Bayesian Skyline Plot* |
| **CBS** | *Coalescent Bayesian Skyline* |
| **CEP** | *Coalescent Exponential Population* |
| **ESS** | Tamanho efetivo da amostra (*Effective Sample Size*) |
| **FEL** | *Fixed Effects Likelihood* |
| **FUBAR** | *Fast Unconstrained Bayesian Approximation* |
| **HPLC** | Cromatografia líquida de alta performance (*High-Performance Liquid Chromatography*) |
| **IEDB** | Banco de Dados de Epítopos Imunológicos (*Immune Epitope Database*) |
| **MCC** | Árvore de Máxima Credibilidade (*Maximum Clade Credibility*) |

| | |
|---|---|
| **MCMC** | Cadeia de Markov Monte Carlo (*Markov Chain Monte Carlo*) |
| **DGZ** | Centro de saúde animal holandês (*Dierengezondheidszorg*) |
| **EMBRAPA** | Empresa Brasileira de Pesquisa Agropecuária |
| **ABCS** | Associação Brasileira dos Criadores de Suínos |
| **ABPA** | Associação Brasileira de Proteína Animal |
| **SMEDI** | Falha reprodutiva dos suínos |
| **DNA** | Ácido desoxirribonucleico |
| **RNA** | Ácido ribonucleico |
| **ORF** | Fase de Leitura Aberta (*Open Reading Frame*) |
| **TLR** | Receptor Toll-like (*Toll-like Receptor*) |
| **CRM** | Exportina |
| **NS** | Amostragem Aninhada (*Nested Sampling*) ou Proteína não estrutural (*Non-structural*) |
| **ORC** | Modelo de Relógio Relaxado Otimizado (*Optimized Relaxed Clock*) |
| **PLS** | Algoritmo de mínimos quadrados parciais (*Partial Least Squares*) |
| **RMSD** | *Root Mean Square Deviation* |
| **SLAC** | *Single-Likelihood Ancestor Counting* |
| **Å** | Angstrom (unidade de comprimento) |
| **dS** | Taxa de substituições sinônimas |
| **dN** | Taxa de substituições não sinônimas |
| **ω (Omega)** | Razão dN/dS, que indica a pressão seletiva sobre uma proteína |

# 1. INTRODUÇÃO

A suinocultura desempenha um papel crucial na produção de proteína animal em todo o mundo, contribuindo significativamente para a segurança alimentar e a economia global. Atualmente, o Brasil é o quarto maior produtor de carne suína do mundo (ABPA, 2024) com cerca de 58 milhões de cabeças abatidas, gerando 151 mil empregos diretos formais, 1.1 milhões de empregos indiretos, 56,2 bilhões de reais de valor bruto e 3 bilhões de dólares em exportações no ano de 2024, que apresenta um crescimento de 10% no volume exportado em comparação a 2023 (ABCS 2024; ABPA, 2025). A região sul do Brasil é responsável por aproximadamente 93% de todas as exportações de carne suína do país (ABPA, 2024). Portanto, as dimensões dessa cadeia de produção de carne suína inevitavelmente exigem um conjunto rigoroso de abordagens em relação à saúde animal e ao desempenho reprodutivo (Vargas et al., 2007).

Um dos patógenos mais comuns e importantes que têm impacto substancial na suinocultura é o parvovírus suíno tipo 1 (PPV1 – do inglês, *Protoparvovirus ungulate1*). Na América Latina, o primeiro trabalho relacionado ao PPV foi realizado por Gouveia (1982), que encontrou uma frequência média de 55,3% de animais soropositivos distribuídos em todos os rebanhos testados. Em granjas situadas nos estados de Santa Catarina e Rio Grande do Sul, cerca de 80% dos animais eram soropositivos (Martins, 1984). Atualmente, o parvovírus suíno continua sendo isolado em granjas no sul do país, geralmente associados à infecções simultâneas com o circovírus suíno tipo 2 (PCV2) (Herdt et al., 2019; Pescador et al., 2007).

O PPV1 é um vírus altamente resistente que pode perdurar por até 4 meses no ambiente, infectando suínos de todas as idades e causando falhas reprodutivas nos rebanhos. A doença é conhecida pelo acrônimo SMEDI (do inglês, *stillbirth, mummification, embryonic death, and infertility*), e seus sinais clínicos são caracterizadas pelo retorno ao estro, atraso do parto, gestações prolongadas, infertilidade, redução de leitões por leitegada e presença de fetos mumificados e natimortos (Christianson et al., 1993; Mengeling; Lager; Vorwald, 2000).

A infecção por PPV1 geralmente resulta em altas taxas de perdas reprodutivas e significativo impacto econômico na suinocultura, e até o momento não há um tratamento específico disponível contra a infecção pelo vírus, o que torna medidas estritas de biosseguridade e vacinação a única forma de controle do vírus (Mészáros et al., 2017b). No entanto, estudos evidenciaram que o PPV1 possui uma alta taxa de mutação, se comparando aos vírus RNA, o que pode comprometer a eficácia vacinal (Af et al., 2011). As falhas na imunização são uma das principais razões para circulação do PPV1 globalmente. Essas falhas

podem ser atribuídas a várias causas, incluindo a divergência genética entre cepas vacinais e de campo, a presença de diferentes linhagens genéticas do vírus, e a alta quantidade de animais não vacinados ou a execução inadequada dos protocolos de vacinação (Vereecke et al., 2022; Zeeuw et al., 2007).

A cepa NADL-2, utilizada como base para muitas vacinas comerciais há aproximadamente 50 anos e sendo a mais amplamente empregada no Brasil, tem demonstrado limitações significativas na proteção contra cepas geneticamente heterólogas de PPV1, particularmente contra os isolados 27a-like que se tornaram predominantes em muitas regiões (Cho et al., 2022; Vereecke et al., 2022). Estudos de neutralização cruzada têm revelado que vacinas baseadas na cepa NADL-2 apresentam atividade neutralizante consideravelmente reduzida contra a cepa 27a, com títulos de anticorpos neutralizantes 100 a 1000 vezes menores em comparação com a resposta homóloga (Zeeuw et al., 2007). A persistência de problemas reprodutivos em rebanhos vacinados, evidenciada pelo aumento na detecção de PPV1 em casos de síndrome SMEDI de 0,5% em 2017 para 9,2% em 2019 na Bélgica, e tendências similares observadas na Dinamarca, demonstra claramente as consequências práticas dessas limitações vacinais (Vereecke et al., 2022). O aumento da circulação viral indica que as vacinas disponíveis não estão fornecendo proteção adequada aos animais contra as variantes em circulação atual (Oh et al., 2017; Vereecke et al., 2022).

No Brasil, os primeiros estudos de epidemiologia molecular do PPV1 foram conduzidos por Soares et al. (2003), que analisaram sequências parciais do gene VP2 de 29 isolados de campo provenientes de diferentes regiões do país. Esse estudo revelou a existência de pelo menos duas linhagens virais distintas circulando no território brasileiro, contendo 26 sítios polimórficos nucleotídicos e 22 sítios polimórficos na sequência de aminoácidos traduzida, com sua grande maioria localizada na superfície externa do capsídeo viral, precisamente em regiões de interação antigênica (Soares et al., 2003). Além disso, trabalhos anteriores relatam alta soroprevalência do PPV1 (> 80%) em granjas comerciais, demonstrando que o vírus circula em rebanhos de suínos brasileiro (Bersano et al., 1993; Rodriguez et al., 2003). Recentemente, estudos realizados em granjas do sul do Brasil demonstraram positividade de 68% para PPV1 em fetos mumificados, frequentemente associados com o PCV2, mesmo em rebanhos vacinados, o que levanta questões sobre a eficácia das estratégias de controle atualmente empregadas e a necessidade de vigilância molecular contínua (Herdt et al., 2019).

# 2. OBJETIVOS

## 2.1 Objetivo geral

Avaliar a dinâmica evolutiva do PPV1 e suas implicações para a eficácia vacinal, com foco na análise genética do gene VP2 e na identificação de potenciais falhas na cobertura imunológica atual.

## 2.2 Objetivos específicos

1. Construir um banco de dados com sequências completas do gene VP2 do PPV1 obtidas através de bancos genômicos públicos.
2. Detectar e excluir eventos de recombinação genética, validando a adequação temporal para análises filogenéticas.
3. Realizar análises filogenéticas e de dinâmica populacional para caracterizar linhagens virais emergentes do PPV1.
4. Detectar sítios sob seleção positiva no gene VP2 e avaliar sua relação com regiões antigênicas e potencial de escape imune.
5. Prever epítopos de células B e T e realizar simulações de docking molecular com alelos SLA a fim de validar a afinidade do epítopo ao MHC e avaliar possíveis falhas imunológicas.
6. Correlacionar a diversidade genética do PPV1 com a eficácia vacinal, destacando a necessidade de atualização de imunógenos para abranger linhagens emergentes .

# 3. REVISÃO DA LITERATURA

## 3.1 Parvovírus Suíno Tipo 1

### 3.1.1 Histórico

Até a década de 60 as perdas reprodutivas em granjas comerciais de suínos eram altas e as causas desconhecidas (Lawson, 1961). Tais perdas reprodutivas eram comumente associadas a fatores ambientais, genéticos, nutricionais e agentes tóxicos (Rasbech, 1969). Ao final dos anos 60, com o avanço das pesquisas em virologia, a correlação de problemas reprodutivos com agentes infecciosos passou a ser bem mais compreendida e descrita (Dunne, 1970). Entre a lista de doenças virais associados a estes problemas reprodutivos estavam a peste suína clássica (Young et al., 1955), a doença de Aujeszky (Gordon e Luke, 1955), os enterovírus suínos (Dunne et al., 1965) e, pela primeira vez, o PPV1 foi descrito (Cartwright; Lucas; Huck, 1969), sendo posteriormente isolado de leitões nascituros em diversos países (Bachmann, 1969; Genov et al., 1971; Morimoto et al., 1972; Coackey e Smith, 1972; Mengeling, 1972; Johnson, 1973; Narita et al., 1975; Redman; Bohl; Ferguson, 1974).

Ao longo dos anos, o PPV1 foi considerado o principal patógeno responsável pela síndrome e até hoje segue sendo relatado em todos os continentes, sempre correlacionado a falhas reprodutivas nos planteis (Gillick, 1977; Rivera et al., 1995; Song et al., 2010; Molini et al., 2022). No Brasil, o parvovírus suíno tem sido uma constante preocupação para a indústria suína ao longo das décadas, sendo isolado em vários estados e municípios (Barthasson et al., 2009; Herdt et al., 2019; Pescador et al., 2007; Silva, 2015; Trindade et al., 2011), gerando perdas econômicas para os suinocultores e afetando a economia do país.

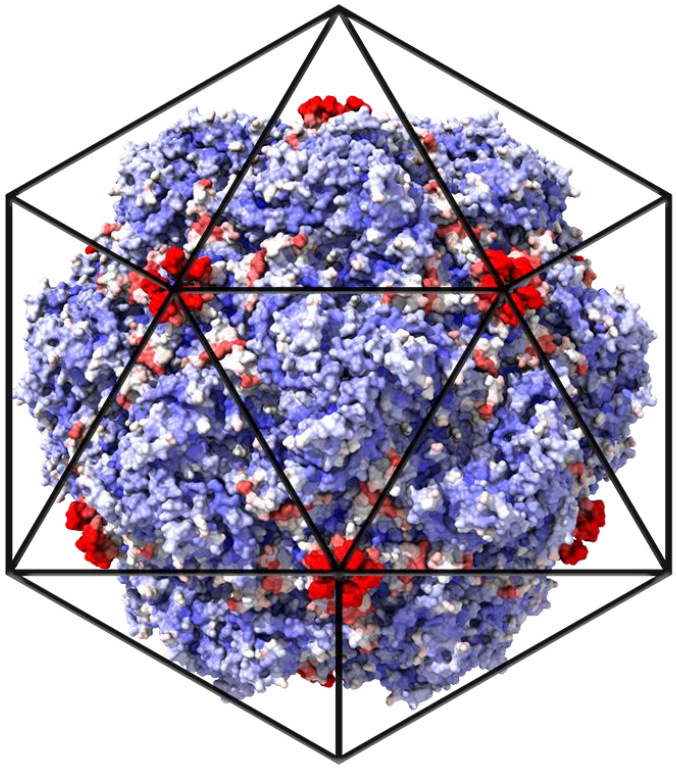
### 3.1.2 Etiologia

O Parvovírus suíno tipo 1 (PPV1), formalmente denominado *Protoparvovirus ungulate1*, pertence à família Parvoviridae da subfamília *Parvovirinae*, gênero *Protoparvovirus* (https://ictv.global/taxonomy, atualizado em 30 de maio, 2025). Outros membros do mesmo gênero incluem parvovírus de bovinos, felinos, caninos, gansos, ratos, camundongos, coelhos, visons, galinhas e guaxinins. Embora o PPV seja distinguível dos parvovírus de todas as outras espécies, ele possui semelhanças antigênicas a alguns deles (Mengeling; Ridpath; Vorwald, 1988).

### 3.1.3 Caracterização Estrutural

Os integrantes da família *Parvoviridae* consistem em vírus considerados pequenos, que oscilam entre 18 e 26 nm, dependendo da espécie viral. O peso molecular varia de 5,5 a 6,2 x106 daltons, com aproximadamente metade do peso dividido em sua massa proteica e outra metade composta por seu DNA (Horwitz et al., 1996). São vírus de DNA linear fita simples que possuem vírions pequenos, simetria icosaédrica e são desprovidos de envelope viral (Figura 1) (Pénzes et al., 2020), o que os torna extremamente resistentes à inativação, resistindo a grandes variações de pH, de 3,0 a 9,0, e de temperatura, como 56ºC por 60 minutos (Horwitz, 1996).

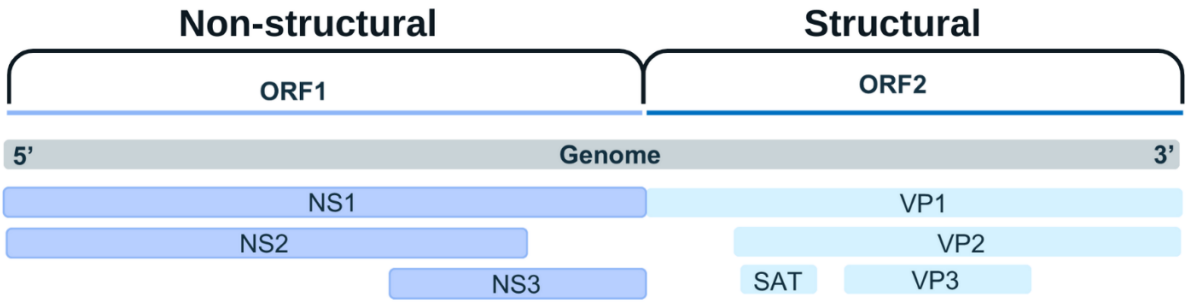
**Figura 1. Modelo em 3D da estrutura do capsídeo do PPV1 gerado a partir de simulação computacional via I-Tasser, editado através do software ChimeraX para melhor visualização.**



Fonte: Autoria pessoal.

O genoma do PPV1 possui em torno de 5000 bases que codificam duas fases abertas de leitura (ORFs – do inglês, Open Reading Frames), como demonstrado na Figura 2. A ORF1, situada à esquerda do genoma (5'), codifica as proteínas não estruturais (NS – do inglês, *non structural*), sendo elas denominadas NS1, NS2 e NS3. A proteína NS1 é uma proteína multifuncional necessária para replicação viral e produção de vírions infecciosos, estando também associada com a indução de apoptose celular, interrupção do ciclo celular, a supressão das respostas mediadas por interferon tipo I (IFN-1). NS1 também está relacionada com a inibição do receptor de patógenos TLR2 (Toll-like receptor 2), que tem como função reconhecer o PPV1 e induzir por sua via de sinalização intrínseca a resposta imune inata (Jin et al., 2020). A proteína NS2 também suprime as respostas mediadas por IFN-1 e participa da saída viral do núcleo da célula hospedeira para o citoplasma por interagir com a exportina CRM1 (Chen et al., 2021a). A proteína NS3 ainda não tem função conhecida, contudo, acredita-se que está igualmente relacionada à replicação viral (Sun et al., 2015).

**Figura 2. Representação esquemática do genoma do PPV1, mostrando as regiões codificadoras de proteínas não-estruturais (NS1, NS2 e NS3) da ORF1, proteínas estruturais (VP1, VP2, VP3) e proteína não-estrutural tardia (SAT) da ORF2.**




Fonte: Autoria pessoal.

A ORF2 está situada à direita do genoma (3') e codifica três proteínas estruturais (VPs), denominadas VP1, VP2 e VP3, além da proteína SAT (*Small Alternatively Translated*). Todas essas três proteínas estruturais podem induzir anticorpos inibidores da hemaglutinação (HI), considerados anticorpos neutralizantes (Xie et al., 2010). As proteínas VP1 e VP2 são proteínas importantes do capsídeo viral do PPV1, que são codificadas por um único gene que sofre *splicing* alterativo durante a transcrição do mRNA, gerando duas isoformas proteicas com domínios N-terminais distintos. A VP1 contém uma única extensão de cerca de 150 aminoácidos em rua região amino-terminal que inclui o domínio da fosfolipase $A_2$ ($PLA_2$) (Fernandes; Boisvert; Tijssen, 2011; López-Astacio et al., 2023). Esse domínio é responsável por facilitar a entrada do vírus na célula hospedeira e promover a liberação do genoma viral no citoplasma. Já a proteína VP2 é resultante da remoção da região estendida dessa região via

processamento do mRNA, que constitui aproximadamente 83% da superfície do capsídeo viral e abriga os principais epítopos neutralizantes, que são organizados em trimeros que formam protuberâncias icosaédricas essenciais para a ligação a receptores celulares (Vereecke et al., 2022; Zhou; Yao; Cui, 2010; Zimmermann et al., 2006). Essa proteína é o principal determinante do tropismo celular e da especificidade de hospedeiros do vírus, além de abrigar domínios responsáveis pelas propriedades hemaglutinantes, fundamentais para a interação viral a célula hospedeira (Zhou; Yao; Cui, 2010). A VP3, por sua vez, é gerada através da clivagem proteolítica da VP2 durante a maturação viral, mantendo a estabilidade estrutural do capsídeo e participando de estágios tardios da montagem viral (Chen; Stehle; Harrison, 1998). Paralelamente, a proteína SAT (SATp), uma proteína não-estrutural tardia, é sintetizada a partir de um quadro de leitura alternativo do mesmo mRNA que codifica a VP2, acumulando-se no retículo endoplasmático (ER) e modulando a resposta ao estresse do ER para acelerar a liberação de partículas virais (Mészáros et al., 2017a; Zádori; Szelei; Tijssen, 2005). A SAT não está diretamente associada ao capsídeo, porém possui funções importantes na indução de necrose celular e na otimização da disseminação viral.

### 3.1.4 Epidemiologia

O PPV1 é um agente viral mundialmente disseminado, estando presente na sua grande maioria em animais domésticos e asselvajados (Herdt et al., 2019; Jakov et al., 2021; Kaden et al., 2009; Montagnaro et al., 2010). Apesar dos esforços significativos da indústria suinícola na prevenção com planos de manejo adequados, uso de desinfetantes e vacinas, o número de relatos de animais infectados continua crescendo ao redor do mundo. Segundo o relatório da DGZ de Flandres; organização dedicada a saúde animal, bem-estar, segurança alimentar localizada na Bélgica, publicado em 2020, o total de incidências de SMEDI positivos para PPV aumentou de 0,5% em 2017 para 9.2% em 2019. Da mesma forma, na Dinamarca, antes de 2015, menos de 5% dos fetos testaram positivo para o PPV, mas a partir de 2015 até 2021, uma média de 17,4% eram soropositivos (Vereecke et al., 2022). No Brasil, estudos em granjas comerciais de suínos indicam que o vírus já está estabelecido no país por pelo menos há quatro décadas, gerando problemas reprodutivos (Martins et al., 1984; Gouveia, 1982). Um dos fatores que contribuem para a sua ampla distribuição é seu fator de resistência ambiental, por resistir a grandes variações de temperatura e muitos dos desinfetantes (Mengeling; Paul, 1986).

A transmissão do vírus ocorre principalmente por meio de contato oro-nasal direto, uma vez que o vírus é eliminado em secreções (ex.: saliva, secreções nasais, sêmen) e excreções (ex.: fezes e urina) a partir de 3 a 7 dias após infecção (Johnson; Donaldson-Wood; Allender,

1976), podendo persistir por até quatro meses em matéria orgânica. O vírus também pode estar presente em natimortos, fetos mumificados, membranas fetais, sêmen, fluidos vaginais e pode ser transportado através de fômites, como luvas, roupas, botas e equipamentos. Além disso, roedores podem carregar o vírus, atuando como vetores mecânicos (Truyen; Streck, 2019).

As fêmeas nulíparas são as mais suscetíveis a apresentar os sinais clínicos da infecção, porém a maioria já possui anticorpos contra o vírus antes mesmo da primeira vacinação, indicando exposição natural prévia (Gava et al., 2017). Os anticorpos maternos persistem por aproximadamente 3 meses em leitões, com meia-vida de 30 dias, desaparecendo completamente aos 87 dias de idade (Gava et al., 2017). Já as fêmeas multíparas apresentam menores problemas causados pelo vírus devido ao desenvolvimento de imunidade ativa (Mengeling; Lager; Vorwald, 2000).

Em leitões, a detecção do vírus é rara, uma vez que são considerados imunes devido à presença de anticorpos adquiridos através do colostro (Mengeling; Lager; Vorwald, 2000). Contudo, a duração dessa proteção é variável. Embora estimativas clássicas apontem para um período de quatro a seis meses (Paul; Mengeling; Pirtle, 1982), a persistência real dos anticorpos depende de fatores como o status imune da matriz e o volume de colostro ingerido pelo leitão. Em condições experimentais, pesquisadores inocularam fêmeas com o vírus durante o início da gestação e após o parto, alguns neonatos sobreviventes apresentavam o vírus, mas não demonstravam a presença de anticorpos, sugerindo uma reação de imunotolerância (Cartwright; Lucas; Huck, 1971). No entanto, a existência de animais imunotolerantes é extremamente rara, com poucos casos conhecidos a campo (Mengeling et al., 1999).

Os machos reprodutores infectados podem atuar como uma importante fonte de introdução do vírus em granjas, disseminando o vírus através do sêmen. Em estudos experimentais, machos reprodutores foram inoculados com o vírus e observou-se que o vírus estava presente tanto nos testículos quanto nos linfonodos escrotais, sugerindo a sua susceptibilidade à infecção e subsequentemente possibilidade da transmissão do vírus através do sêmen (Lucas; Cartwright; Wrathall, 1974; Mengeling; Lager; Vorwald, 2000).

Os javalis (*Sus scrofa*) são uma das principais preocupações na suinocultura. Eles podem servir como reservatórios de diversos patógenos que afetam seres humanos e animais, principalmente em países em desenvolvimento (Beltran-Alcrudo et al., 2019; Fredriksson-Ahomaa, 2019; Meng; Lindsay; Sriranganathan, 2009). O PPV1 foi isolado em javalis selvagens no mundo todo, evidenciando a importância destes animais na transmissão do vírus (De Maio et al., 2023; Jakov et al., 2021; Pacini et al., 2021; Park et al., 2021).

### 3.1.5 Patogenia

A doença pode afetar animais de todas as idades, no entanto as fêmeas nulíparas são as mais suscetíveis a apresentar os sinais clínicos da infecção devido à baixa titulação de anticorpos contra o parvovírus durante sua gestação, pois em alguns casos, os anticorpos maternos podem persistir por até seis meses ou mais em alguns dos animais, interferindo na imunização ativa da fêmea após a vacinação ou contato natural com o vírus (MacLachlan; Dubovi, 2017).

Caso expostas ao PPV1 durante os primeiros 30 dias de sua gestação, é provável que haja infecção transplacentária, que ocorre a partir de 12 a 18 dias após a infecção inicial das fêmeas gestantes, resultando em morte embrionária ou fetal dependendo do tempo de gestação e levando ao retorno do estro (Joo et al., 1976; Mengeling; Paul; Brown, 1980; Mengeling et al., 1999). Se houver sobreviventes, os leitões nascerão fracos e de baixo peso (Mengeling; Cutlip, 1976). Caso a infecção ocorrer após os 35 dias da gestação, haverá deposição de cálcio nos ossos fetais. Neste estágio, a infecção pelo vírus também resulta na morte do feto, porém a reabsorção é impedida devido à formação do esqueleto, o que leva à mumificação fetal (Johnson; Collings, 1971). No entanto, fetos imunocompetentes infectados após aproximadamente 70 dias de gestação conseguem produzir anticorpos e geralmente sobrevivem à infecção (Joo et al., 1976).

A forma em que o vírus ultrapassa a barreira transplacentária e infecta o feto ainda não foi completamente elucidada, visto que devido ao seu tamanho, o PPV não conseguiria atravessá-la sem auxílio. No entanto, estudos sugerem que o transporte do vírus se dá pela capacidade que o PPV1 tem de infectar monócitos e macrófagos sem comprometer sua infectividade. Contudo, ainda não há evidência direta que esses macrófagos migrem da mãe para o feto. A placenta dos suínos é caracterizada corioalantóide e epiteliocorial difusa (Santos, 2002). Dentre suas várias funções, ela é responsável por assegurar a nutrição do concepto em todas as fases de desenvolvimento por meio da passagem de nutrientes, gases, água, excreção de resíduos do metabolismo fetal no sangue materno e adaptação do metabolismo da mãe por meio de hormônios (Cetin; Alvino, 2009; Riquelme, 2009). Foi observado que fetos subdesenvolvidos podem provocar o alargamento das junções celulares em sua placenta, o que possibilita um maior fluxo de nutrientes e moléculas, podendo facilitar a infiltração do vírus (Vallet; Freking, 2007). Além disso, os capilares fetais e maternos da placenta frequentemente atravessam o tecido conjuntivo e penetram as camadas epiteliais. Dessa forma, mesmo com várias camadas entre elas, ambos os sangues podem entrar em contato, possivelmente colaborando com a passagem do vírus da mãe para o concepto (Vallet; Miles; Freking, 2009).

Portanto, como em outros vírus, a passagem do patógeno da mãe para o feto pode estar relacionada com a passagem de fluídos sanguíneos e linfáticos; células de defesa como linfócitos e macrófagos ou por replicação progressiva através dos tecidos que envolvem o feto (Mengeling; Lager; Vorwald, 2000).

A infecção transplacentária por replicação viral progressiva através da barreira placentária é bastante improvável. Essa barreira é composta por seis camadas de tecido que separa a circulação materna da fetal e não foi encontrada nenhuma evidência de que o vírus consiga se replicar no epitélio uterino ou no trofectoderma (Mészáros et al., 2017b). É possível que o vírus infecte os macrófagos maternos e utilize a passagem das células do sistema imunológico como meio de infecção, embora a replicação viral em macrófagos não tenha sido observada (Paul; Mengeling; Brown, 1979). Além do mais, estudos anteriores sugerem que os macrófagos peritoneais e monócitos periféricos conseguem fagocitar o vírus (Mengeling; Lager; Vorwald, 2000).

O PPV possui uma preferência pelos tecidos  do embrião e seus envoltórios, devido a sua alta taxa mitótica, o vírus invade as células por meio de uma série de interações que culminam na liberação do material genético viral em um compartimento celular no qual a replicação pode ocorrer (Harbison; Chiorini; Parrish, 2008; Truyen; Streck, 2019). No decorrer da infecção intrauterina, o vírus é transmitido de um feto para o outro, afetando-os em momentos distintos. Dessa forma, a infecção de toda a leitegada não ocorre de forma simultânea, o que justifica a presença de fetos mumificados em diferentes estágios de desenvolvimento frequentemente misturados com fetos morfologicamente normais, como representado pela Figura 3 (Moraes; Costa, 2007).

**Figura 3. Exemplo de ninhada SMEDI, classificados por tamanho. Os fetos foram pesados (g), medidos (cm) e atribuídos como fetos mumificados e natimortos.**

Fonte: Eddicks et al. (2023).

A composição genética do vírus exerce influência fundamental sobre o desfecho da infecção fetal. As variantes de menor patogenicidade e cepas utilizadas em vacinas como a NADL-2, 143a, IDT (MSV) não conseguem atravessar a barreira placentária com a mesma eficácia das cepas de maior patogenicidade, como a 27a e Kresse, que tem capacidade de matar até mesmo fetos imunocompetentes (Johnson; Donaldson-Wood; Allender, 1976; Kresse et al., 1985; Zeeuw et al., 2007). Portanto, efeitos adversos durante a gestação provocados pelas variantes de menor patogenicidade não são tão frequentemente detectadas comparadas com as cepas de alta patogenicidade (Mészáros et al., 2017b; Zeeuw et al., 2007). O PPV possui uma preferência pelos tecidos do embrião e seus envoltórios e quando o vírus atinge o concepto, ele encontra um ambiente propício para replicação devido à elevada taxa de mitose que a maioria destes tecidos apresenta (Moraes; Costa, 2007; Truyen; Streck, 2019). Analisando a distribuição do PPV em vários tecidos fetais, o vírus foi identificado no duodeno, tonsilas, jejuno, coração, fígado, pulmão, rim, baço, timo, gônadas, estômago e cérebro (Oraveerakul; Choi; Molitor, 1993; Wilhelm et al., 2005). Para fins de diagnóstico, o tecido de eleição em animais adultos é a tonsila, enquanto em animais jovens o coração representa a alternativa preferencial. Ademais, as cepas de menor virulência (NADL-2, 143a e IDT) demonstraram menor distribuição, estando localizadas principalmente nos rins, enquanto a cepa de maior virulência (27a) foi encontrada em todos os órgãos (Mészáros et al., 2017b; Wilhelm et al., 2005).

### 3.1.6 Relação Sistema Imune e Hospedeiro

A relação entre o sistema imunológico do hospedeiro e a capacidade do PPV de gerar os sintomas SMEDI depende diretamente do sistema imune do hospedeiro. Segundo Roth (1999), a primeira resposta contra o PPV1 se dá por meio de três tipos de barreiras: físicas, químicas e microbianas. As defesas físicas compreendem processos de autolimpeza, como tosse, espirros, descarga de muco no trato respiratório, além de diarreia e vômito no trato gastrointestinal. As barreiras químicas incluem o pH ácido intestinal e enzimas presentes em ambos os sistemas. Por fim, a barreira microbiana atua no intestino e no muco respiratório (Roth, 1999; Tizard, 2014). Possivelmente, por apresentar um capsídeo simples e compacto, capaz de suportar alterações bruscas de temperatura e pH, o PPV1 consegue ultrapassar esta primeira barreira com relativa facilidade (Streck, 2009).

Durante a infecção inicial por PPV1, o sistema imune inato é ativado através do reconhecimento de padrões moleculares associados a patógenos (PAMPs) via receptores de reconhecimento de padrões (PPRs) (Gao et al., 2022). A proteína NS1 do PPV1 atua como um destes PAMPs, sendo especificamente reconhecida pelo TLR2 (Chen et al., 2021b; Jin et al., 2020; Zhou et al., 2017). Essa ligação desencadeia uma cascata de sinalização que promove a fosforilação e degradação do IκB fator nuclear kappa B (IκB), liberando o fator de transcrição NF-κB (Jin et al., 2020; Zhou et al., 2017). Após translocar para o núcleo, o NF-κB induz a expressão de genes pró-inflamatórios, elevando citocinas como a interleucina-6 (IL-6) e o fator de necrose tumoral alfa (TNF-α) (Haddad; Abdel-Karim, 2011; Hayden; Ghosh, 2011). Além de recrutar células de defesa para o local da infecção, essa resposta inflamatória, combinada com a apoptose celular induzida pela NS1, contribui tanto para o combate do vírus quanto para a patogenia associada à infecção. Além disso, a interação da NS1 com o sistema imune não se limita à ativação de vias pró-inflamatórias. Estudos recentes revelaram um mecanismo de evasão imune específico mediado pela interação direta da proteína NS1 com uma proteína do hospedeiro chamada subunidade épsilon do complexo coatomer (COPε), que desativa a via de sinalização de interferon tipo I (IFN-I), suprimindo a resposta de IFN-β (Chen et al., 2021b). A COPε é um componente do complexo COPI (*Coat Protein Complex I*), que medeia a formação de vesículas envolvidas no transporte de proteínas e lipídios entre o Golgi e o retículo endoplasmático (RE) (Zhang et al., 2021).

Após o primeiro contato com o agente, a resposta imune adaptativa, que é a principal responsável pela proteção contra a doença, entra em ação através da ativação de células dendríticas que servem como apresentadoras de antígenos primárias, responsáveis por iniciar as respostas de células T (Morón et al., 2002). Essas células apresentadoras de antígenos (APC)

capturam antígenos virais através de mecanismos como a fagocitose das células infectadas e captação direta de partículas virais, o que leva à apresentação de peptídeos através das vias do complexo principal de histocompatibilidade (MHC), tanto de classe I quanto de classe II na superfície celular (Morón et al., 2002). Os peptídeos derivados desses antígenos são então apresentados por moléculas de MHC, que funcionam como plataformas de sinalização para os linfócitos T (Cruvinel et al., 2010; Tizard, 2014). A apresentação via MHC de classe I é especializada em exibir peptídeos de origem intracelular, como os virais, sinalizando uma infecção ativa diretamente para os linfócitos T citotóxicos (CD8+). Ao reconhecerem o complexo peptídeo-MHC, essas células T CD8+ ativadas controlam a infecção liberando mediadores citotóxicos, como perforinas e granzimas, que induzem a apoptose da célula infectada (Tizard, 2014).

### 3.1.7 Sinais Clínicos

As manifestações clínicas do parvovírus suíno podem ser caracterizados pelo acrônimo SMEDI (*stillbirth, mummification, embryonic death and infertility*). A doença aguda em fêmeas gestantes é subclínica, no entanto, podem apresentar uma leve leucopenia transitória dentro de 10 dias após a exposição ao vírus (Helke et al., 2015). Os sinais da doença dependem da categoria do animal infectado, bem como sua imunidade, tempo de gestação e patogenicidade da cepa em questão. Tais sinais são manifestados como retorno ao cio, atraso do parto, gestações prolongadas, infertilidade, redução de leitões por ninhada e presença de fetos mumificados e natimortos (Christianson, 1992; Mengeling; Lager; Vorwald, 2000). Os machos são assintomáticos e não apresentam nenhuma alteração clínica reprodutiva.

Não há evidências concretas, porém o PPV1 já foi isolado e relacionado a outros sinais clínicos. Dentre eles estão o isolamento do vírus nos pulmões de suínos que apresentavam pneumonia (Darbyshire; Roberts, 1968), diarreia (Yasuhara et al., 1989), lesões cutâneas em leitões (Choi et al., 1987; Kresse et al., 1985; Lager; Mengeling, 1994; Whitaker; Neu; Pace, 1990), miocardite não-supurativa (Bolt et al., 1997) e nefrite intersticial (Drolet et al., 2002).

### 3.1.8 Diagnóstico

O diagnóstico de PPV1 pode ser feita através de várias técnicas laboratoriais e deve ser considerado quando houver falhas reprodutivas dentro das granjas, principalmente em casos de mumificação fetal e natimortos. A identificação de fetos mumificados com comprimento cabeça-rabo ≤ 17cm e em diferentes estágios de desenvolvimento é um forte indicador de que o PPV1 é o agente infeccioso em questão (Helke et al., 2015). Os materiais enviados para os

laboratórios devem consistir em soro de fêmeas, fetos abortados, natimortos que não ingeriram colostro, fluidos fetais e sangue total, de acordo com o método de diagnóstico.

Os testes sorológicos estão entre os mais usados devido à necessidade de equipamentos especializados e complexidade das demais técnicas, mas são recomendadas apenas quando tecidos de fetos mumificados não estão disponíveis (Helke et al., 2015). A técnica de inibição da hemaglutinação (HI) mede a titulação de anticorpos que o animal apresenta e já foi descrita por vários autores, sendo uma das técnicas sorológicas mais usadas no Brasil para a detecção do PPV1 devido a capacidade do vírus em aglutinar eritrócitos de uma grande variedade de animais como ratos, macacos, galinhas, porquinhos da índia e humanos do grupo sanguíneo O (Fujisaki, 1975; Joo et al., 1976; Siegl, 1976). No entanto, não permite diferenciar animais vacinados dos infectados naturalmente e não existe uma padronização internacional de parâmetros como temperatura de incubação, espécie, idade, doador de hemáticas e tratamento prévio da amostra, o que pode interferir nos resultados (Mengeling; Lager; Vorwald, 2000). O teste sorológico de ELISA (*enzyme-linked immunosorbent assay*) é uma técnica indireta e possui uma eficácia equivalente ao teste de inibição da hemaglutinação. Ela dispensa o tratamento prévio das amostras; pode ser padronizada, automatizada e replicada, promovendo uma alternativa rápida para uma análise de muitas amostras. Além disso, também pode ser utilizada como método de monitoramento do perfil imunológico dos rebanhos suínos.

O diagnóstico do antígeno viral por imunofluorescência (IF) é um procedimento confiável para o diagnóstico de PPV1 (Mengeling; Cuptlip, 1975), no entanto, em matrizes a amostra de soro deve ser colhida no momento da falha reprodutiva, seguido de uma segunda amostra de 2 a 4 semanas depois (Truyen; Streck, 2019). Existem outras técnicas sorológicas, porém não são tão amplamente utilizados como os descritos anteriormente. Entre estes outros métodos estão o de micro aglutinação em tubo (Joo et al., 1975), imunodifusão em gel de ágar (Too et al., 1983) e aglutinação rápida em placa (Lü et al., 2006).

O isolamento viral é uma técnica altamente sensível e pode ser usado para diagnóstico do PPV1, porém não é muito eficaz visto que normalmente as amostras de tecidos chegam ao laboratório em autólise. A técnica basicamente consiste na replicação do vírus em células permissivas (ex.: PK-15 e ST) (Oraveerakul; Choi; Molitor, 1992; Wang et al., *[S.d.]*), resultando em grandes quantidades do agente viral. Vale ressaltar que é uma técnica complexa e que demanda bastante tempo de cultivo, resultando em um diagnóstico tardio (Roehe; Sobestiansky; Barcellos 2007).

Ultimamente com os avanços da biologia molecular, novas técnicas baseadas na detecção de sequência de nucleotídeos foram sendo desenvolvidas, e a qPCR (*real-time*

*polymerase chain reaction*) é uma das principais delas. A qPCR no Brasil tem a desvantagem de ser uma técnica mais cara devido ao custo elevado dos equipamentos, porém possui grandes vantagens que devem ser levadas em consideração. Em comparação com a PCR convencional, a qPCR possui maior sensibilidade e especificidade (McKillen et al., 2007). Além disso, automatizam a leitura das amostras e possibilitam o uso de sondas moleculares fluorescentes (sonda TaqMan e SYBR Green) que permitem a quantificação dos produtos amplificados a partir das amostras de soro, tecidos e sangue total dos animais infectados (McKillen et al., 2007; Wilhelm et al., 2006). Recentemente, pesquisas empregando a técnica com a sonda TaqMan para detecção e quantificação de PPV1 revelaram eficiência no diagnóstico, destacando-se pela rapidez, alta sensibilidade e especificidade (Song et al., 2010; Streck et al., 2015).

### 3.1.9 Manejo Clínico

Atualmente não existe tratamento comprovado para o PPV1. Hoje em dia as vacinas são o único método específico de prevenção, cujo objetivo é estimular a imunidade do plantel a desenvolver proteção contra o PPV1 e devem ser atreladas a boas medidas de manejo, a fim de promover um bom estado sanitário, uma vez que não existem agentes antivirais específicos aprovados para uso veterinário contra o vírus (Mengeling, 1999; Roehe; Sobestiansky; Barcellos 2007). A abordagem terapêutica se concentra na prevenção de complicações secundárias, especialmente em fêmeas gestantes afetadas pela síndrome SMEDI (Eddicks et al., 2023; Ma et al., 2020).

Em fêmeas que apresentam os sinais clássicos de falha reprodutiva associada ao PPV1, o manejo clínico inclui a avaliação do status reprodutivo através de exames ultrassonográficos seriados, a fim de monitorar a viabilidade fetal (Botero; Martinat-Botté; Bariteau, 1986). Quando detectada morte fetal, pode ser necessária a indução do parto para evacuar o conteúdo uterino e prevenir complicações como metrite ou septicemia, fazendo o uso de prostaglandinas ou outros agentes indutores de parto, que devem ser avaliados pelo médico veterinário responsável (Pressing et al., 1987).

Pesquisas recentes identificaram compostos naturais com potencial terapêutico contra o PPV1, utilizando o ácido ferúlico, que demonstrou capacidade de inibir a apoptose induzida pelo vírus através da supressão da via NF-κB e modulação da resposta inflamatória (Ma et al., 2020). Os estudos *in vitro* demonstraram que o ácido ferúlico reduz significativamente a expressão da proteína NS1 viral e diminui a produção de espécies reativas de oxigênio (ROS), que são moléculas altamente reativas derivadas do oxigênio e associadas ao estresse oxidativo

e danos celulares, sugerindo potencial terapêutico (Ma et al., 2020). No entanto, estes achados ainda requerem validação em ensaios clínicos controlados.

O manejo nutricional adequado também é essencial para fêmeas gestantes expostas ao PPV1, o que inclui a suplementação vitamínica e mineral apropriada para suporte do sistema imunológico, seguido da administração de probióticos e prebióticos, que podem auxiliar na manutenção da modulação da resposta imune (Ma et al., 2022). Em casos de coinfecções bacterianas secundárias, o uso de antimicrobianos pode ser necessário, e deve ser criteriosamente avaliado, respeitando os princípios de uso racional de medicamentos.

### 3.1.10 Controle

As estratégias de manejo sanitário representa a base para o controle do PPV1, considerando a sua alta resistência viral a condições ambientais adversas e a ausência de tratamentos específicos eficazes (Alarcón; Allepuz; Mateu, 2021; Otake; Yoshida; Dee, 2024). A implementação de protocolos rigorosos de biosseguridade externas e internas constituem a primeira linha de defesa contra a introdução e disseminação viral em rebanho suínos (Dee et al., 2024; Dewulf et al., 2019; Otake; Yoshida; Dee, 2024).

A biosseguridade externa tem como principal objetivo impedir a entrada do PPV1 no rebanho, utilizando barreiras físicas, desinfecção rigorosa de veículos com soluções virucidas, como o hipoclorito de sódio a 2% em rodolúvios, e estabelecendo quarentena mínima de 72 horas para animais recém-chegados e visitantes, práticas que, segundo estudos, reduzem em até 68% a introdução de novos agentes virais (Alarcón; Allepuz; Mateu, 2021; Filippitzi et al., 2018). Os equipamentos e materiais utilizados pelos funcionários devem ser tratados com desinfetantes de amplo espectro, como fenóis ou glutaraldeído, selecionados pela eficácia comprovada contra vírus não envelopados (Juszkiewicz et al., 2020; Rabenau et al., 2014).

No âmbito interno, a estratégia se concentra em evitar a propagação do vírus dentro da propriedade, adotando medidas como segregação rigorosa dos animais por idade, fluxo unidirecional de pessoas e animais, e a utilização do sistema "*all-in/all-out*", que prevê a desinfecção completa das instalações entre os lotes (Alarcón; Allepuz; Mateu, 2021; Filippitzi et al., 2018). Este processo inclui a aplicação de vapor a 80ºC durante 15 minutos, seguida do uso de desinfetantes oxidantes, como peróxido de hidrogênio a 5%, protocolo capaz de eliminar até 99,8% dos patógenos residuais (Filippitzi et al., 2018). Além disso, é de suma importância realizar a remoção mecânica de toda matéria orgânica antes da desinfecção, pois resíduos proteicos podem inativar agentes químicos e comprometer a eficácia do procedimento (Alarcón; Allepuz; Mateu, 2021; Rhee et al., 2023).

O controle de água e ração, apesar de subestimado, é essencial e deve receber atenção especial, uma vez que estes podem servir como veículos de transmissão viral (CABI, 2019). Sistemas de tratamento de água (cloração, ozonização ou UV) e controle de qualidade microbiológica da ração, associado ao manejo adequado de dejetos e carcaças, incluindo compostagem ou incineração, previnem a contaminação ambiental e quebra o ciclo epidemiológico (CABI, 2019).

### 3.1.11 Profilaxia

A profilaxia contra o PPV1 baseia-se na imunização ativa através de vacinas comerciais, representando a estratégia mais eficaz na prevenção da SMEDI e suas consequências econômicas (Ling et al., 2023; Paul; Mengeling, 1986). Os programas de vacinação devem ser cuidadosamente planejados considerando a imunidade do rebanho, pressão de infecção ambiental e sistemas de produção utilizados nos rebanhos (Paul; Mengeling, 1984, 1986).

As primeiras vacinas foram desenvolvidas durante a década de 70, usando o vírus inativado (Suzuki; Fujisaki, 1976; Joo; Johnson, 1977; Mengeling, 1979), se mostrando seguras e eficazes na prevenção da infecção transplacentária (Thomson; Prozesky, 1994). Na década de 80, a cepa NADL-2 passou a ser a mais utilizada para a formulação de vacinas inativadas contra o PPV1 (Paul; Mengeling, 1984; Zeeuw et al., 2007). No cenário brasileiro, predominam-se vacinas inativadas monovalentes ou combinadas com antígenos contra outras patologias, como a leptospirose e erisipela, embora estudos recentes apontem para o desenvolvimento de vacinas de subunidade baseadas na proteína VP2 (Ling et al., 2023; Rocha; Alberton; Santos, 2010). Geralmente, o protocolo vacinal envolve a aplicação de duas doses em nulíparas, 30 dias antes da primeira cobertura e 15 dias após a cobertura.

No entanto, a evolução viral acelerada do PPV1, com taxa estimada em $4{,}71 \times 10^{-5}$ substituições nucleotídica por sítio por ano, tem levantado preocupações sobre a eficácia prolongada das vacinas tradicionais, se comparando com as taxas de vírus RNA (Oh et al., 2017; Vereecke et al., 2022). Além do mais, em outro estudo foi verificado a atividade de neutralização gerada pelas cepas vacinais, que não demonstraram uma efetividade satisfatória frente as novas cepas circulantes na Europa, o que sugere que o vírus pode ser disseminado no plantel mesmo em rebanhos imunizados com a vacina (Duffy; Shackelton; Holmes, 2008; Shackelton et al., 2007; Simpson et al., 2002; Zeeuw et al., 2007). Essa falha de imunização foi causada por mutações em regiões críticas da VP2, particularmente nos loops antigênicos envolvidos na ligação a receptores celulares (Foerster et al., 2016; Oh et al., 2017). Portanto, programas de sanidade animal que envolvem a monitoração e caracterização das cepas

circulantes no campo são de grande importância para a saúde do plantel, evidenciando se há a necessidade da revacinação ou desenvolvimento de novas vacinas.

## 4. ARTIGO PUBLICADO EM PERIÓDICO

A seguir é apresentado o manuscrito resultante de nossas análises. Este manuscrito será submetido à revista *Viruses* (MDPI).

# *IN SILICO* INSIGHTS INTO THE DYNAMIC EVOLUTION OF PORCINE PARVOVIRUS LINEAGES AND VACCINE EFFICACY EVALUATION

Pedro Augusto Freire de Sá Pontes, Nicole Amoedo Luvison, Rafael Sartori Flores, Vagner Ricardo Lunge, Scheila de Avila e Silva, André Felipe Streck.

## ABSTRACT

Porcine parvovirus type 1 (PPV1) is globally recognized as the etiological agent responsible for one of the primary diseases causing reproductive failures in swine herds, known by the acronym SMEDI. Although it has been identified since the mid-1960s, PPV1 continues to be isolated worldwide, leading to significant financial losses. Vaccination remains the primary preventive measure against the virus. However, the emergence of new variants globally raises concerns regarding the efficacy of currently commercialized vaccines. This study aimed to elucidate the evolutionary dynamics of porcine parvovirus type 1 (PPV1) through a detailed bioinformatics analysis of 200 complete sequences of the gene encoding the VP2 protein, the primary structural component of the viral capsid spanning the years 1963 to 2021. The methodology involved data collection, genetic sequence analysis, followed by phylogenetic tree construction, simulation of different VP2 proteins, and prediction of positive selection sites and B and T cell epitope regions. Phylogenetic analysis revealed a total of five lineages. Two of those groups did not contain vaccine reference variants. The first group is predominantly composed of strains isolated in Europe, and the second is mainly divided between Asian and European strains. Predictions and simulations confirmed structural differences at various protein positions among the five groups identified in the phylogenetic tree and their three reference vaccine variants (NADL-2, 27a, and PPV014), which were classified as epitope regions and positive selection sites, suggesting that the strains used as vaccine references are possibly not providing protection against the new variants.

**Keywords:** PPV1; Protoparvovirus ungulate1; SMEDI; epidemiology; epitope prediction;

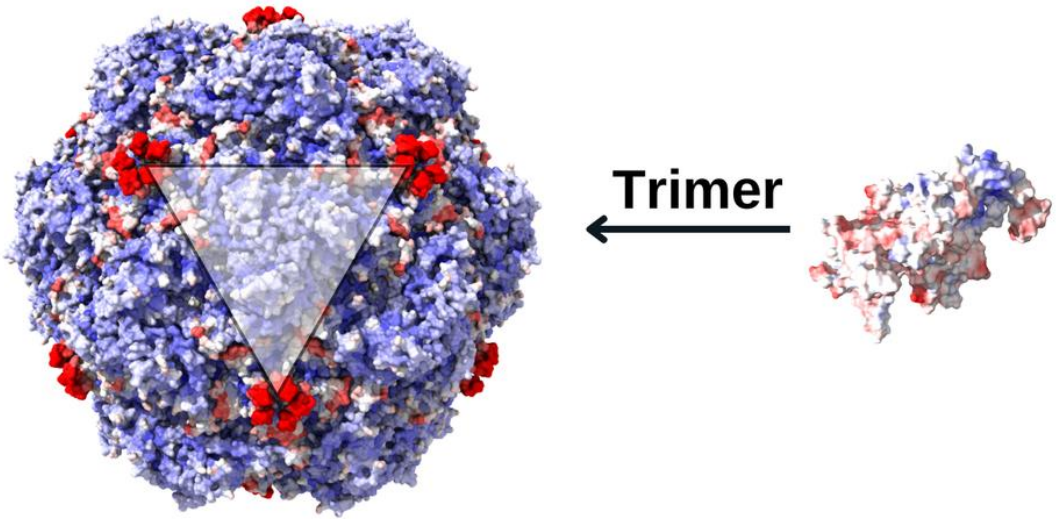
# 1. INTRODUCTION

Porcine parvovirus 1 (PPV1) is considered one of the most important infectious agents related to reproductive disorders in sows. It was first identified in Germany in 1965 as a contaminant of porcine primary cell cultures used for classical swine fever virus propagation (Streck; Canal; Truyen, 2015), PPV1 clinically manifests predominantly as SMEDI syndrome, which is an acronym for stillbirth, fetal mummification, embryonic death, and infertility. Beyond reproductive issues, PPV has been linked to cases of diarrhea, skin lesions, and joint inflammation in swine (Chen et al., 2009). Although the virus has been identified since the mid-sixties, it continues to be isolated worldwide. In recent years, infection has spread widely, and the virus spreads quickly, which further aggravates its impact on the reproductive performance of sows, severely affecting the swine industry and causing huge economic losses worldwide (Franzo et al., 2023; Ouh et al., 2024; Parthiban et al., 2022).

PPV1, currently named *Protoparvovirus ungulate1* (https://global/taxonomy, accessed on May 21, 2024), is icosahedral-shaped, having a single-stranded, linear DNA, non-enveloped, approximately 5 kb in length (Shackelton et al., 2007). Its genome contains two main coding regions, designated ORF1 and ORF2. ORF1 is located in the 5' region and is responsible for encoding three non-structural proteins: NS1, NS2, and NS3. ORF2, located in the 3' region, encodes three structural proteins: VP1, VP2, and VP3, , with VP3 via cleavage from VP2 **(Mészáros et al., 2017b)**. Moreover, the same VP2 mRNA differentiates a non-structural protein, SAT, initiated seven nucleotides downstream from the VP2 start codon (Zádori; Szelei; Tijssen, 2005).

The viral capsid is composed of 60 identical subunits of VP1 and VP2 proteins in a 1:5 ratio. Each VP1/VP2 trimer assembles into a structure that forms extrusions at the three-fold symmetry axis and dimples at the two-fold axis, as shown in Figure 01. These three-fold extrusions serve as key sites for receptor binding and neutralizing antibody attachment, while the two-fold dimples are crucial for oligosaccharide recognition and tissue tropism.

**Figure 1. Structural representation of the viral capsid, composed of 60 identical subunits of VP1 and VP2 proteins. The trimer forms protrusions at the three-fold symmetry axis, indicated by the triangular region.**




Source: Own image.

Alterations in specific VP1 amino acids, such as T214, G378, and P436, have been associated with variations in host specificity, immune response, and hemagglutination activity, as observed in the attenuated NADL-2 strain. However, no definitive correlation between these mutations and altered antigenicity has been established (Bergeron; Hébert; Tijssen, 1996; Cotmore; Tattersall, 2007; Streck; Canal; Truyen, 2015). Despite being a DNA virus, which have lower mutation rates compared to RNA viruses, PPV1 has exhibited a relatively high mutation rate, similar to that seen in RNA viruses (Af et al., 2011). This heightened mutability contributes to significant genetic variability, particularly in the viral capsid proteins, which are key targets for immune recognition and vaccine efficacy. The first vaccines against PPV1 date back to the 1980s, when inactivated or attenuated virus-based vaccines were developed (Streck; Canal; Truyen, 2015). These vaccines have been widely used to prevent SMEDI, and other clinical manifestations associated with PPV1. However, variants arising due to the high viral mutation rate can alter the epitopes of these proteins, rendering existing vaccines less effective or even ineffective against emerging strains. Mutations in these regions may potentially result in antigenic drift, allowing PPV1 variants to escape the neutralizing antibodies induced by existing vaccines.

This study aimed to investigate the evolutionary dynamics of Porcine parvovirus type 1 (PPV1), focusing on phylogenetic analysis, identification of emerging lineages, and characterization of sites under positive selection in the VP2 gene, the primary structural

component of the viral capsid. Using in silico approaches, we evaluated antigenic divergence between circulating variants and reference vaccine strains (NADL-2, 27a, and PPV014), identifying alterations in critical regions for immune recognition, such as B and T cell epitopes. Additionally, molecular docking analysis was performed between predicted epitopes and three major swine histocompatibility complex class I (MHC-I) alleles (SLA-1*08:01, SLA-2*02:01, and SLA-3*04:01) to elucidate potential antigen-receptor interactions associated with immune presentation. The study further sought to correlate PPV1 genetic diversity with implications for vaccine efficacy, providing insights for the rational design of updated immunogens capable of conferring cross-protection against contemporary globally circulating lineages.

## 2. MATERIALS AND METHODS

The analyses and programs were run between August 2023 and March 2025. All sequences are available for analysis in the supplementary material.

### 2.1 GenBank Data Collection and Sequence Alignment

A total of 203 complete VP2 coding sequences spanning the period of 1963 to 2021 were analyzed in this study, comprising 200 sequences from distinct viral isolates and 3 reference vaccine strains (NADL-2, PPV014, and 27a). All samples were obtained from the NCBI genetic database (https://www.ncbi.nlm.nih.gov/). Sequence alignment was performed using the MUSCLE algorithm (Multiple Sequence Comparison by Log-Expectation). The alignment was carried out in MEGA v11.0.3 (Tamura; Stecher; Kumar, 2021).

### 2.2 Detection of Recombinant Variants

Since our focus is on naturally occurring mutations, recombinant variants need to be excluded from the analysis. The detection of recombinant variants was conducted with the RDP v5.5 software (Martin et al., 2020), using the algorithms GENECONV, Chimaera, MaxChi, 3Seq, BootScan, SiScan, and RDP, all configured with the default parameters. Each of these algorithms detects recombination events using slightly different approaches, ensuring a comprehensive evaluation of potential recombination. Putative recombination events were considered significant when a p-value $\leq 0.01$ was observed for the same event in three or more algorithms. Sequences identified as putative recombinants were excluded from further analysis to maintain the integrity of the dataset (Sabir et al., 2016).

## 2.3 Substitution Model Selection

In order to ensure the accuracy of the phylogenetic analysis, the most appropriate substitution model was determined by using JModelTest v2.1.10 (Darriba et al., 2012). The best model, according to both Bayesian Information Criterion (BIC) and Akaike Information Criterion (BIC) scores, proved to be "GTR+G+I" (General Time Reversible with gamma distribution for rate heterogeneity among sites and a proportion of invariant sites). While the BIC score is biased towards simpler models with heavier penalties on its complexity, AIC balances model accuracy and model complexity, therefore fitting the data more flexibly (Liu et al., 2023).

## 2.4 Temporal Data Analysis and Quality Control

To ensure the reliability of molecular clock estimations, it is essential to identify and exclude potential outliers that could distort the analysis. For this purpose, temporal data analysis and data quality control were conducted using TempEst v1.5.3 (formerly known as "Path-O-Gen") (Rambaut et al., 2016). Initially, the program requires a phylogenetic tree without estimated dates, so for this purpose, IQ-TREE v2.3.1 (Nguyen et al., 2015) was employed to generate a tree by Maximum Likelihood (ML), using the previously selected “GTR+G+I” model. No outliers were detected.

## 2.5 Estimation of Time-Scaled Phylogenetic Tree

A time-scaled phylogenetic tree was estimated using BEAST2 v2.7.7 (Bouckaert et al., 2019), using the best-selected nucleotide substitution model (GTR+G+I), together with an Optimized Relaxed Clock (ORC) model with lognormal distribution and a Coalescent Exponential Population (CEP) model. This approach allows for variation in evolutionary rates among branches, while accurately scaling the phylogeny to time. The ORC and CEP models were compared using the Nested Sampling (NS) method, which provides Marginal Likelihood (ML) estimates for Bayesian model selection. The model with the highest ML was ultimately selected. The Bayesian Markov Chain Monte Carlo (MCMC) analysis was conducted with 100.000.000 iterations, sampling every 10.000 steps. The convergence was checked in Tracer v1.7.2 (Rambaut et al., 2018), with an effective sample size (ESS) threshold of > 250 after a 10% burn-in (Drummond et al., 2006). Finally, a maximum clade credibility tree was generated using TreeAnnotator v1.10.4 (Helfrich et al., 2018).

## 2.6 Population Dynamics Inference

For the inference of PPV1 population dynamics, the Coalescent Bayesian Skyline (CBS) model (Drummond et al., 2005) was applied, also using BEAST2 software (Bouckaert et al., 2019). The best-fitting nucleotide substitution model (GTR+G+I) and molecular clock model were selected as per the previous NS analysis. The MCMC chains were run for 100.000.000 iterations, sampling every 10.000 steps. Tracer v1.7 (Rambaut et al., 2018) was used to assess if there was enough convergence by checking if the value of the effective sample size ESS for all estimated parameters with a threshold of > 250 after a 10% burn-in.

## 2.7 Detection of Sites Under Positive Selection

The identification of sites under positive selection allows us to pinpoint regions of evolutionary interest, helping to locate areas potentially associated with specific biological functions and, ultimately, contributing to our understanding of the virus's evolution. To enhance accuracy in identifying sites under positive selection, two distinct analytical approaches were employed. The first method utilized the algorithms SLAC (Single-Likelihood Ancestor Counting), FEL (Fixed Effects Likelihood), MEME (Mixed Effects Model of Evolution), and FUBAR (Fast Unconstrained Bayesian Approximation), all accessible through the Datamonkey web server (https://datamonkey.org).

A site was considered to be under positive selection only if it was detected by at least two of these algorithms. The second method involved using the phylogenetic tree previously generated by IQ-TREE in conjunction with CODEML, part of the PAML v4.10.7 package (Phylogenetic Analysis by Maximum Likelihood; (Yang, 2007)) by comparing the rate of nonsynonymous substitutions ($d_N$) to the rate of synonymous substitutions ($d_S$) as an additional approach for identifying evidences of natural selection (M0 vs M3) and detecting sites under positive selection (M1a vs M2a) using four models (Table 4) (Álvarez-Carretero; Kapli; Yang, 2023; Anisimova; Bielawski; Yang, 2001; Anisimova; Nielsen; Yang, 2003). The model M0 is an evolutionary model where all amino acids sites have a single ω value. M1 is a neutral model, and assumes two classes of sites (conserved, $\omega = 0$; neutral, $\omega = 1$). In M2 (selection), an additional class characterized by $\omega_2$ is introduced. This class can take on any nonnegative value and applies to a proportion, $p_2$, of sites, subject to the constraint that $p_0 + p_1 + p_2 = 1$. M3 uses a general discrete distribution with three classes of sites, with 3 proportions and 3 ω-ratios. Finally, to test the hypothesis of variable selective pressure, our data set was fit to these four models and compared by LRT (Likelihood Ratio Test).

## 2.8 Amino Acid Evaluation

Based on the analysis and identification of five major clusters, five consensus amino acid sequences were generated by considering nucleotides present in more than 50% of positions for each cluster. These consensus sequences were then compared to three reference vaccine strains (NADL-2, 27a, and PPV014) and the virulent Kresse strain. The SnipIt v1.6 software was subsequently used to identify amino acid mutation sites (O'Toole; Aziz; Maloney, 2024). Additionally, a consensus sequence comprising all five clusters was created for further downstream analysis.

## 2.9 Protein Structure Prediction

Protein structure predictions were conducted using the I-TASSER (Iterative Threading Assembly Refinement) web server, a well-established tool developed by Zhang Lab at the University of Michigan (Yang; Zhang, 2015). I-TASSER constructs 3D models based on multiple threading alignments and iterative structural assembly simulations. The protein sequences of the three previously mentioned reference vaccine strains and five consensus sequences representing the identified porcine parvovirus (PPV) groups (PPV1a – PPV1e) were used as input for the structural prediction. To enhance the accuracy of these predictions, the crystal structure of the porcine parvovirus capsid protein (PDB ID: 1K3V), crystallized by Simpson et al. in 2002, was chosen as the template for the simulations. The resulting I-TASSER models were evaluated using C-scores, which reflect the confidence of the predicted models, as well as TM-scores and RMSD (Root Mean Square Deviation) values to ensure accurate and reliable predictions. Additionally, our simulated protein were further refined with GalaxyRefine (Heo; Park; Seok, 2013). The peptides used to perform the docking simulations were modeled using PEP-FOLD3 (Lamiable et al., 2016), with the simulated structures from I-TASSER as a reference. Finally, the refined protein was subjected to structural validation through the Multimetric Model Validation – Iris module, available in the CCP4i2 package (Potterton et al., 2018; Rochira; Agirre, 2021). This analysis employs established metrics for evaluating the quality of protein models, integrating tools such as MolProbity to provide a comprehensive overview of the model's geometry and stereochemistry (Joseph et al., 2022).

During validation, indicators such as the percentage of residues in disallowed regions of the Ramachandran plot (Ramachandran outliers), the percentage of residues in favored regions (Ramachandran favored), the occurrence of side-chain conformations outside common

rotamers (Rotamer outliers), Cβ deviations, atomic clash-score, as well as the root mean square deviations of bond lengths (RMS bonds) and bond angles (RMS angles) were generated. The MolProbity score, a widely used composite metric adopted by the wwPDB, was also calculated as a global indicator of model quality (Joseph et al., 2022; Moroño; Alejandro, 2021; Wlodawer, 2017). These metrics were automatically obtained by Iris, which integrates and presents the results in a compact and interactive manner, enabling a rapid and detailed assessment of the geometric and stereochemical aspects of the refined model.

## 2.10 Linear B Cell Epitope Prediction

B cells are considered a central component of the adaptive immune system, as they could recognize and provide long-term protection against infectious pathogens or cancer cells (Jespersen et al., 2017). To perform the prediction of B-cell epitopes, the web-software BepiPred v2.0 was used with the default threshold (0.5) to obtain only the top epitopes with higher confidence. Additionally, the analysis tool from the Immune Epitope Database (IEDB) was utilized and the following algorithms were applied: Parker hydrophilicity, Karplus–Schulz flexibility, Emini surface accessibility, Kolaskar–Tongaonkar antigenicity, and Chou–Fasman secondary structure prediction to identify all potential epitopes.

The Parker approach is based on the retention time of peptides in high-performance liquid chromatography (HPLC) to infer hydrophilic regions associated with epitopes (Parker; Guo; Hodges, 1986). The Kolaskar & Tongaonkar antigenicity method employs physicochemical properties of amino acids, such as charge and polarity to predict antigenic regions (Kolaskar; Tongaonkar, 1990). The Emini scale estimates the probability of specific residues being exposed on the surface of the protein, indicating potential binding sites (Emini et al., 1985). The Karplus & Schulz flexibility quantifies the conformational mobility of protein segments, while the Chou & Fasman method predicts the formation of β-turns, structures frequently associated with epitopes (Chou; Fasman, 1978; Karplus; Schulz, 1985).

## 2.11 T Cell Epitope Prediction

The analyzed MHC class I molecules were the swine leukocyte antigen (SLA) alleles SLA-1*08:01, SLA-2*02:01, and SLA-3*04:01. These alleles were chosen because of their high prevalence in commercial pig breeds, as shown in Table 1, making them relevant for studying immune responses in swine populations (Techakriengkrai et al., 2021). These molecules play an essential role in antigen presentation to the cellular immune system, mediating responses against pathogens

The web-based software NetMHCpan v4.1 (Reynisson et al., 2020) was the tool used to predict peptide bindings to MHC molecules using artificial neural networks (ANNs) selecting 9mer peptides. Each predicted epitope is given a score, which is a value that quantifies the strength of a peptide's binding to the MHC molecule based on the comparison of its performance with other peptides and the lower the ranking, the stronger the predicted epitope binding affinity. All analyses were performed using default parameters, prioritizing strong binding peptides (percent rank < 0.5%). Additionally, weak binding peptides (percent rank between 0.5% and 2%), comprising 320 and 436 binding sites, were also included for subsequent molecular docking analysis.

**Table 1. Allele frequencies of selected SLA (Swine Leukocyte Antigen) alleles based on Techakriengkrai et al. Only alleles with a frequency of 10% or higher in the studied boars and/or gilts were included in this study. The table presents the specific SLA allele groups and their chosen alleles, with the frequencies shown separately for boars (2n = 64), gilts (2n = 252), and the total population (2n = 316). Frequencies are expressed as percentages, and missing data for certain alleles are indicated by blank cells. Chosen alleles were highlighted in bold.**

| Allele Group | Chosen Alleles | Boar (2n = 64) | Gilt (2n = 252) | Total (2n = 316) |
|---|---|---|---|---|
| **SLA-1** | SLA-1:0401 | 53.1% | 1.2% | 11.7% |
| | SLA-1:0701<br>SLA-1:0702 | 10.9% | 23.8% | 21.2% |
| | **SLA-1:0801** | 32.8% | 29.4% | **30.1%** |
| **SLA-2** | **SLA-2:0201**<br>SLA-2:0202 | 9.4% | 20.6% | **18.4%** |
| | SLA-2:0401<br>SLA-2:0402 | 51.6% | 2.8% | 12.7% |
| | SLA-2:0501<br>SLA-2:0502 | 7.8% | 9.9% | 9.5% |
| | SLA-2:1001<br>SLA-2:1002 | 17.2% | 13.9% | 14.6% |
| | SLA-2:1201 | 4.7% | 15.1% | 13.0% |
| **SLA-3** | SLA-3:0101 | | 15.5% | 12.3% |
| | **SLA-3:0401** | 62.5% | 27.4% | **34.5%** |
| | SLA-3:0501<br>SLA-3:0502<br>SLA-3:0503 | 21.9% | 25.0% | 24.4% |
| | SLA-3:0601<br>SLA-3:0602 | 6.3% | 16.3% | 14.2% |

Source: Techakriengkrai et al. (2019).

## 2.12 Assessment of Antigenicity, Allergenicity and Toxicity

Antigenicity was evaluated using VaxiJen v3.0 with a threshold of 0.4 (default). The allergenicity of the predicted T-cell and B-cell epitopes was analyzed with AllerTOP v2.1 to distinguish between allergen and non-allergen epitopes. Additionally, toxicity was assessed using the web tool ToxinPred, employing the SVM (Swiss-Prot) + Motif-based prediction method with an e-value cutoff of 10 for the motif-based approach. All physicochemical properties were displayed in the analysis.

The VaxiJen webserver is a bioinformatic tool for in silico identification of protective antigens of different origins, and it uses an alignment-independent method based on auto and cross-covariance (ACC) transformation of protein sequences into uniform equal-length vectors and a partial least squares algorithm (PLS) for antigen prediction (Doneva; Dimitrov, 2024). Likewise, AllerTOP also uses ACC to predict protein allergenicity. It processes amino acid sequences in FASTA file format by extracting features via ACC transformations encoding physicochemical properties and evolutionary descriptors (E-descriptors) to capture structural and phylogenetic patterns. Then, a k-nearest neighbors (k=1) classifier with cosine distance compares these features against AllergenOnline (http://www.allergenonline.org/) database (v23) (Dimitrov et al., 2014). Similarly, ToxinPred also employs machine learning to predict peptide toxicity but has a different methodology from the previous tools. It integrates amino acid composition (AAC), dipeptide composition (DPC), and motif analysis (MERCI) for feature extraction, analyzed by an Extra-Trees (Extremely Randomized Trees) algorithm trained on existing toxic and non-toxic peptides (Rathore et al., 2024).

### 2.13 Molecular Docking with MHC-I Receptor

To evaluate the binding affinities between the predicted epitopes to swine leukocyte antigen (SLA) molecules, molecular docking simulations were performed. The three-dimensional structures of SLA alleles SLA-1*08:01 (Accession: SLA06115), SLA-2*02:01 (Accession: SLA08466) and SLA-3*04:01 (Accession: SLA06198) were downloaded from the Immuno Polymorphism Database (IPD) MHC database (https://www.ebi.ac.uk/ipd/mhc). Prior to docking, the molecules were preprocessed to standardize conditions: all water molecules and heteroatoms were removed to eliminate potential steric or electrostatic interference, followed by the addition of polar hydrogen atoms to correct protonation states and Kollman charges to parameterize atomic interactions. For the three-dimensional structural modeling of the predicted epitopes, the PEP-FOLD3 web server was utilized (Lamiable et al., 2016), an algorithm for peptide structure prediction based on a *de novo* approach. The torsion trees (rotatable bonds) were assessed using AutoDockTools (MGLTools v.1.5.7) to optimize conformational flexibility, ensuring accurate representation of epitope dynamics during molecular docking simulations (Morris et al., 2009). Molecular docking was carried out by AutoDock Vina v1.2.7 (Trott; Olson, 2010), with the grid box centered on six predefined binding pocket regions (A – F) within the SLA alleles, which were then augmented by ±4 Å in x, y, and z directions. These pockets were selected based on their structural relevance to epitope binding, as inferred from prior crystallographic studies of SLA-peptide complexes (Fan et al.,

2016; Zhang et al., 2011). Ligand selection criteria encompassed both high-affinity candidates (minimal binding energies) and low-affinity variants distributed across the 320- and 436-site frameworks, with structural validation requiring absolute absence of steric clashes across all analyzed complexes, but molecular docking analysis of low-affinity T-cell epitopes focused on two peptide cohorts: 320 epitopes with isoleucine (I) and threonine (T) residues at MHC anchor positions, and 436 epitopes incorporating serine (S), threonine (T), and proline (P) residues essential for interaction stability.

## 3. RESULTS

### 3.1 Recombinant Detection and Temporal Correlation Analysis of the Dataset

A single recombination event was identified, with three sequences confirmed as recombinants and subsequently removed from the database. These recombinants were detected using the MAXCHI, 3SEQ, and SISCAN methods, as detailed in Table 2.

**Table 2. Recombinant sequences detected by the RDP (Recombination Detection Program) using the MaxChi, SiScan, and 3Seq algorithms. The table lists the recombinant sequences (Recomb.), along with their major and minor parent strains (Major P. and Minor P.). The detection methods used by the RDP program include RDP, Geneconv, BootScan, MaxChi, Chimaera, SiScan, and 3Seq, with the presence or absence of detection indicated by a '+' (detected) or '-' (not detected).**

| **Recombinants** | | | **Detection Method** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Recomb. | Major P. | Minor P. | RDP | Geneconv | BootScan | MaxChi | Chimaera | SiScan | 3Seq |
| PPV1-JBNU003 | 21620005-1h | IDT | - | - | - | + | - | + | + |

None of the sequences were identified as outliers, demonstrating adequate temporal signal for inclusion in our dataset. Additionally, considerable data dispersion was identified, suggesting that the “relaxed clock” molecular clock model is the most appropriate for our analysis (Rambaut et al., 2016).

### 3.2 Phylogenetic Analysis

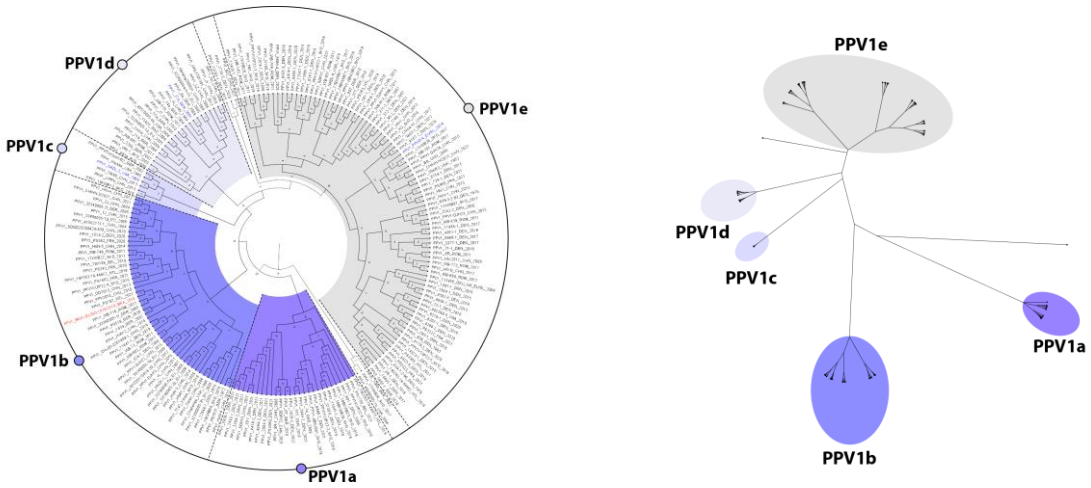
The Maximum Clade Credibility (MCC) tree generated using VP2 sequences showed five major lineages, each with distinct geographic distributions and molecular signatures. These lineages were well-defined in both the circular and radial representations of the tree (Figure 2a/b).

The PPV1a lineage predominantly comprises European strains (70%, n = 19/27), with the oldest isolate, SR-1 (China, 2006) and is mostly composed of recent variants from Denmark (e.g., 3928-1, 2021). Notably, PPV1a lacks representation of vaccine reference strains (NADL-2, 27a, PPV014), suggesting independent evolution from immunologically targeted variants. In

contrast, the PPV1b lineage is characterized by a dual geographic origin, with 50% European strains (n=25/50), 48% Asian strains (n=24/50) and the sole Brazilian isolate (BRA-UEL/GO-1074/2018, 2018). The ancestral node traces to PS1831 (Germany, 1972), while 12 recent variants (2019-2021) highlight sustained circulation in Asia and Europe. The compact PPV1c clade (n=6) includes the NADL-2 vaccine strain (USA, 1964), a non-pathogenic variant associated with transient viremia known for its inability to cross the placental barrier (Paul; Mengeling, 1980).

The PPV1d lineage consists predominantly of European strains (n=11/22), followed by Asian strains (n=9/22), one American strain, and a single Oceania-sourced strain (Australia, 2009). Its circulation spans nearly three decades, from the ancestral variant N (China, 1989) to strain HNLY201301 (China, 2013). The oldest lineage, PPV1e, originates from the ancestral strain 59e/63 (UK, 1963) and comprises 71% European strains (n=63/89). This lineage additionally includes a vaccine reference strain (PPV014, Europa Island, 2014).

**Figure 2. Phylogenetic tree generated in BEAST2 used for analysis. The five identified lineage clades (PPV1a – PPV1e) are color-coded, with each lineage represented by a distinct color. Vaccine reference strains are highlighted in blue, while the single Brazilian strain is marked in red. (a) Polar plot (circular tree format) illustrating the relationships among lineages; (b) Radial plot, where branch lengths are proportional to genetic distances, providing a clearer view of evolutionary divergence.**



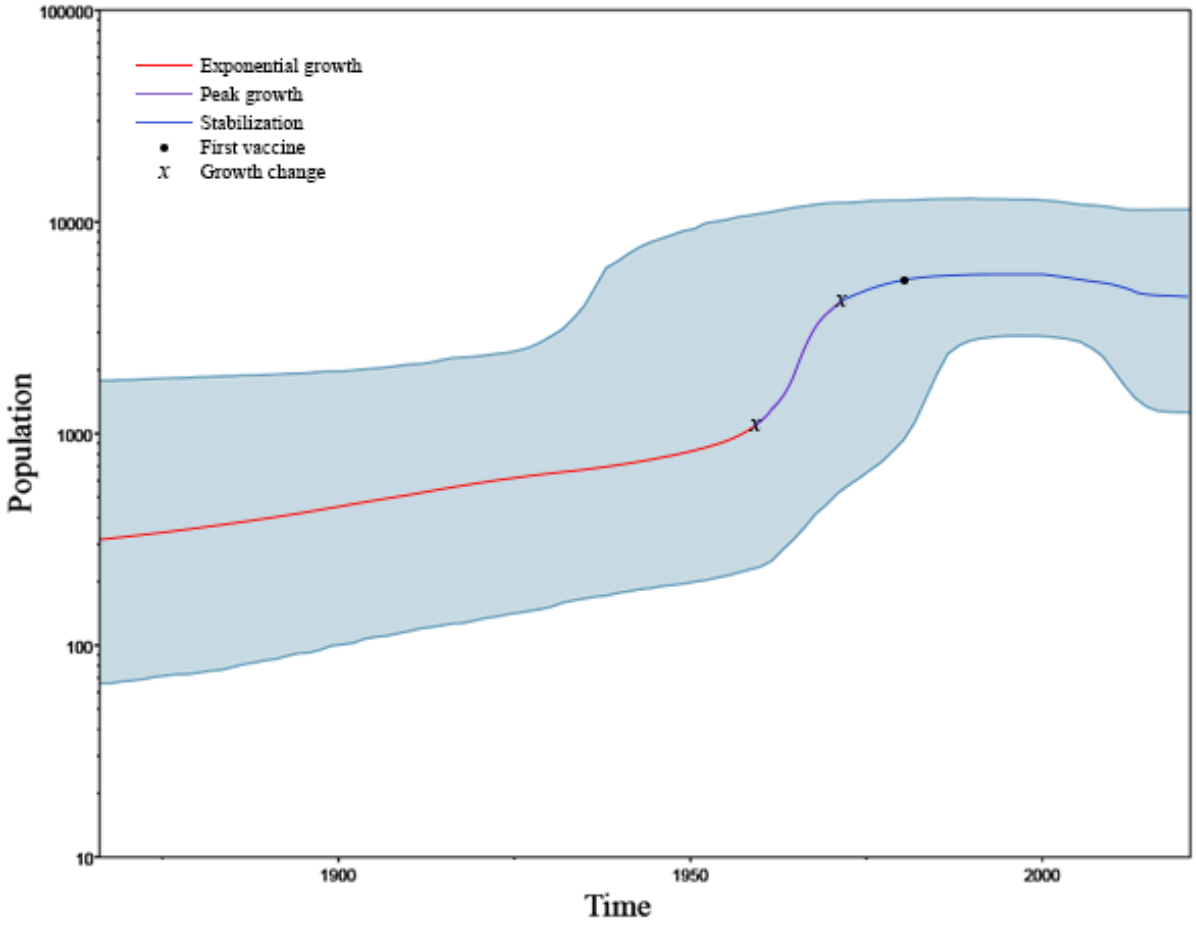

**(a)** **(b)**

## 3.3 Phylodynamic Inference

The phylodynamic analysis estimated the evolutionary rate to be around $1.57 \times 10^{-5}$ nucleotide substitutions per site per year. The populational dynamics of PPV1 were analyzed (Figure 3), and the skyline plot reveals three distinct phases: The first phase (1863–1960), depicted in dark red, shows exponential growth, with the trend line rising sharply. The second

phase (1960–1968) marks the peak of viral population growth before stabilization begins. Finally, the third phase (1968–2021), represented by the dark blue line, signals the onset of stabilization, with the curve flattening over time.

**Figure 3. Skyline plot of PPV1 population dynamics, with the vertical axis (y) representing viral population, and the horizontal axis (x) representing time (in years). The red, purple and dark blue lines represent the first, second and third phases, respectively. The "*X*" marks the phases shifts or growth changes, and the black circle marks the moment when the first vaccine was introduced (~1980). The blue area is the limit of 95% higher posterior probability density.**




## 3.4 Analysis of Positive Selection Sites

Datamonkey analysis identified sites 320 and 436 as being under positive selection across multiple algorithms, providing strong evidence of selective pressure at these positions. Site 320 was consistently detected by all four algorithms, while site 436 was confirmed by SLAC and FUBAR (Table 3).

**Table 3. Results from the Datamonkey analysis, highlighting positively selected sites identified using four different algorithms: SLAC, FEL, MEME, and FUBAR. Sites were considered under positive selection if detected by at least two algorithms. Each algorithm used specific reference values for p-values.**

| Algorithm | Reference Value | Positively Selected Sites | *P*-value |
|---|---|---|---|
| SLAC | $p < 0.10$ | 320; 436 | 0.013; 0.027 |
| FEL | $p < 0.10$ | 320 | 0.02 |
| MEME | $p < 0.05$ | 320 | 0.03 |
| FUBAR | $p > 0.90$ | 320; 436; 414; 378; 555; 565; 144; 419; 564 | 0.996; 0.965; 0.953; 0.949; 0.949; 0.941; 0.931; 0.911; 0.900 |

Following the second method, several sites were identified by the CODEML algorithm to be under strong positive selection, also emphasizing sites 320 and 436 (Table 4). In M0, the estimated ω was 0.20292, suggesting that the sequence is primarily under purifying selection. In contrast, M3 resulted in three categories of ω: $\omega_1 = 10.0671$, $\omega_2 = 1.23674$, and $\omega_3 = 5.60110$, distributed among the proportions $p_0 = 0.89010$, $p_1 = 0.10513$, and $p_2 = 0.00477$, respectively, reflecting strong heterogeneity among sites. In M1a, the maximum likelihood estimates were $p_0 = 0.87214$, representing the proportion of sites under purifying selection with $\omega_1 = 0.05365$ and $p_1 = 0.12786$, sites under neutral evolution with $\omega_2 = 1.00000$. By contrast, M2a accounted for selection pressures, identifying three distinct classes of ω: $\omega_1 = 0.05719$ for purifying selection, $\omega_2 = 1.00000$ for neutral sites, and $\omega_3 = 5.17563$ for sites under positive selection, with respective proportions of $p_0 = 0.86858$, $p_1 = 0.12593$, and $p_2 = 0.00549$. M2a, as indicated by $\omega_3 > 1$ and a small percentage of sites under this class, $p_2 = 0.00549$ provides evidence of positive selection.

Interestingly, sites 320 and 436 were identified by all analysis methods and all sites identified by FUBAR algorithm were also detected by CODEML's M3 model with confidence interval > 99%. While FUBAR uses a Bayesian approach, the other models employ a maximum likelihood method, and since they use different assumptions and statistical methods, the agreement between them reflects the robustness of the results, regardless of the methodological differences, ensuring that the selection of the detected sites is not an artifact of a single type of analysis.

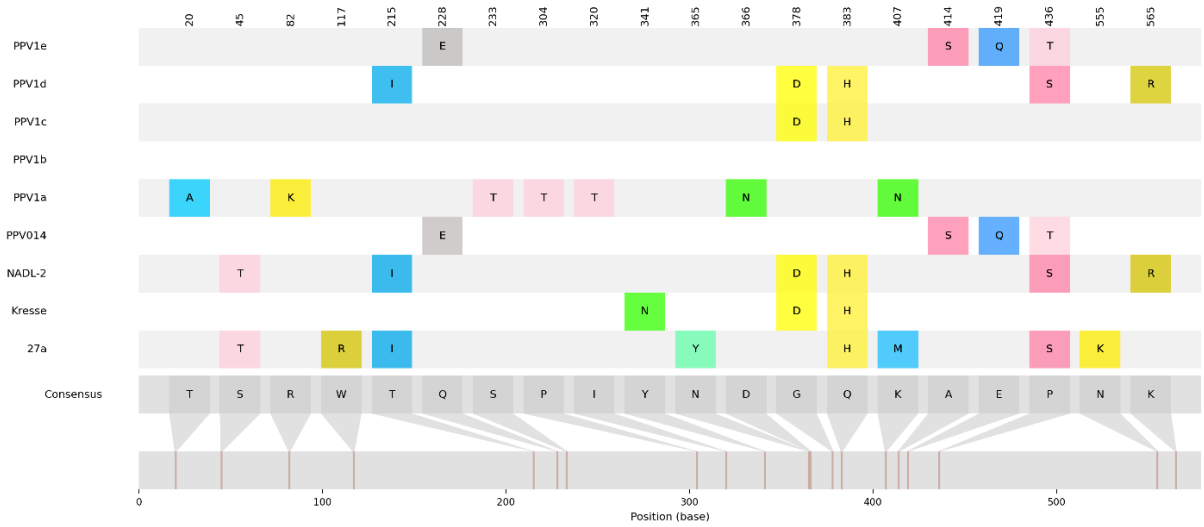
**Table 4. Results of sites under positive selection analyzed with CODEML (PAML package). Several sites were identified by the CODEML algorithm to be under positive selection. The table compares models M0, M1a, M2a, and M3, highlighting parameter estimates, likelihood values, and likelihood ratio test (LRT) p-values, with positively selected sites identified across models.**

| Model | np | InL | Estimative of parameters | Models compared | LRT *p*-value | Positively selected sites |
|---|---|---|---|---|---|---|
| M3 *(discrete)* | 404 | -5417.924 | $p_0 = 0.89010$<br>$p_1 = 0.10513$<br>$p_2 = 0.00477$<br>$\omega_1 = 10.0671$<br>$\omega_2 = 1.23674$<br>$\omega_3 = 5.60110$ | M0 Versus M3 | 0.00E-0 | 13A*, 14G, 20T**, 45S**, 82R*, 107T, 117W, 144E**, 155E, 164I, 215T**, 226Q, 228E**, 233S, 238L, 295E, 304P**, 311N, 320I**, 366D*, 378G**, 383Q**, 391L, 394Y, 407K**, 414S**, 419Q**, 436T**, 441M, 506F, 521N, 540W, 550A, 554G*, 555N**, 564I**, 565K** |
| M0 *(one-ratio)* | 400 | -5520.734 | $\omega = 0.20292$ | | | Not allowed |
| M2a *(positive-selection)* | 403 | -5418.515 | $p_0 = 0.86858$<br>$p_1 = 0.12593$<br>$p_2 = 0.00549$<br>$\omega_1 = 0.05719$<br>$\omega_2 = 1.00000$<br>$\omega_3 = 5.17563$ | M1a Versus M2a | 4.85E-5 | 320I**, 436T* |
| M1a *(nearly-neutral)* | 401 | -5428.449 | $p_0 = 0.87214$<br>$p_1 = 0.12786$<br>$\omega_1 = 0.05365$<br>$\omega_2 = 1.00000$ | | | Not allowed |

## 3.5 Amino acid Evaluation

To determine the molecular characteristics of the different viral lineages, the consensus amino acid sequences of the VP2 coding regions were analyzed against the consensus sequence of all variants. The results of the analysis performed using the SnipIt software are presented in Figure 4.

**Figure 4. Comparative overview of amino acid substitutions at specific positions (20, 45, 82, 117, 215, 228, 233, 304, 320, 341, 365, 366, 378, 383, 407, 414, 419, 436, 555, and 565) across several PPV lineages (PPV1e, PPV1d, PPV1c, PPV1b, PPV1a), vaccine reference strains (PPV014, NADL-2, 27a) and virulent strain Kresse relative to our consensus sequence (Figure 5). Each colored box highlights a residue that differs from the consensus at a given position, allowing us to visualize patterns of conservation and variation among the lineages and strains.**




## 3.6 Predicted Protein Structures Prediction and Comparison

The structural validation of eight protein models (PPV1a–PPV1e, NADL-2, PPV014, and 27a) was performed using the Multimetric Model Validation - Iris module in CCP4i2 (Figure 5), which integrates MolProbity analyses. The validation metrics revealed high-quality models across all evaluated parameters.

**Figure 5. Circular validation plot generated by Iris of the consensus protein structure. The concentric rings represent different validation metrics, with the outer rings corresponding to rings for atomic clashes, Ramachandran plot (backbone geometry), and rotamer (side-chain conformation) assessments. The predominance of green across the clashes, Ramachandran, and rotamer rings indicates that most residues are free from steric clashes, are in favored regions of the Ramachandran plot, and adopt preferred rotamer conformations, respectively. Small colored segments (yellow and red) interspersed within the green rings correspond to isolated residues with minor deviations, highlighting the overall high quality of the model.**


Ramachandran plot analysis showed that most models exhibited excellent backbone geometry, with outlier percentages ranging from 0.35% to 2.43% (Figure 6, Table 5). Models PPV1b, PPV1c, and NADL-2 displayed particularly low outlier values (<1%), which is consistent with high-quality protein structures (Chen et al., 2010; Williams et al., 2018). The percentage of residues in favored regions exceeded 93% for all models, surpassing the recommended threshold of 90% for reliable protein structures (Chen et al., 2010). Rotamer analysis indicated excellent side-chain conformations across all models, with outlier percentages consistently below 1%, suggesting proper modeling of side-chain geometries (Williams et al., 2018). All models demonstrated optimal atomic packing, with clash scores of 0.0, indicating no serious atomic overlaps; this metric, representing the number of serious

clashes per 1,000 atoms, is a critical indicator of model quality (Chen et al., 2010; Word et al., 1999). The root mean square deviations (RMSD) for bond lengths ranged from 0.0593 to 0.592 Å, while RMSD for bond angles ranged from 2.28° to 2.55°, values that are slightly above the ideal limits for high-resolution structures but remain acceptable for models of this nature (Wlodawer, 2017). The MolProbity scores, which provide a composite assessment of protein quality, ranged from 0.79 to 0.91 for all models; these values are below 1.0, indicating excellent overall quality that would be expected for structures of high resolution (Williams et al., 2018).

**Figure 6. Ramachandran plot representation of the consensus sequence. The plot shows the dihedral Phi and Psi angles for all residues, colored according to Ramachandran's criterion. The favored and allowed residues are presented by the green and yellow dots, respectively and outliers are represented as red crosses.**

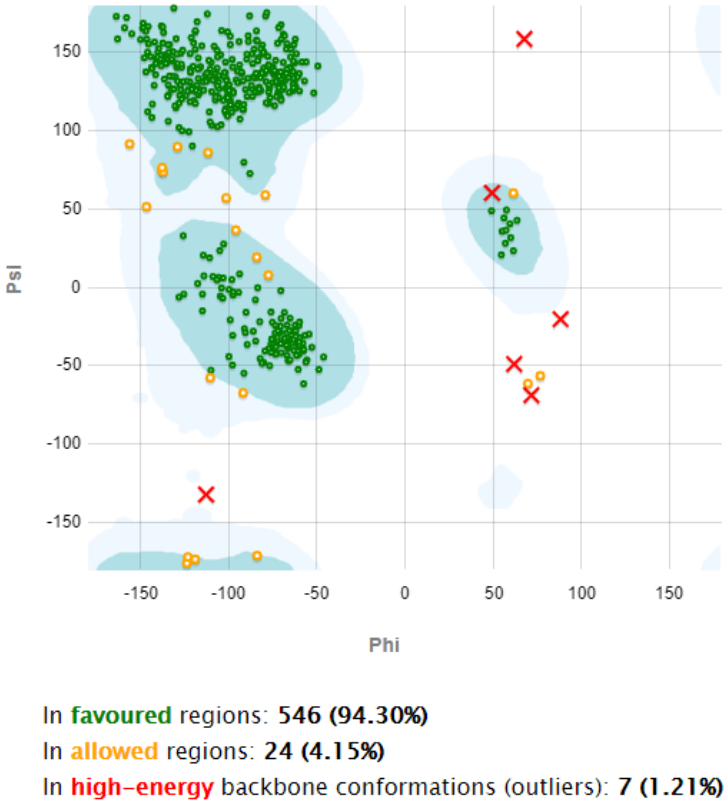
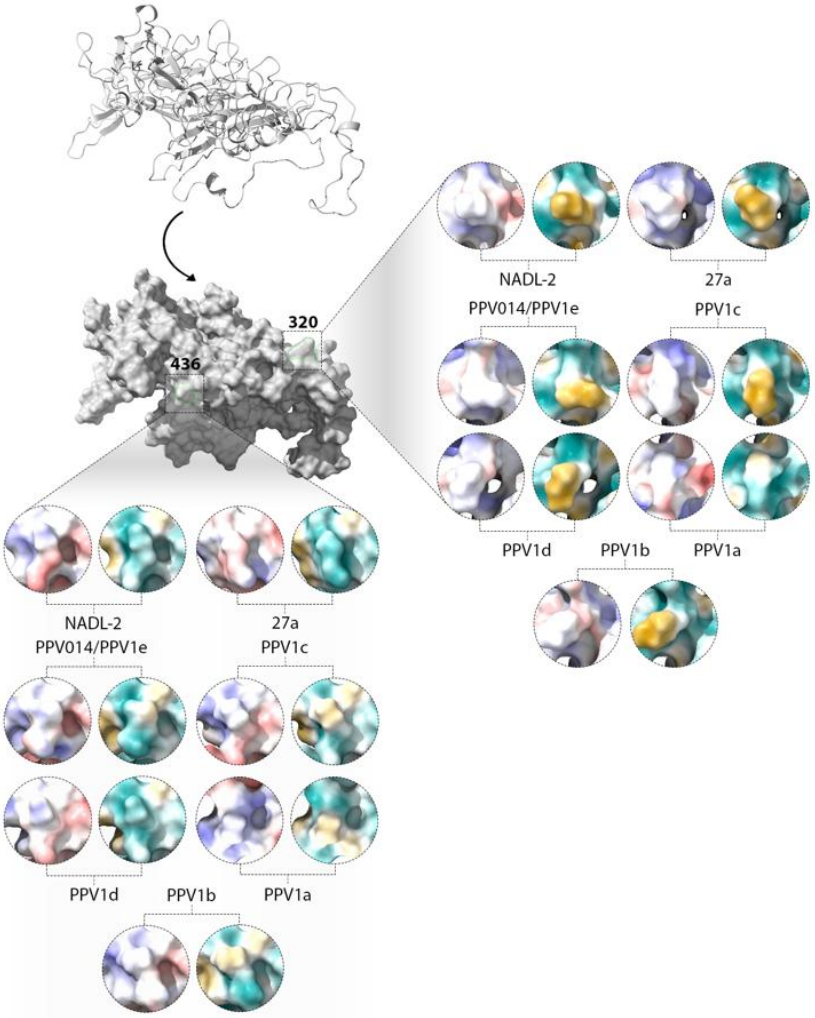



**Table 5. Results of the structural validation statistics obtained for the capsid models of the PPV lineages (PPV1a–PPV1e) and the reference strains (NADL-2, PPV014 and 27a).**

| Lineage | RMSD Outliers | RMSD favoured | Rotamer Outliers | CBeta Deviations | Clashscore | RMS bonds (Å) | RMS angles (°) | MolProbity score |
|---|---|---|---|---|---|---|---|---|
| PPV1a | 1.39% | 94.97% | 0.2% | 14 | 0.0 | 0.0593 | 2.38 | 0.85 |
| PPV1b | 0.35% | 95.84% | 0.6% | 16 | 0.0 | 0.0593 | 2.32 | 0.79 |
| PPV1c | 0.87% | 94.45% | 0.4% | 13 | 0.0 | 0.592 | 2.37 | 0.88 |
| PPV1d | 1.21% | 95.49% | 0.0% | 15 | 0.0 | 0.0593 | 2.33 | 0.81 |
| PPV1e | 2.43% | 94.97% | 0.6% | 22 | 0.0 | 0.0594 | 2.42 | 0.85 |
| NADL-2 | 0.87% | 95.49% | 0.6% | 18 | 0.0 | 0.592 | 2.28 | 0.81 |
| PPV014 | 2.43% | 94.97% | 0.6% | 22 | 0.0 | 0.0594 | 2.42 | 0.85 |
| 27a | 2.25% | 93.76% | 0.6% | 19 | 0.0 | 0.0593 | 2.55 | 0.91 |

The predicted structures of the VP2 protein across porcine parvovirus lineages (PPV1a to PPV1e) and reference strains (NADL-2, 27a, PPV014) revealed distinct structural variations. The 320 and 436 sites under positive pressure selection are highlighted in the surface representation of the protein, allowing a detailed comparison of surface topology, electrostatic potential, and hydrophobicity (Figure 7).

**Figure 7. Predicted protein structure comparison. The 3D protein structure highlights two key sites under positive selection, 320 and 436, identified in the analysis. The surface structure surrounding these sites is shown for multiple porcine parvovirus lineages (PPV1a to PPV1e) and reference strains (NADL-2, 27a, PPV014). Each zoomed-in section illustrates the surface topology, electrostatic surface potential, and hydrophobicity of the protein around these positively selected sites, allowing for a detailed comparison of structural variation across different strains and lineages.**




## 3.7 Prediction of Linear B-Cell and T-Cell Epitopes

BepiPred v2.0 identified a total of 10 B-cell epitopes of varying lengths above the threshold score (0.5) from our consensus sequence derived from the five lineages (PPV1a – e), as shown in Table 6. These candidate epitopes were subsequently clustered with predictions

from multiple computational algorithms, including Parker, Karplus-Schulz, Emini, Kolaskar-Tongaonkar, and Chou-Fasman, to enhance prediction reliability. Only regions predicted by a minimum of three independent algorithms were retained, yielding four high-confidence B-cell epitopes that underwent further characterization for antigenicity, allergenicity, and toxicity properties (Table 7).

T-cell epitopes predicted using NetMHCpan v4.1 are presented in Table 8. Analysis across swine leukocyte antigen alleles SLA-1*08:01, SLA-2*02:01, and SLA-2:0202 identified 14, 16, and 9 high-affinity epitope regions (%Rank < 0.5), respectively, yielding a comprehensive repertoire of 39 potential T-cell epitopes.

**Table 6. Results of linear B-cell epitopes predicted by BepiPred v2.0 from VP2 consensus sequence.**

| Epitope Sequence | Start | End | Length |
|---|---|---|---|
| VEQHNPINAGTELSATGNESGGGGGGGGRGAGGVGVSTGSFNNQTEFQYLGE | 5 | 57 | 53 |
| VLNSESGVAGQMVQDDA | 85 | 101 | 17 |
| ESATSPPTKIYNN | 155 | 167 | 13 |
| RSETL | 191 | 195 | 5 |
| CTRNLNPPTYTGQSQQITDSIQTGLHS | 214 | 240 | 27 |
| FDTKPLKLTHSWQTNRSLG | 269 | 287 | 19 |
| TEPTTEGDQHPGTLPAANTRKGYHQTINNSYTEATAIRPAQVGYNTPYMNFEYSNGGPF | 294 | 352 | 59 |
| PTADTQYNDDEPN | 358 | 370 | 13 |
| GYQHGQLTTSSQELERYTFNPQSKCGRAPKQQFNQQAPLNLENTNNGTLLPSDPIGGKPNMHFMNTLNTYGPLTALNNTAP | 378 | 458 | 81 |
| DTDLKPRLH | 471 | 479 | 9 |
| TDDFNADSPQQ | 503 | 513 | 11 |
| RSSNMWNPIQQHTTTAENIGN | 535 | 555 | 21 |
| KMFPEYSQLIP | 565 | 575 | 11 |

**Table 7. Results of antigenicity, allergenicity, toxicity, and clustered algorithms for linear B-cell epitopes.**

| Start | End | Sequence | Clustered | Antigenicity | Allergenicity | Toxicity |
|---|---|---|---|---|---|---|
| 5 | 57 | VEQHNPINAGTELSATGNESGGGGGGGGRGAGGVGVSTGSFNNQTEFQYLGE | Parker Karplus Kolaskar Chou | Antigenic (0.7891) | Non-allergen | Non-toxic |
| 294 | 352 | TEPTTEGDQHPGTLPAANTRKGYHQTINNSYTEATAIRPAQVGYNTPYMNFEYSNGGPF | Parker Karplus Chou | Antigenic (0.7777) | Non-allergen | Non-toxic |
| 358 | 370 | PTADTQYNDDEPN | Parker Chou Emini | Antigenic (0.5416) | Allergen | Non-toxic |
| 378 | 458 | GYQHGQLTTSSQELERYTFNPQSKCGRAPKQQFNQQAPLNLENTNNGTLLPSDPIGGKPNMHFMNTLNTYGPLTALNNTAP | Parker Karplus Emini | Antigenic (0.5829) | Non-allergen | Non-toxic |

**Table 8. NetMHCpan v4.1 predicted T-cell results. The IC50 (BA) represents the peptide concentration required to inhibit 50% of the binding of a reference peptide to MHC. %Rank (BA) classifies the peptide relative to a set of random natural peptides, expressing its relative position as a percentile.**

| SLA Allele | Sequence (9mer) | Position | End | IC50 (BA) | % Rank (BA) |
|---|---|---|---|---|---|
| SLA-1*08:01 | AQVGYNTPY | 333 | 341 | 206.08 | 0.04 |
| | ALNNTAPVF | 452 | 460 | 339.26 | 0.05 |
| | GLHSDIMFY | 237 | 245 | 515.41 | 0.08 |
| | GAIRFTMGY | 371 | 379 | 738.34 | 0.12 |
| | TTAENIGNY | 548 | 556 | 905.72 | 0.15 |
| | AIRPAQVGY | 329 | 337 | 1309.45 | 0.2 |
| | ATSPPTKIY | 157 | 165 | 1495.04 | 0.23 |
| | KMFPEYSQL | 565 | 573 | 1761.92 | 0.26 |
| | FEYSNGGPF | 344 | 352 | 1790.81 | 0.27 |
| | HLLRTGDEF | 254 | 262 | 1860.19 | 0.28 |
| | YTIENAVPI | 245 | 253 | 1919.17 | 0.3 |
| | RIITYSNFW | 515 | 523 | 2250.84 | 0.37 |
| | GSFNNQTEF | 44 | 52 | 2944.52 | 0.49 |
| | VQDDAHTQM | 97 | 105 | 2947.33 | 0.49 |
| SLA-2*02:01 | TQMVTPWSL | 103 | 111 | 834.03 | 0.01 |
| | QQFNQQAPL | 408 | 416 | 2339.51 | 0.03 |
| | VQDDAHTQM | 97 | 105 | 2344.55 | 0.03 |
| | KMFPEYSQL | 565 | 573 | 3349.42 | 0.06 |
| | IENAVPIHL | 247 | 255 | 4133.72 | 0.09 |
| | FEYSNGGPF | 344 | 352 | 4262.03 | 0.09 |
| | WQTNRSLGL | 280 | 288 | 5313.45 | 0.13 |
| | EQEIFNVVL | 142 | 150 | 5745.85 | 0.15 |
| | SQELERYTF | 388 | 396 | 6677.21 | 0.2 |
| | LENTNNGTL | 418 | 426 | 7221.77 | 0.25 |
| | YTIENAVPI | 245 | 253 | 7504.15 | 0.26 |
| | SESGVAGQM | 88 | 96 | 7792.85 | 0.28 |
| | SETLGFYPW | 192 | 200 | 7865.79 | 0.29 |
| | WFNPADWQL | 120 | 128 | 7874.98 | 0.29 |
| | YNNDLTASL | 165 | 173 | 9310.15 | 0.4 |
| | ALNNTAPVF | 452 | 460 | 9455.32 | 0.41 |
| SLA-3*04:01 | RSSNMWNPI | 535 | 543 | 765.58 | 0.04 |
| | ASRLIHLNM | 66 | 74 | 1554.44 | 0.11 |
| | SNFWWKGTL | 520 | 528 | 1634.35 | 0.12 |
| | RSLGLPPKL | 284 | 292 | 1792.36 | 0.13 |
| | HSWQTNRSL | 278 | 286 | 2347.75 | 0.19 |
| | RIITYSNFW | 515 | 523 | 3531.1 | 0.37 |

| | | | | |
|---|---|---|---|---|
| TQMVTPWSL | 103 | 111 | 3864.14 | 0.43 |
| CGRAPKQQF | 402 | 410 | 3955.12 | 0.44 |
| KNNPPGQLF | 487 | 495 | 4090.96 | 0.47 |

## 3.8 Molecular Docking

Molecular docking simulations of T-cell epitopes were performed using AutoDock Vina (Figure 8), generating nine models per ligand (n = 39). The ligands which demonstrated high binding affinities (minimal binding energies), and no steric clashes were selected via structural analysis in ChimeraX. The molecular docking results revealed varying binding affinities between predicted T-cell epitopes and swine leukocyte antigen (SLA) alleles, with scores ranging from -9.4 to -5.6. The docking results are summarized in Table 9 and 10.

**Figure 8. Docked complex of the ligand FEYSNGGPF (positions 344-352) and the SLA-2*02:01 immune receptor. The peptide is represented in pink and the SLA molecule in blue. The grey area represents the binding pockets (A – F) of the SLA allele.**

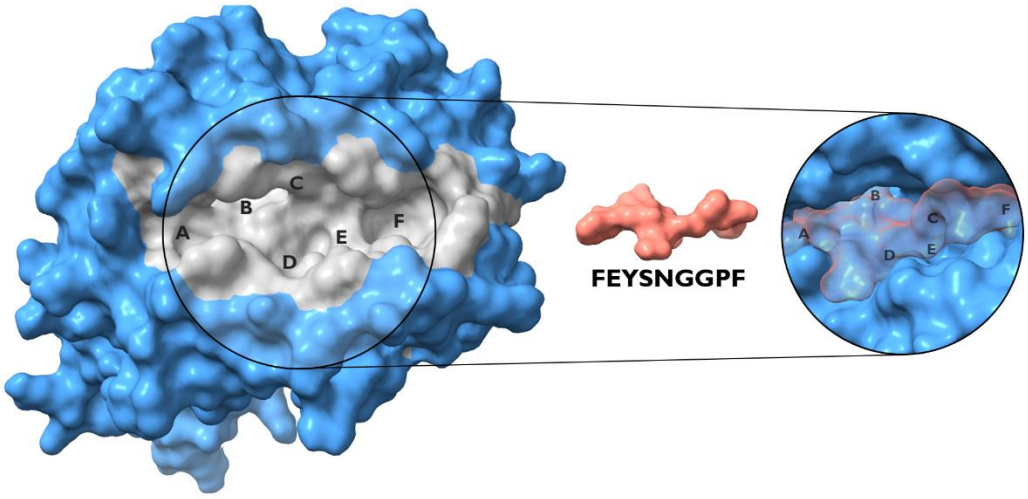


**Figure 9. Figure illustrating the spatial separation between the FEYSNGGPF epitope (positions 344-352, pink) and residues 320 (34.677 Å) and 436 (21.119 Å, red) in the VP2 protein of the PPV1b lineage.**

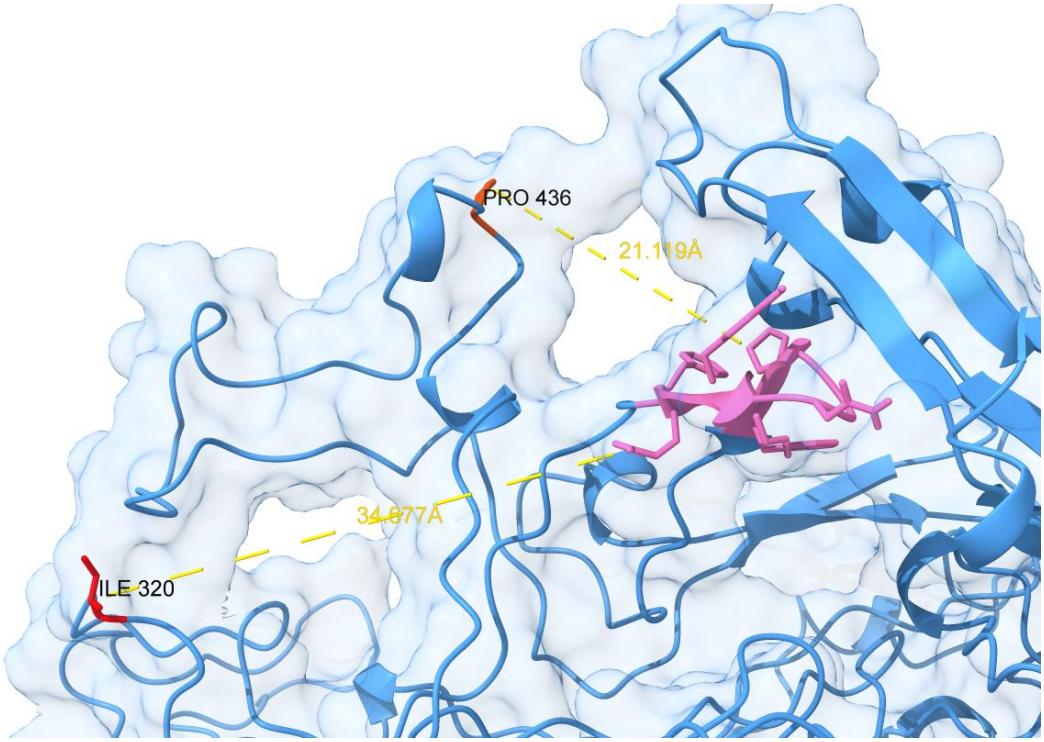



**Table 9. Molecular docking analysis of all high-affinity (<0.5%) T-cell epitopes. Binding affinities exhibit an inverse relationship with docking scores; whereby lower numerical values correspond to stronger protein-peptide interactions. Hydrogen bond counts represent the quantity of stabilizing intermolecular interactions within the binding interface.**

| Macromolecule | Ligands | Start | End | Score | H-Bonds |
|---|---|---|---|---|---|
| SLA-1*08:01 | GSFNNQTEF | 44 | 52 | -8,4 | 8 |
| | HLLRTGDEF | 254 | 262 | -8,1 | 2 |
| | FEYSNGGPF | 344 | 352 | -7,8 | 3 |
| | KMFPEYSQL | 565 | 573 | -7,7 | 3 |
| | RIITYSNFW | 515 | 523 | -7,6 | 4 |
| | YTIENAVPI | 245 | 253 | -7,3 | 2 |
| | ALNNTAPVF | 452 | 460 | -7,2 | 3 |
| | AIRPAQVGY | 329 | 337 | -7,2 | 1 |
| | VQDDAHTQM | 97 | 105 | -6,8 | 2 |
| | AQVGYNTPY | 333 | 341 | -6,6 | 5 |
| | GAIRFTMGY | 371 | 379 | -6,5 | 4 |
| | TTAENIGNY | 548 | 556 | -6,5 | 5 |
| | ATSPPTKIY | 157 | 165 | -6,5 | 3 |
| | GLHSDIMFY | 237 | 245 | -5,7 | 2 |
| SLA-2*02:01 | FEYSNGGPF | 344 | 352 | -9,4 | 4 |
| | YNNDLTASL | 165 | 173 | -8,2 | 4 |
| | WQTNRSLGL | 280 | 288 | -8,1 | 3 |
| | WFNPADWQL | 120 | 128 | -8 | 1 |
| | TQMVTPWSL | 103 | 111 | -7,5 | 1 |
| | QQFNQQAPL | 408 | 416 | -7,4 | 3 |
| | ALNNTAPVF | 452 | 460 | -7,4 | 1 |
| | YTIENAVPI | 245 | 253 | -7,2 | 1 |
| | KMFPEYSQL | 565 | 573 | -7 | 1 |
| | SETLGFYPW | 192 | 200 | -6,8 | 1 |
| | IENAVPIHL | 247 | 255 | -6,7 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| | EQEIFNVVL | 142 | 150 | -6,6 | 4 |
| | SQELERYTF | 388 | 396 | -6,6 | 2 |
| | SESGVAGQM | 88 | 96 | -6 | 0 |
| | LENTNNGTL | 418 | 426 | -5,7 | 4 |
| | VQDDAHTQM | 97 | 105 | -5,6 | 3 |
| | RIITYSNFW | 515 | 523 | -7,3 | 7 |
| | SNFWWKGTL | 520 | 528 | -7,3 | 7 |
| | CGRAPKQQF | 402 | 410 | -7,1 | 5 |
| | KNNPPGQLF | 487 | 495 | -7,0 | 8 |
| SLA-3*04:01 | HSWQTNRSL | 278 | 286 | -6,5 | 8 |
| | TQMVTPWSL | 103 | 111 | -6,4 | 3 |
| | ASRLIHLNM | 66 | 74 | -6,3 | 2 |
| | RSSNMWNPI | 535 | 543 | -6,2 | 4 |
| | RSLGLPPKL | 284 | 292 | -5,6 | 4 |

**Table 10. Molecular docking analysis of weak-binding (0.5 to 2%) T-cell epitopes. Amino acid substitutions at positions 320 and 436 significantly modulate both binding affinity scores and hydrogen bond formation patterns, revealing that residue positions play in determining peptide recognition specificity and binding stability.**

| Macromolecule | Position | Amino Acid | Ligands | Start | End | Score | H-Bonds |
|---|---|---|---|---|---|---|---|
| SLA-1*08:01 | 320 | I | YHQT**I**NNSY | 316 | 324 | -7,6 | 2 |
| | | T | YHQT**T**NNSY | 316 | 324 | -7.1 | 4 |
| SLA-2*02:01 | 436 | S | **S**NMHFMNTL | 436 | 444 | -5,6 | 3 |
| | | T | **T**NMHFMNTL | 436 | 444 | -6,6 | 0 |
| | | P | **P**NMHFMNTL | 436 | 444 | -6,8 | 1 |
| SLA-3*04:01 | 320 | I | TRKGYHQT**I** | 312 | 320 | -7 | 1 |
| | | T | TRKGYHQT**T** | 312 | 320 | -7,1 | 7 |
| | 436 | S | IGGK**S**NMHF | 432 | 440 | -6,6 | 1 |
| | | T | IGGK**T**NMHF | 432 | 440 | -6,4 | 2 |
| | | P | IGGK**P**NMHF | 432 | 440 | -5,8 | 3 |
| | | S | GGK**S**NMHFM | 433 | 441 | -5,9 | 2 |
| | | T | GGK**T**NMHFM | 433 | 441 | -5,4 | 5 |
| | | P | GGK**P**NMHFM | 433 | 441 | -6,5 | 1 |
| | | S | K**S**NMHFMNT | 435 | 443 | -5,9 | 2 |
| | | T | K**T**NMHFMNT | 435 | 443 | -6,2 | 2 |
| | | P | K**P**NMHFMNT | 435 | 443 | -6,8 | 4 |
| | | S | **S**NMHFMNTL | 436 | 444 | -6,6 | 1 |
| | | T | **T**NMHFMNTL | 436 | 444 | -6,6 | 5 |
| | | P | **P**NMHFMNTL | 436 | 444 | -6,7 | 3 |

# 4. DISCUSSION

Porcine Parvovirus Type 1 (PPV1) remains a leading cause of reproductive failure in swine herds worldwide, responsible for the SMEDI syndrome, which results in substantial economic losses for the pork industry. Outbreaks of PPV1 can reduce reproductive efficiency, and recent epidemiological surveys in Europe have reported fetal infection rates as high as 17.4% even in vaccinated herds (Vereecke et al., 2022). The economic repercussions extend beyond decreased numbers of viable piglets, encompassing increased costs associated with herd management, culling of breeding sows, and production disruptions.

The ongoing evolution of PPV1 has led to the emergence of antigenic variants, such as the 27a-like group that partially evade immune responses induced by conventional vaccines based on the NADL-2 strain. Recent studies have demonstrated that antibodies elicited by commercial vaccines can exhibit up to 100-fold lower neutralizing titers against heterologous strains, revealing critical limitations in cross-protective efficacy (Cho et al., 2022; Vereecke et al., 2022). Moreover, coinfection with other pathogens, such as porcine circovirus type 2 (PCV2), further exacerbates clinical outcomes and complicates disease management (Kennedy et al., 2000). Consequently, if the 27a-like variant is representative of the currently circulating PPV1 strains, this strongly suggests that vaccines in use for the past three decades may no longer provide full protection, highlighting the urgent need for updated immunization strategies (Zeeuw et al., 2007).

In 2011, the first evolutionary analysis of PPV, a pathogen recognized for exhibiting a comparatively conserved genome among parvoviruses and single-stranded DNA viruses was conducted through the investigation of viral strain in swine under intensive farming systems (Af et al., 2011), followed by the application of analogous methodological approaches to assess those circulating in wild boars population (Cadar et al., 2012). The results were unexpected, since they observed high mutation rates ($3–5 \times 10^{-4}$) of the VP genes. A recent study also conducted a robust phylodynamic analysis, identifying lower substitution rates ($2.1–7.6 \times 10^{-5}$) (Vereecke et al., 2022), which align with the findings of our analysis ($1.57 \times 10^{-5}$). Despite these comparatively lower substitution rates, they remain relatively fast, considering they fall within the range observed in certain RNA viruses ($10^{-3}–10^{-5}$ substitutions/site/year) (Streck; Canal; Truyen, 2015).

The Bayesian Skyline Plot (BSP) revealed three distinct temporal patterns in the population dynamics of PPV1. Between 1863 and 1960, a gradual increase in the effective viral population size was observed, reflecting the virus's sustained circulation in swine populations under extensive farming systems and the accumulation of moderate genetic diversity. This

period aligns with the early industrialization of pig farming, characterized by the absence of structured sanitary interventions, which may have allowed ancestral lineages to persist (Maes et al., 2020; Niesten; Raymaekers; Segers, 2003). From 1960, a rapid population expansion occurred (Kim et al., 2024). Maybe this phase is linked to the post-World War II intensification of global pork production, driven by the adoption of high-yield commercial breeds and increased host density (Maes et al., 2020). The lack of vaccines until the 1980s facilitated the uncontrolled spread of emerging variants, adapted to high-density environments, alongside transcontinental dissemination via trade of infected animals and contaminated equipment (Joo; Johnson, 1977; Mengeling et al., 1979). After 1968, the viral population stabilized, with a slight decline in the final period (2019–2021), this likely reflects reduced genetic diversity among vaccine-targeted clades, rather than eradication. The initial stabilization coincides with the introduction of inactivated vaccines in the 1980s and the adoption of biosecurity practices, which may have restricted the circulation of vaccine-sensitive lineages (Renault; Humblet; Saegerman, 2021). However, selective pressure from vaccination favored the persistence of adapted variants capable of evading partial immune responses. The recent decline in emphasizes progress in control measures, and stabilization (2019 – 2021) shows the need for updated vaccines targeting conserved epitopes and emerging lineages (PPV1a – b). Additionally, continuous surveillance, particularly in high-density swine regions (e.g., China, United States, Brazil), remains critical to mitigate risks of viral resurgence and adaptation.

Several polymorphic sites were identified along the VP2 amino acid sequences, and some amino acid mutations in the VP2 protein could distinguish the different lineages (PPV1a – e). For example, viruses of the PPV1a lineage exhibited lineage-specific alterations at T20A, R82K, S233T, P304T, I320T, D336N, and K407N, whereas PPV1e, which includes the vaccine strain PPV014, presents exclusive alterations at Q228E, A414S, E419Q, and P436T. These two lineages are the only ones with exclusive alterations and, interestingly, they are phylogenetically very distant from each other. A study by Sun et al. (2015) demonstrated that the S233T substitution in the linear epitope $^{228}$QQITDA$^{233}$ of the PPV1 VP2 protein correlates with antigenic variability, potentially facilitating immune evasion and viral adaptation in swine populations, and site 228, which is located near the triple shoulder of the capsid unit (Simpson et al., 2002), is relevant for humoral immune response, presenting substitutions (Q→E in PPV1e and PPV014) which may impact recognition by neutralizing antibodies (Sun et al., 2015).

As in many viruses, in PPV, the determinants of cellular and tissue tropism, hemagglutination properties, and host range are in the capsid proteins. Although the biological

impact of most variations is unknown, some amino acids considered important in the VP2 protein have been identified through comparison between infectious clones of the NADL-2 and Kresse strains (Bergeron; Hébert; Tijssen, 1996; Bergeron; Menezes; Tijssen, 1993), combined with the structural resolution of viral capsids using X-ray crystallography (Simpson et al., 2002). The three-dimensional analysis of the capsid structure demonstrated that some of these residues are exposed on the surface of the viral particle, such as residue 215, inserted in loop II, residues 378 and 383 in loops III and IV respectively, and residue 565 in the C-terminal region. The 215T substitution, which is present in the virulent Kresse strain, was also observed in PPV1a, PPV1b, PPV1c, PPV1e, and PPV014, but was absent in the vaccine strains NADL-2 and 27a, and this I215T substitution has been previously associated with increased pathogenicity in highly virulent field isolates and is considered a molecular marker of virulence of various parvoviruses (Chapman; Rossmann, 1993). The D378G and H383Q mutations are associated with increased pathogenicity, hemagglutination, and cellular tropism; they are located close to the 2-fold axis on the surface of the virus capsid and may facilitate transposition of the placental barrier (Bergeron; Hébert; Tijssen, 1996). Residue 436, identified as a positive selection site by all algorithms applied in this study, is located at the top of the 3-fold spike, within loop IV, being in one of the most accessible regions of the capsid and possibly related to viral tropism, with the 436P/T variation associated with increased pathogenicity and cellular tropism (Li et al., 2021; Zeeuw et al., 2007), while the R565K substitution has been observed in highly virulent field isolates and may influence hemagglutination activity (Bergeron; Hébert; Tijssen, 1996). Studies indicate that monoclonal antibodies neutralize viruses carrying the isolated S436T substitution but fail against complex mutational combinations, suggesting synergy between *loci* (Streck; Canal; Truyen, 2022). Additionally, the 320T site, also identified as a positive selection site by all algorithms, is a characteristic feature of Brazilian isolates and represents a critical epitope with high antibody binding potential, suggesting its importance in host immune recognition and viral evolution under selective pressure (Deng et al., 2023; Streck et al., 2013). Considering the available molecular and phylogenetic data, it is plausible to infer that PPV1a, PPV1b, and PPV1e lineages exhibit higher pathogenic potential compared to PPV1c and PPV1d, as they accumulate specific mutations linked to virulence markers, expanded cellular tropism, pathogenesis, and potential immune evasion, features less evident in the other lineages.

The molecular docking results revealed varying binding affinities between predicted T-cell epitopes and swine leukocyte antigen (SLA) alleles, with scores ranging from -9.4 to -5.4. The epitope $^{344}$FEYSNGGPF$^{352}$ exhibited a strong binding to SLA-2*02:01 (score: -9.4) and

SLA-1*08:01 (score: -7.8), supported by hydrogen bond interactions, identifying it as a highly promising immunogenic candidate for multi-epitope vaccine design. Additionally, as illustrated in Figure 9, this epitope is positioned at a considerable distance from residues 320 (34.677 Å) and 436 (21.119 Å). This spatial separation is important, as proximity between these regions could allow mutations at critical residues (e.g., the I320T substitution observed in circulating PPV1a lineages) to alter surface topology or electrostatic properties, potentially impairing antibody neutralization or T-cell receptor (TCR) engagement, even if the epitope itself remains conserved. For instance, I320T could destabilize hydrogen bonds or introduce steric hindrance, diminishing immunogenicity. This immune evasion mechanism was exemplified by epidemiological observations from Thai isolates, where phylogenetic analysis revealed that seven distinct lineages maintained isoleucine at position 320 (consistent with the NADL-2 vaccine strain genotype), whereas five emergent lineages acquired threonine through non-synonymous substitution (I320T) (Saekhow; Sriphannam; Yamsakul, 2022). A parallel trend occurs at residue 436, a positively selected site within an exposed capsid loop influencing cellular tropism, where substitutions (proline, serine, threonine) have been observed. Importantly, lineages absent from current vaccine strains (e.g., PPV1a, PPV1b) displayed unique substitutions near key epitopes, potentially modulating peptide binding affinity and T-cell response efficacy. For example, the Q228E substitution in PPV1e modifies surface topology near the 3-fold spike—a region critical for antibody recognition—which may reduce humoral response effectiveness in vaccines targeting other strains. For example, the Q228E substitution in PPV1e alters the surface topology near the 3-fold spike, a region implicated in antibody recognition, potentially reducing the effectiveness of humoral responses elicited by vaccines based on other strains. Collectively, these results emphasize that antigenic drift in VP2, driven by selective pressure, may affect both B-cell epitope antigenicity and T-cell epitope presentation. While our *in silico* analysis suggests potential alterations in those epitopes may induce immune evasion, it is important to clarify that no direct evidence of immune evasion has been demonstrated. Experimental studies including neutralization assays and T-cell activation experiments are necessary to confirm these theoretical predictions.

A promising strategy to overcome this challenge would be the development of multi-epitope vaccines incorporating immunoinformatics approaches to broaden coverage against divergent variants. Recent studies have demonstrated the efficacy of this strategy, showing an excellent antigenic profile, low allergenicity, and potential to induce immune responses in preclinical swine virus assays (Chen; Li; Wang, 2024), suggesting applicability to PPV1. The epitope selection should prioritize highly conserved and immunodominant regions, including

high-affinity epitopes such as $^{344}$FEYSNGGPF$^{352}$, in conformations resistant to structural alterations induced by substitutions at adjacent positively selected sites.

## 5. CONCLUSIONS

In summary, our *in silico* analyses indicated that the highly adaptable PPV1 may be under selective pressure potentially influenced by vaccines, leading to the emergence of genetically divergent lineages, such as PPV1a, which exhibit predictive features of antigenic escape against commercial formulations. The identification of positively selected sites (320 and 436), mutations potentially associated with altered antigenicity and immune invasion (e.g., I320T, P436T), and lineage-specific substitutions in PPV1a (e.g., S233T, previously linked to antigenic variability in studies) suggests a possible progressive loss of vaccine efficacy. Additionally, the high genetic diversity of PPV1 observed during our analyses emphasizes the need for further epidemiological studies and experimental validation of these findings, including *in vitro* neutralization assays and *in vivo* challenge studies, alongside the implementation of integrated genomic surveillance to monitor emerging variants and guide strategic updates to immunogens.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/, Table S1. B-cell epitope prediction results; Table S2. Docking results; Table S3. Phylogenetic tree data; Table S4. Background information about selected strains; Table S5. T-cell epitope prediction and docking results; Figure S1. Structural representation of the viral capsid; Figure S2a. Phylogenetic tree in polar plot; Figure S2b. Phylogenetic tree in radial plot; Figure S3. Skyline plot of PPV1; Figure S4. Lineages amino acid substitutions; Figure S5. Circular validation plot generated by Iris; Figure S6. Ramachandran plot representation; Figure S7. Predicted protein structure comparison; Figure S8. Docked complex of the ligand FEYSNGGPF (pos. 344-352) and SLA-2*02:01 immune receptor; Figure S9. Spatial separation between FEYSNGGPF ligand and residues 320 and 436; Figure S10.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, P.A.F.S.P., A.F.S; methodology, P.A.F.S.P.; software, P.A.F.S.P.; validation, P.A.F.S.P.; formal analysis, P.A.F.S.P.; investigation, P.A.F.S.P., R.S.F., N.A.L.; resources, P.A.F.S.P.; data curation, P.A.F.S.P.; writing—original draft preparation, P.A.F.S.P.; writing—review and editing, P.A.F.S.P., A.F.S., V.R.L., S.A.S.; visualization, P.A.F.S.P., V.R.L., S.A.S.; supervision, A.F.S.; project administration, P.A.F.S.P., A.F.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) – Finance Code 001.

**Informed Consent Statement:** Not applicable

**Acknowledgments:** We thank our graduate students, undergraduate students and researchers who assisted in the making of this article.

**Conflicts of Interest:** The authors declare no conflicts of interest regarding the publication of this article.

## 6. REFERENCES

STRECK, André Felipe *et al.* High rate of viral evolution in the capsid protein of porcine parvovirus. **The Journal of general virology**, v. 92, n. Pt 11, nov. 2011.

ALARCÓN, Laura Valeria; ALLEPUZ, Alberto; MATEU, Enric. Biosecurity in pig farms: a review. **Porcine Health Management**, v. 7, n. 1, p. 5, 4 jan. 2021.

ÁLVAREZ-CARRETERO, Sandra; KAPLI, Paschalia; YANG, Ziheng. Beginner's Guide on the Use of PAML to Detect Positive Selection. **Molecular Biology and Evolution**, v. 40, n. 4, p. msad041, 25 abr. 2023.

ANISIMOVA, M.; BIELAWSKI, J. P.; YANG, Z. Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution. **Molecular Biology and Evolution**, v. 18, n. 8, p. 1585–1592, ago. 2001.

ANISIMOVA, Maria; NIELSEN, Rasmus; YANG, Ziheng. Effect of Recombination on the Accuracy of the Likelihood Method for Detecting Positive Selection at Amino Acid Sites. **Genetics**, v. 164, n. 3, p. 1229–1236, 1 jul. 2003.

BAI, Yihan *et al.* Designing a Candidate Multi-Epitope Vaccine against Transmissible Gastroenteritis Virus Based on Immunoinformatic and Molecular Dynamics. **International Journal of Molecular Sciences**, v. 25, n. 16, p. 8828, jan. 2024.

BARTHASSON, D. L. *et al.* Ocorrência de infecção por parvovírus suíno e gastrenterite transmissível em suínos, criados de forma extensiva, em Goiás. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 61, p. 1227–1229, out. 2009.

BELTRAN-ALCRUDO, Daniel *et al.* Transboundary spread of pig diseases: the role of international trade and travel. **BMC veterinary research**, v. 15, n. 1, p. 64, 22 fev. 2019.

BERGERON, J.; HÉBERT, B.; TIJSSEN, P. Genome organization of the Kresse strain of porcine parvovirus: identification of the allotropic determinant and comparison with those of NADL-2 and field isolates. **Journal of Virology**, v. 70, n. 4, p. 2508–2515, abr. 1996.

BERGERON, J.; MENEZES, J.; TIJSSEN, P. Genomic organization and mapping of transcription and translation products of the NADL-2 strain of porcine parvovirus. **Virology**, v. 197, n. 1, p. 86–98, nov. 1993.

BOLT, D. M. *et al.* Non-suppurative myocarditis in piglets associated with porcine parvovirus infection. **Journal of Comparative Pathology**, v. 117, n. 2, p. 107–118, ago. 1997.

BOTERO, O.; MARTINAT-BOTTÉ, F.; BARITEAU, F. Use of ultrasound scanning in swine for detection of pregnancy and some pathological conditions. **Theriogenology**, v. 26, n. 3, p. 267–278, 1 set. 1986.

BOUCKAERT, Remco *et al.* BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. **PLOS Computational Biology**, v. 15, n. 4, p. e1006650, 8 abr. 2019.

BROWN, T. T.; WHITACRE, M. D.; ROBISON, O. W. Use of an inactivated vaccine for prevention of parvovirus-induced reproductive failure in gilts. **Journal of the American Veterinary Medical Association**, v. 190, n. 2, p. 179–181, 15 jan. 1987.

CABI. porcine parvovirus infection. **CABI Compendium**, v. CABI Compendium, p. 68583, 21 nov. 2019.

CADAR, Dániel *et al.* Phylogeny and evolutionary genetics of porcine parvovirus in wild boars. **Infection, Genetics and Evolution**, v. 12, n. 6, p. 1163–1171, 1 ago. 2012.

CARTWRIGHT, S. F.; LUCAS, M.; HUCK, R. A. A small haemagglutinating porcine DNA virus. I. Isolation and properties. **Journal of Comparative Pathology**, v. 79, n. 3, p. 371–377, jul. 1969.

CARTWRIGHT, Sheila F.; LUCAS, Margaret; HUCK, R. A. A small haemagglutinating porcine DNA virus: II. Biological and serological studies. **Journal of Comparative Pathology**, v. 81, n. 1, p. 145–155, 1 jan. 1971.

CETIN, I.; ALVINO, G. Intrauterine growth restriction: implications for placental metabolism and transport. A review. **Placenta**, v. 30 Suppl A, p. S77-82, mar. 2009.

CHAPMAN, M. S.; ROSSMANN, M. G. Structure, sequence, and function correlations among parvoviruses. **Virology**, v. 194, n. 2, p. 491–508, jun. 1993.

CHEN, Hao-tai *et al.* Rapid detection of porcine parvovirus DNA by sensitive loop-mediated isothermal amplification. **Journal of Virological Methods**, v. 158, n. 1–2, p. 100–103, jun. 2009.

CHEN, Songbiao *et al.* SYNCRIP facilitates porcine parvovirus viral DNA replication through the alternative splicing of NS1 mRNA to promote NS2 mRNA formation. **Veterinary Research**, v. 52, p. 73, 2021a.

CHEN, Songbiao *et al.* Coatomer protein COPƐ, a novel NS1-interacting protein, promotes the replication of Porcine Parvovirus via attenuation of the production of type I interferon. **Veterinary Microbiology**, v. 261, p. 109188, 1 out. 2021b.

CHEN, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. **Acta Crystallographica Section D: Biological Crystallography**, v. 66, n. 1, p. 12–21, 1 jan. 2010.

CHEN, Xiaojiang S.; STEHLE, Thilo; HARRISON, Stephen C. Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. **The EMBO Journal**, v. 17, n. 12, p. 3233–3240, 15 jun. 1998.

CHEN, Xiaowei; LI, Yongfeng; WANG, Xiao. Multi-epitope vaccines: a promising strategy against viral diseases in swine. **Frontiers in Cellular and Infection Microbiology**, v. 14, 20 dez. 2024.

CHO, Kyou-Nam *et al.* A Plant-Produced Porcine Parvovirus 1-82 VP2 Subunit Vaccine Protects Pregnant Sows against Challenge with a Genetically Heterologous PPV1 Strain. **Vaccines**, v. 11, n. 1, p. 54, 26 dez. 2022.

CHOI, C. S. *et al.* Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses. **Veterinary Microbiology**, v. 15, n. 1–2, p. 19–29, out. 1987.

CHOU, P. Y.; FASMAN, G. D. Prediction of the secondary structure of proteins from their amino acid sequence. **Advances in Enzymology and Related Areas of Molecular Biology**, v. 47, p. 45–148, 1978.

CHRISTIANSON, W. T. *et al.* Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. **Canadian Journal of Veterinary Research = Revue Canadienne De Recherche Veterinaire**, v. 57, n. 4, p. 262–268, out. 1993.

CHRISTIANSON, William T. Stillbirths, Mummies, Abortions, and Early Embryonic Death. **Veterinary Clinics of North America: Food Animal Practice**, v. 8, n. 3, p. 623–639, 1 nov. 1992.

COTMORE, Susan F.; TATTERSALL, Peter. Parvoviral Host Range and Cell Entry Mechanisms. *In*: **Advances in Virus Research**. *[S.l.]*: Academic Press, 2007. v. 70 p. 183–232.

CRUVINEL, Wilson de Melo *et al.* Immune system: Part I. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. **Revista Brasileira de Reumatologia**, v. 50, p. 434–447, ago. 2010.

DARBYSHIRE, J. H.; ROBERTS, D. H. Some respiratory virus and mycoplasma infections of animals. **Journal of Clinical Pathology. Supplement (College of Pathologists)**, v. 2, p. 61–92, 1968.

DARRIBA, Diego *et al.* jModelTest 2: more models, new heuristics and parallel computing. **Nature Methods**, v. 9, n. 8, p. 772–772, ago. 2012.

DE MAIO, Federico Andrés *et al.* Detection of porcine circovirus 2, porcine parvovirus 1, and torque teno sus virus k2a in wild boars from northeastern Patagonia, Argentina. **Archives of Virology**, v. 168, n. 8, p. 208, 18 jul. 2023.

DEE, Scott *et al.* Further Evidence That Science-Based Biosecurity Provides Sustainable Prevention of Porcine Reproductive and Respiratory Syndrome Virus Infection and Improved Productivity in Swine Breeding Herds. **Animals**, v. 14, n. 17, p. 2530, jan. 2024.

DENG, Huiwei *et al.* Isolation, characterization, and phylogenetic analysis of two new porcine parvovirus 1 isolates from Northern China. **Virus Research**, v. 339, p. 199247, 8 nov. 2023.

DEWULF, J. *et al.* Transmission of pig diseases and biosecurity in pig production. *In*: **Biosecurity in animal production and veterinary medicine: from principles to practice**. CABI Books. *[S.l.: S.n.]*. p. 295–328.

DIMITROV, Ivan *et al.* AllerTOP v.2--a server for in silico prediction of allergens. **Journal of Molecular Modeling**, v. 20, n. 6, p. 2278, jun. 2014.

DONEVA, Nikolet; DIMITROV, Ivan. Viral Immunogenicity Prediction by Machine Learning Methods. **International Journal of Molecular Sciences**, v. 25, n. 5, p. 2949, jan. 2024.

DROLET, R. *et al.* Infectious agents identified in pigs with multifocal interstitial nephritis at slaughter. **Veterinary Record**, v. 150, n. 5, p. 139–143, 2002.

DRUMMOND, A. J. *et al.* Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. **Molecular Biology and Evolution**, v. 22, n. 5, p. 1185–1192, 1 maio 2005.

DRUMMOND, Alexei J. *et al.* Relaxed phylogenetics and dating with confidence. **PLoS biology**, v. 4, n. 5, p. e88, maio 2006.

DUFFY, Siobain; SHACKELTON, Laura A.; HOLMES, Edward C. Rates of evolutionary change in viruses: patterns and determinants. **Nature Reviews. Genetics**, v. 9, n. 4, p. 267–276, abr. 2008.

DUNNE, H. W. *et al.* Porcine reproductive failure associated with a newly identified "SMEDI" group of picorna viruses. **American Journal of Veterinary Research**, v. 26, n. 115, p. 1284–1297, nov. 1965.

EDDICKS, Matthias *et al.* Examination on the Occurrence of Coinfections in Diagnostic Transmittals in Cases of Stillbirth, Mummification, Embryonic Death, and Infertility (SMEDI) Syndrome in Germany. **Microorganisms**, v. 11, n. 7, p. 1675, 27 jun. 2023.

EMINI, E. A. *et al.* Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. **Journal of Virology**, v. 55, n. 3, p. 836–839, set. 1985.

FAGBOHUN, Olusegun A. *et al.* Contriving a multi-epitope vaccine against African swine fever utilizing immunoinformatics. **Biomedical Research and Therapy**, v. 11, n. 8, p. 6642–6660, 31 ago. 2024.

FAJARDO, Lauren Emily *et al.* Computational multi-epitope based design of a multivalent subunit vaccine against co-infecting African swine fever virus and porcine circovirus type 2 : https://doi.org/10.12982/VIS.2025.065. **Veterinary Integrative Sciences**, v. 23, n. 3, p. 1–30, 2025.

FAN, Shuhua *et al.* Structural and Biochemical Analyses of Swine Major Histocompatibility Complex Class I Complexes and Prediction of the Epitope Map of Important Influenza A Virus Strains. **Journal of Virology**, v. 90, n. 15, p. 6625–6641, 1 ago. 2016.

FERNANDES, Sandra; BOISVERT, Maude; TIJSSEN, Peter. Genetic Elements in the VP Region of Porcine Parvovirus Are Critical to Replication Efficiency in Cell Culture. **Journal of Virology**, v. 85, n. 6, p. 3025–3029, mar. 2011.

FILIPPITZI, M. E. *et al.* Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. **Transboundary and Emerging Diseases**, v. 65, n. 2, p. 381–398, 2018.

FOERSTER, Tessa *et al.* An inactivated whole-virus porcine parvovirus vaccine protects pigs against disease but does not prevent virus shedding even after homologous virus challenge. **Journal of General Virology**, v. 97, n. 6, p. 1408–1413, 2016.

FRANZO, Giovanni *et al.* A Phylogeographic Analysis of Porcine Parvovirus 1 in Africa. **Viruses**, v. 15, n. 1, p. 207, jan. 2023.

FREDRIKSSON-AHOMAA, Maria. Wild Boar: A Reservoir of Foodborne Zoonoses. **Foodborne Pathogens and Disease**, v. 16, n. 3, p. 153–165, mar. 2019.

GAO, Linyi Alex *et al.* Prokaryotic innate immunity through pattern recognition of conserved viral proteins. **Science**, v. 377, n. 6607, p. eabm4096, 12 ago. 2022.

GAVA, Danielle *et al.* Atualização sobre a parvovirose na suinocultura. **Acta Scientiae Veterinariae**, v. 37, n. 1, p. s105–s115, 2009.

GAVA, Danielle *et al.* Dynamics of vanishing of maternally derived antibodies of Ungulate protoparvovirus 1 suggests an optimal age for gilts vaccination. **Tropical Animal Health and Production**, v. 49, n. 5, p. 1085–1088, 1 jun. 2017.

GOUVEIA, Aurora Maria Guimaraes. Alterações reprodutivas e prevalência de anticorpos inibidores da hemoaglutinação para o parvovírus suino no estado de Minas Gerais. 23 abr. 1982.

HADDAD, John J.; ABDEL-KARIM, Nisreen E. NF-κB cellular and molecular regulatory mechanisms and pathways: therapeutic pattern or pseudoregulation? **Cellular Immunology**, v. 271, n. 1, p. 5–14, 2011.

HARBISON, Carole E.; CHIORINI, John A.; PARRISH, Colin R. The parvovirus capsid odyssey: from the cell surface to the nucleus. **Trends in Microbiology**, v. 16, n. 5, p. 208–214, maio 2008.

HAYDEN, Matthew S.; GHOSH, Sankar. NF-κB in immunobiology. **Cell Research**, v. 21, n. 2, p. 223–244, fev. 2011.

HELFRICH, Philipp *et al.* TreeAnnotator: Versatile Visual Annotation of Hierarchical Text Relations. *In*: CALZOLARI, Nicoletta *et al.* (orgs.). LREC 2018. **Proceedings of the Eleventh International Conference on Language Resources and Evaluation (LREC 2018)**. Miyazaki, Japan: European Language Resources Association (ELRA), maio 2018. Disponível em: <https://aclanthology.org/L18-1308>. Acesso em: 17 set. 2024

HELKE, Kristi L. *et al.* Chapter 16 - Biology and Diseases of Swine. *In*: FOX, James G. *et al.* (Orgs.). **Laboratory Animal Medicine (Third Edition)**. American College of Laboratory Animal Medicine. Boston: Academic Press, 2015. p. 695–769.

HEO, Lim; PARK, Hahnbeom; SEOK, Chaok. GalaxyRefine: protein structure refinement driven by side-chain repacking. **Nucleic Acids Research**, v. 41, n. Web Server issue, p. W384–W388, jul. 2013.

HERDT, Geslaine *et al.* High prevalence of porcine circovirus 2, porcine parvovirus, and pathogenic leptospires in mummified swine fetuses in Southern Brazil. **Ciência Rural**, v. 49, p. e20180965, 11 abr. 2019.

JAKOV, Nišavić *et al.* Genetic Analysis and Distribution of Porcine Parvoviruses Detected in the Organs of Wild Boars in Serbia. **Acta Veterinaria**, v. 71, n. 1, p. 32–46, 28 fev. 2021.

JESPERSEN, Martin Closter *et al.* BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. **Nucleic Acids Research**, v. 45, n. Web Server issue, p. W24–W29, 3 jul. 2017.

JIN, Xiaohui *et al.* Porcine parvovirus nonstructural protein NS1 activates NF-κB and it involves TLR2 signaling pathway. **Journal of Veterinary Science**, v. 21, n. 3, p. e50, maio 2020.

JOHNSON, R. H.; COLLINGS, D. F. Transplacental Infection of Piglets with a Porcine Parvovirus. **Research in Veterinary Science**, v. 12, n. 6, p. 570–572, 1 nov. 1971.

JOHNSON, R. H.; DONALDSON-WOOD, C.; ALLENDER, U. Observations on the epidemiology of porcine parvovirus. **Australian Veterinary Journal**, v. 52, n. 2, p. 80–84, fev. 1976.

JOO, H. S.; JOHNSON, R. H. Serological responses in pigs vaccinated with inactivated porcine parvovirus. **Australian Veterinary Journal**, v. 53, n. 11, p. 550–552, nov. 1977.

JOSEPH, Agnel Praveen *et al.* Atomic model validation using the CCP-EM software suite. **Acta Crystallographica. Section D, Structural Biology**, v. 78, n. Pt 2, p. 152–161, 1 fev. 2022.

JÓŹWIK, A. *et al.* Vaccination against porcine parvovirus protects against disease, but does not prevent infection and virus shedding after challenge infection with a heterologous virus strain. **The Journal of General Virology**, v. 90, n. Pt 10, p. 2437–2441, out. 2009.

JUSZKIEWICZ, Małgorzata *et al.* Effectiveness of Chemical Compounds Used against African Swine Fever Virus in Commercial Available Disinfectants. **Pathogens**, v. 9, n. 11, p. 878, 24 out. 2020.

KADEN, V. *et al.* Retrospective serological survey on selected viral pathogens in wild boar populations in Germany. **European Journal of Wildlife Research**, v. 55, n. 2, p. 153–159, 1 abr. 2009.

KARPLUS, P. A.; SCHULZ, G. E. Prediction of chain flexibility in proteins. **Naturwissenschaften**, v. 72, n. 4, p. 212–213, 1 abr. 1985.

KENNEDY, S. *et al.* Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. **Journal of Comparative Pathology**, v. 122, n. 1, p. 9–24, jan. 2000.

KIM, Sung Woo *et al.* - Invited Review - Current status of global pig production: an overview and research trends. **Animal Bioscience**, v. 37, n. 4, p. 719–729, abr. 2024.

KOLASKAR, A. S.; TONGAONKAR, P. C. A semi-empirical method for prediction of antigenic determinants on protein antigens. **FEBS letters**, v. 276, n. 1–2, p. 172–174, 10 dez. 1990.

KRESSE, J. I. *et al.* Parvovirus infection in pigs with necrotic and vesicle-like lesions. **Veterinary Microbiology**, v. 10, n. 6, p. 525–531, 1 dez. 1985.

LAGER, K. M.; MENGELING, W. L. Porcine parvovirus associated with cutaneous lesions in piglets. **Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc**, v. 6, n. 3, p. 357–359, jul. 1994.

LAMIABLE, Alexis *et al.* PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. **Nucleic Acids Research**, v. 44, n. Web Server issue, p. W449–W454, 8 jul. 2016.

LI, Jixiang *et al.* A Systematic Investigation Unveils High Coinfection Status of Porcine Parvovirus Types 1 through 7 in China from 2016 to 2020. **Microbiology Spectrum**, v. 9, n. 3, p. e0129421, 22 dez. 2021.

LING, Zhanye *et al.* A Subunit Vaccine Based on the VP2 Protein of Porcine Parvovirus 1 Induces a Strong Protective Effect in Pregnant Gilts. **Vaccines**, v. 11, n. 11, p. 1692, 5 nov. 2023.

LIU, Qin *et al.* Performance of Akaike Information Criterion and Bayesian Information Criterion in Selecting Partition Models and Mixture Models. **Systematic Biology**, v. 72, n. 1, p. 92–105, 1 jan. 2023.

LÓPEZ-ASTACIO, Robert A. *et al.* The Structures and Functions of Parvovirus Capsids and Missing Pieces: the Viral DNA and Its Packaging, Asymmetrical Features, Nonprotein Components, and Receptor or Antibody Binding and Interactions. **Journal of Virology**, v. 97, n. 7, p. e00161-23, 27 jun. 2023.

LÜ, J. *et al.* A slide latex agglutination test for the rapid detection of antibodies in serum against porcine parvovirus. **Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health**, v. 53, n. 2, p. 59–61, mar. 2006.

LUCAS, M. H.; CARTWRIGHT, S. F.; WRATHALL, A. E. Genital infection of pigs with porcine parvovirus. **Journal of Comparative Pathology**, v. 84, n. 3, p. 347–350, jul. 1974.

MA, Xia *et al.* Ferulic Acid Protects against Porcine Parvovirus Infection-Induced Apoptosis by Suppressing the Nuclear Factor-κB Inflammasome Axis and Toll-Like Receptor 4 via Nonstructural Protein 1. **Evidence-Based Complementary and Alternative Medicine**, v. 2020, n. 1, p. 3943672, 2020.

MA, Xia *et al.* Phytochemical Constituents of Propolis Flavonoid, Immunological Enhancement, and Anti-porcine Parvovirus Activities Isolated From Propolis. **Frontiers in Veterinary Science**, v. 9, 8 abr. 2022.

MACLACHLAN, N. James; DUBOVI, Edward J. **Fenners Veterinary Virology (pp. 269-297)**. Cambridge, USA: Academic Press Elsevier, , 2017.

MAES, Dominiek G. D. *et al.* A critical reflection on intensive pork production with an emphasis on animal health and welfare. **Journal of Animal Science**, v. 98, n. Suppl 1, p. S15–S26, 18 ago. 2020.

MARTIN, Darren P. *et al.* RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. **Virus Evolution**, v. 7, n. 1, p. veaa087, 12 abr. 2020.

MAYR, A.; MAHNEL, H. Cultivation of hog cholera virus in pig kidney cultures with cytopathogenic effect. **Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale**, v. 195, n. 2, p. 157–166, 1964.

MAYR, A.; MAHNEL, H. [Further studies on the cultivation of swine plague virus in cell cultures with a cytopathogenic effect]. **Zentralblatt Fur Bakteriologie, Parasitenkunde, Infektionskrankheiten Und Hygiene. 1. Abt. Medizinisch-Hygienische Bakteriologie, Virusforschung Und Parasitologie. Originale**, v. 199, n. 4, p. 399–407, abr. 1966.

MCKILLEN, John *et al.* Molecular beacon real-time PCR detection of swine viruses. **Journal of Virological Methods**, v. 140, n. 1–2, p. 155–165, mar. 2007.

MENG, X. J.; LINDSAY, D. S.; SRIRANGANATHAN, N. Wild boars as sources for infectious diseases in livestock and humans. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 364, n. 1530, p. 2697–2707, 27 set. 2009.

MENGELING, W. L. Prenatal infection following maternal exposure to porcine parvovirus on either the seventh or fourteenth day of gestation. **Canadian Journal of Comparative Medicine: Revue Canadienne De Medecine Comparee**, v. 43, n. 1, p. 106–109, jan. 1979.

MENGELING, W. L. *et al.* Efficacy of an inactivated virus vaccine for prevention of porcine parvovirus-induced reproductive failure. **American Journal of Veterinary Research**, v. 40, n. 2, p. 204–207, fev. 1979.

MENGELING, W. L.; CUTLIP, R. C. Reproductive Disease Experimentally Induced by Exposing Pregnant Gilts to Porcine Parvovirus. 1 dez. 1976.

MENGELING, W. L.; LAGER, K. M.; VORWALD, A. C. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. **Animal Reproduction Science**, v. 60–61, p. 199–210, 2 jul. 2000.

MENGELING, W. L.; PAUL, P. S. Interepizootic survival of porcine parvovirus. 1 jun. 1986.

MENGELING, W. L.; PAUL, P. S.; BROWN, T. T. Transplacental infection and embryonic death following maternal exposure to porcine parvovirus near the time of conception. **Archives of Virology**, v. 65, n. 1, p. 55–62, 1 mar. 1980.

MENGELING, W. L.; RIDPATH, J. F.; VORWALD, A. C. Size and Antigenic Comparisons among the Structural Proteins of Selected Autonomous Parvoviruses. **Journal of General Virology**, v. 69, n. 4, p. 825–837, 1988.

MÉSZÁROS, István *et al.* The SAT Protein of Porcine Parvovirus Accelerates Viral Spreading through Induction of Irreversible Endoplasmic Reticulum Stress. **Journal of Virology**, v. 91, n. 16, p. e00627-17, 15 ago. 2017a.

MÉSZÁROS, István *et al.* Biology of Porcine Parvovirus (Ungulate parvovirus 1). **Viruses**, v. 9, n. 12, p. 393, dez. 2017b.

MONTAGNARO, Serena *et al.* Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (Sus scrofa) in Campania Region, Italy. **Journal of Wildlife Diseases**, v. 46, n. 1, p. 316–319, jan. 2010.

MORÓN, Gabriel *et al.* CD8alpha- CD11b+ dendritic cells present exogenous virus-like particles to CD8+ T cells and subsequently express CD8alpha and CD205 molecules. **The Journal of Experimental Medicine**, v. 195, n. 10, p. 1233–1245, 20 maio 2002.

MOROÑO, Roncero; ALEJANDRO, David. **Creation, refinement, and evaluation of conformational ensembles of proteins using the Torsional Network Model**. masterThesis—*[S.l.: S.n.]*.

MORRIS, Garrett M. *et al.* AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. **Journal of computational chemistry**, v. 30, n. 16, p. 2785–2791, dez. 2009.

NARITA, M. *et al.* Histopathological changes of the brain in swine fetuses naturally infected with procine parvovirus. **National Institute of Animal Health Quarterly**, v. 15, n. 1, p. 24–28, 1975.

NGUYEN, Lam-Tung *et al.* IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. **Molecular Biology and Evolution**, v. 32, n. 1, p. 268–274, 1 jan. 2015.

NIESTEN, Eddie; RAYMAEKERS, Jan; SEGERS, Yves. **Lekker dier!? Dierlijke productie en consumptie in de 19de en 20ste eeuw.** Leuven: CAG Centrum voor agrarische geschiedenis, 2003.

OH, Woo-Taek *et al.* Perspectives on the Evolution of Porcine Parvovirus. **Viruses**, v. 9, n. 8, p. 196, ago. 2017.

ORAVEERAKUL, K.; CHOI, C. S.; MOLITOR, T. W. Restriction of porcine parvovirus replication in nonpermissive cells. **Journal of Virology**, v. 66, n. 2, p. 715–722, fev. 1992.

ORAVEERAKUL, K.; CHOI, C. S.; MOLITOR, T. W. Tissue tropisms of porcine parvovirus in swine. **Archives of Virology**, v. 130, n. 3, p. 377–389, 1 set. 1993.

OTAKE, Satoshi; YOSHIDA, Mio; DEE, Scott. A Review of Swine Breeding Herd Biosecurity in the United States to Prevent Virus Entry Using Porcine Reproductive and Respiratory Syndrome Virus as a Model Pathogen. **Animals**, v. 14, n. 18, p. 2694, jan. 2024.

O'TOOLE, Áine; AZIZ, Ammar; MALONEY, Daniel. Publication-ready single nucleotide polymorphism visualization with snipit. **Bioinformatics (Oxford, England)**, v. 40, n. 8, p. btae510, 2 ago. 2024.

OUH, In-Ohk *et al.* Prevalence of Porcine Parvovirus 1–6 Detected in South Korean Domestic Pigs. **Archives of Microbiology & Immunology**, v. 8, n. 3, p. 397–409, 5 set. 2024.

PACINI, Maria Irene *et al.* Detection and Characterization of Viral Pathogens Associated with Reproductive Failure in Wild Boars in Central Italy. **Animals**, v. 11, n. 2, p. 304, fev. 2021.

PARK, Gyu-Nam *et al.* Genetic analysis of porcine parvoviruses detected in South Korean wild boars. **Archives of Virology**, v. 166, n. 8, p. 2249–2254, 1 ago. 2021.

PARKER, J. M.; GUO, D.; HODGES, R. S. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. **Biochemistry**, v. 25, n. 19, p. 5425–5432, 23 set. 1986.

PARTHIBAN, S. *et al.* Molecular detection of porcine parvovirus 1–associated reproductive failure in southern India. **Tropical Animal Health and Production**, v. 54, n. 3, p. 195, 2 jun. 2022.

PAUL, P. S.; MENGELING, W. L. Evaluation of a modified live-virus vaccine for the prevention of porcine parvovirus-induced reproductive disease in swine. **American Journal of Veterinary Research**, v. 41, n. 12, p. 2007–2011, dez. 1980.

PAUL, P. S.; MENGELING, W. L. Oronasal and intramuscular vaccination of swine with a modified live porcine parvovirus vaccine: multiplication and transmission of the vaccine virus. **American Journal of Veterinary Research**, v. 45, n. 12, p. 2481–2485, dez. 1984.

PAUL, P. S.; MENGELING, W. L.; BROWN, T. T. Replication of porcine parvovirus in peripheral blood lymphocytes, monocytes, and peritoneal macrophages. **Infection and Immunity**, v. 25, n. 3, p. 1003–1007, set. 1979.

PAUL, P. S.; MENGELING, W. L.; PIRTLE, E. C. Duration and biological half-life of passively acquired colostral antibodies to porcine parvovirus. **American Journal of Veterinary Research**, v. 43, n. 8, p. 1376–1379, ago. 1982.

PAUL, Prem S.; MENGELING, William L. Vaccination of swine with an inactivated porcine parvovirus vaccine in the presence of passive immunity. 15 fev. 1986.

PÉNZES, Judit J. *et al.* Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. **Archives of Virology**, v. 165, n. 9, p. 2133–2146, set. 2020.

PESCADOR, Caroline A. *et al.* Co-infection by porcine circovirus type 2 and porcine parvovirus in aborted fetuses and stillborn piglets in southern Brazil. **Pesquisa Veterinária Brasileira**, v. 27, p. 425–429, out. 2007.

POTTERTON, Liz *et al.* CCP4i2: the new graphical user interface to the CCP4 program suite. **Acta Crystallographica. Section D, Structural Biology**, v. 74, n. Pt 2, p. 68–84, 1 fev. 2018.

PRESSING, A. L. *et al.* Prostaglandin-induced abortion in swine: endocrine changes and influence on subsequent reproductive activity. **American Journal of Veterinary Research**, v. 48, n. 1, p. 45–50, jan. 1987.

RABENAU, Holger F. *et al.* Evaluation of a Virucidal Quantitative Carrier Test for Surface Disinfectants. **PLoS ONE**, v. 9, n. 1, p. e86128, 27 jan. 2014.

RAMBAUT, Andrew *et al.* Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). **Virus Evolution**, v. 2, n. 1, p. vew007, 1 jan. 2016.

RAMBAUT, Andrew *et al.* Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. **Systematic Biology**, v. 67, n. 5, p. 901–904, set. 2018.

RATHORE, Anand Singh *et al.* ToxinPred 3.0: An improved method for predicting the toxicity of peptides. **Computers in Biology and Medicine**, v. 179, p. 108926, set. 2024.

REDMAN, D. R.; BOHL, E. H.; FERGUSON, L. C. Porcine parvovirus: natural and experimental infections of the porcine fetus and prevalence in mature swine. **Infection and Immunity**, v. 10, n. 4, p. 718–723, out. 1974.

RENAULT, Véronique; HUMBLET, Marie-France; SAEGERMAN, Claude. Biosecurity Concept: Origins, Evolution and Perspectives. **Animals: an open access journal from MDPI**, v. 12, n. 1, p. 63, 28 dez. 2021.

REYNISSON, Birkir *et al.* NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. **Nucleic Acids Research**, v. 48, n. W1, p. W449–W454, 2 jul. 2020.

RHEE, Chae Hong *et al.* Chemical stability of active ingredients in diluted veterinary disinfectant solutions under simulated storage conditions. **Frontiers in Chemistry**, v. 11, p. 1204477, 16 jun. 2023.

RIQUELME, G. Placental chloride channels: a review. **Placenta**, v. 30, n. 8, p. 659–669, ago. 2009.

ROCHA, Danilo Leal; ALBERTON, Geraldo Camilo; SANTOS, José Lúcio dos. Identificação do circovírus suíno tipo 2 e do parvovírus suíno em fetos suínos natimortos e mumificados provenientes de granjas no Brasil. **Ciência Animal Brasileira / Brazilian Animal Science**, v. 11, n. 3, p. 600–606, 2 out. 2010.

ROCHIRA, William; AGIRRE, Jon. Iris: Interactive all-in-one graphical validation of 3D protein model iterations. **Protein Science : A Publication of the Protein Society**, v. 30, n. 1, p. 93–107, jan. 2021.

RODRIGUEZ, Cesar Alejandro Rosales *et al.* Soroprevalência de anticorpos anti-parvovírus suíno em suínos do município de Uruará, estado do Pará. **Arquivos do Instituto Biológico**, v. 70, n. 4, p. 501–503, 2003.

SABIR, Jamal S. M. *et al.* Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. **Science (New York, N.Y.)**, v. 351, n. 6268, p. 81–84, 1 jan. 2016.

SAEKHOW, Prayuth; SRIPHANNAM, Chayaphon; YAMSAKUL, Panuwat. Molecular characterization of porcine parvovirus 1 based on partial VP2 gene in the ovaries of Thai pigs: https://doi.org/10.12982/VIS.2022.035. **Veterinary Integrative Sciences**, v. 20, n. 2, p. 475–488, 6 jun. 2022.

SERENA, M. S. *et al.* Detection and molecular characterization of porcine parvovirus in fetal tissues from sows without reproductive failure in Argentina. **Heliyon**, v. 5, n. 11, p. e02874, 22 nov. 2019.

SHACKELTON, Laura A. *et al.* Comparative analysis reveals frequent recombination in the parvoviruses. **The Journal of General Virology**, v. 88, n. Pt 12, p. 3294–3301, dez. 2007.

SIEGL, Günter. Properties of Parvoviruses. *In*: SIEGL, Günter (Org.). **The Parvoviruses**. Vienna: Springer, 1976. p. 6–85.

SILVA, Mariana Soares da. Sequenciamento de última geração aplicado à virologia veterinária. 2015.

SIMBULAN, Alea Maurice *et al.* Immunoinformatics-guided approach for designing a pan-proteome multi-epitope subunit vaccine against African swine fever virus. **Scientific Reports**, v. 14, n. 1, p. 1354, 16 jan. 2024.

SIMPSON, Alan A. *et al.* The structure of porcine parvovirus: comparison with related viruses1. **Journal of Molecular Biology**, v. 315, n. 5, p. 1189–1198, 1 fev. 2002.

SIRA, Ella Mae Joy S. *et al.* In silico design of multi-epitope vaccine candidate based on structural proteins of porcine reproductive and respiratory syndrome virus. **Veterinary Immunology and Immunopathology**, v. 280, p. 110881, fev. 2025.

SOARES, Rodrigo Martins *et al.* Genetic variability of porcine parvovirus isolates revealed by analysis of partial sequences of the structural coding gene VP2. **The Journal of General Virology**, v. 84, n. Pt 6, p. 1505–1515, jun. 2003.

SONG, Cuiping *et al.* Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene. **Virology Journal**, v. 7, n. 1, p. 353, 2 dez. 2010.

STRECK, André Felipe. Detecção e caracterização de amostras de parvovírus suíno. 2009.

STRECK, André Felipe *et al.* Population dynamics and in vitro antibody pressure of porcine parvovirus indicate a decrease in variability. **Journal of General Virology**, v. 94, n. 9, p. 2050–2055, 2013.

STRECK, André Felipe *et al.* A TaqMan qPCR for quantitation of *Ungulate protoparvovirus 1* validated in several matrices. **Journal of Virological Methods**, v. 218, p. 46–50, 15 jun. 2015.

STRECK, André Felipe; CANAL, Cláudio Wageck; TRUYEN, Uwe. Molecular epidemiology and evolution of porcine parvoviruses. **Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases**, v. 36, p. 300–306, dez. 2015.

STRECK, André Felipe; CANAL, Cláudio Wageck; TRUYEN, Uwe. Viral Fitness and Antigenic Determinants of Porcine Parvovirus at the Amino Acid Level of the Capsid Protein. **Journal of Virology**, v. 96, n. 2, p. e01198-21, 26 jan. 2022.

SUN, Jianhui *et al.* Identification of three PPV1 VP2 protein-specific B cell linear epitopes using monoclonal antibodies against baculovirus-expressed recombinant VP2 protein. **Applied Microbiology and Biotechnology**, v. 99, n. 21, p. 9025–9036, nov. 2015.

TAMURA, Koichiro; STECHER, Glen; KUMAR, Sudhir. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. **Molecular Biology and Evolution**, v. 38, n. 7, p. 3022–3027, 1 jul. 2021.

TECHAKRIENGKRAI, Navapon *et al.* Diversity of the Swine Leukocyte Antigen Class I and II in Commercial Pig Populations. **Frontiers in Veterinary Science**, v. 8, p. 637682, 30 abr. 2021.

TOO, H. L. *et al.* Evaluation of a gel diffusion precipitin test for porcine parvovirus. **Australian Veterinary Journal**, v. 60, n. 6, p. 161–165, jun. 1983.

TRINDADE, Isa Maria Sousa *et al.* Pesquisa sorológica do parvovirus suíno em reprodutoras de rebanhos suínos da Bahia. **Pubvet**, v. 5, n. 25, 2011.

TROTT, Oleg; OLSON, Arthur J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. **Journal of computational chemistry**, v. 31, n. 2, p. 455–461, 30 jan. 2010.

TRUYEN, Uwe; STRECK, André Felipe. Parvoviruses. *In*: **Diseases of Swine**. *[S.l.]*: John Wiley & Sons, Ltd, 2019. p. 611–621.

ULLAH, Shaukat *et al.* In Silico Designed Multi-Epitope Vaccine Based on the Conserved Fragments in Viral Proteins for Broad-Spectrum Protection Against Porcine Reproductive and Respiratory Syndrome Virus. **Veterinary Sciences**, v. 12, n. 6, p. 577, jun. 2025.

VALLET, J. L.; FREKING, B. A. Differences in placental structure during gestation associated with large and small pig fetuses. **Journal of Animal Science**, v. 85, n. 12, p. 3267–3275, dez. 2007.

VALLET, J. L.; MILES, J. R.; FREKING, B. A. Development of the pig placenta. **Society of Reproduction and Fertility Supplement**, v. 66, p. 265–279, 2009.

VARGAS, Anamaria Jung *et al.* Que decisão tomar frente a matrizes que apresentam falhas reprodutivas: elas merecem uma nova chance? 2007.

VENKATESWARAN, Dhithya *et al.* Designing a multi-epitope vaccine against African swine fever virus using immunoinformatics approach. **Scientific Reports**, v. 15, n. 1, p. 16044, 8 maio 2025.

VEREECKE, Nick *et al.* Molecular epidemiology of Porcine Parvovirus Type 1 (PPV1) and the reactivity of vaccine-induced antisera against historical and current PPV1 strains. **Virus Evolution**, v. 8, n. 1, p. veac053, 1 jan. 2022.

WANG, Linqing *et al.* Proteomics analysis of PK-15 cells infected with porcine parvovirus and the effect of PCBP1 on PPV replication. **Microbiology Spectrum**, v. 12, n. 6, p. e03914-23, *[S.d.]*.

WHITAKER, H. K.; NEU, S. M.; PACE, L. W. Parvovirus infection in pigs with exudative skin disease. **Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc**, v. 2, n. 3, p. 244–246, jul. 1990.

WILHELM, S. *et al.* Tissue distribution of two field isolates and two vaccine strains of porcine parvovirus in foetal organs after experimental infection of pregnant sows as determined by real-time PCR. **Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health**, v. 52, n. 7–8, p. 323–326, 2005.

WILHELM, Sonja *et al.* Real-time PCR protocol for the detection of porcine parvovirus in field samples. **Journal of Virological Methods**, v. 134, n. 1–2, p. 257–260, jun. 2006.

WILLIAMS, Christopher J. *et al.* MolProbity: More and better reference data for improved all-atom structure validation. **Protein Science: A Publication of the Protein Society**, v. 27, n. 1, p. 293–315, jan. 2018.

WLODAWER, Alexander. Stereochemistry and Validation of Macromolecular Structures. **Methods in molecular biology (Clifton, N.J.)**, v. 1607, p. 595–610, 2017.

WORD, J. Michael *et al.* Visualizing and quantifying molecular goodness-of-fit: small-probe contact dots with explicit hydrogen atoms1. **Journal of Molecular Biology**, v. 285, n. 4, p. 1711–1733, 29 jan. 1999.

XIE, Hong-ling *et al.* The epitope of the VP1 protein of porcine parvovirus. **Virology Journal**, v. 7, n. 1, p. 161, 16 jul. 2010.

YANG, Jianyi; ZHANG, Yang. I-TASSER server: new development for protein structure and function predictions. **Nucleic Acids Research**, v. 43, n. W1, p. W174-181, 1 jul. 2015.

YANG, Ziheng. PAML 4: Phylogenetic Analysis by Maximum Likelihood. **Molecular Biology and Evolution**, v. 24, n. 8, p. 1586–1591, 1 ago. 2007.

YASUHARA, H. *et al.* Characterization of a parvovirus isolated from the diarrheic feces of a pig. **Nihon Juigaku Zasshi. The Japanese Journal of Veterinary Science**, v. 51, n. 2, p. 337–344, abr. 1989.

ZÁDORI, Z.; SZELEI, J.; TIJSSEN, P. SAT: A late NS protein of porcine Parvovirus. **Journal of Virology**, v. 79, n. 20, p. 13129–13138, 2005.

ZEEUW, E. J. L. *et al.* Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. **The Journal of General Virology**, v. 88, n. Pt 2, p. 420–427, fev. 2007.

ZHANG, Liang *et al.* ARFGAP1 binds to classical swine fever virus NS5A protein and enhances CSFV replication in PK-15 cells. **Veterinary Microbiology**, v. 255, p. 109034, 1 abr. 2021.

ZHANG, Nianzhi *et al.* Crystal structure of swine major histocompatibility complex class I SLA-1 0401 and identification of 2009 pandemic swine-origin influenza A H1N1 virus cytotoxic T lymphocyte epitope peptides. **Journal of Virology**, v. 85, n. 22, p. 11709–11724, nov. 2011.

ZHOU, Hongchao; YAO, Guizhe; CUI, Shangjin. Production and purification of VP2 protein of porcine parvovirus expressed in an insect-baculovirus cell system. **Virology Journal**, v. 7, p. 366, 10 dez. 2010.

ZHOU, Yong *et al.* Porcine parvovirus infection activates inflammatory cytokine production through Toll-like receptor 9 and NF-κB signaling pathways in porcine kidney cells. **Veterinary Microbiology**, v. 207, p. 56–62, ago. 2017.

ZHU, Xiaochen *et al.* Design of multi-epitope vaccine against porcine rotavirus using computational biology and molecular dynamics simulation approaches. **Virology Journal**, v. 21, n. 1, p. 160, 22 jul. 2024.

ZIMMERMANN, P. *et al.* VP1 sequences of German porcine parvovirus isolates define two genetic lineages. **The Journal of General Virology**, v. 87, n. Pt 2, p. 295–301, fev. 2006.

# 5. DISCUSSÃO

Desde sua identificação na Alemanha em 1965 (Dunne et al., 1965; Mayr; Mahnel, 1964, 1966), o PPV1 se mantém como um dos patógenos mais importantes para a suinocultura mundial, sendo continuamente detectado em diferentes regiões do planeta. Sua ação provoca falhas reprodutivas associadas à síndrome SMEDI, impactando a eficiência reprodutiva e gerando perdas econômicas para os produtores. Como forma de proteger o rebanho de falhas reprodutivas, as primeiras vacinas foram introduzidas em meados da década de 1980 (Brown; Whitacre; Robison, 1987). Contudo, a evolução contínua do PPV1 levou ao surgimento de variantes antigênicas, como a cepa 27a, variante com capacidade de escapar de respostas imunes induzidas pelas vacinas convencionais baseadas na cepa NADL-2 (Vereecke et al., 2022). Estudos recentes demonstraram, ainda, reduções de até 100 vezes nos títulos de anticorpos neutralizantes frente a estas cepas heterólogas (Cho et al., 2022; Vereecke et al., 2022). Além disso, foi comprovado que a coinfecção com outros patógenos como o circovírus suíno tipo 2 (Altherr et al., 2003; Zeeuw et al., 2007), exacerbam o quadro clínico, ampliando a complexidade no manejo da doença.

No Brasil, a maior parte da produção suinícola está concentrada na região Sul, abrangendo os estados de Santa Catarina, Rio Grande do Sul e Paraná, que juntos somam aproximadamente 61% das matrizes do país (ABCS, 2024). Devido à grande densidade da população de suínos, estas regiões são importantes para a compreensão da evolução e dos impactos econômicos causados pelo PPV1, cuja soroprevalência em granjas comerciais permanece acima de 80% desde 1993 (Bersano et al., 1993; Rodriguez et al., 2003; Trindade et al., 2011). Além disso, o vírus também vem sendo detectado em 68% das amostras de fetos mumificados, vinculando-o diretamente às falhas reprodutivas (Herdt et al., 2019). Do ponto de vista prático, a disseminação da infecção indica que o PPV1 provavelmente contribui significativamente para a incidência do aparecimento de natimortos e fetos mumificados nos rebanhos brasileiros. Além disso, relatos indicam uma incidência crescente de casos de PPV1, correspondendo a aproximadamente 30 a 40% dos natimortos com diagnóstico positivo, bem como a adoção de práticas vacinais esporádicas, frequentemente iniciadas apenas durante a ocorrência de surtos (Streck, 2020). Em suma, ressaltamos que o PPV1 é ubíquo em granjas suinícolas brasileiras.

Nossas análises, combinadas com estudos anteriores, comprovam que o PPV1 está evoluindo rapidamente, precisamente a uma taxa de substituição de nucleotídeos estimada em $1{,}57 \times 10^{-5}$ substituições/sítio/ano, valor comparável ao observado em muitos vírus de RNA, o

que implica uma rápida renovação genética (Af et al., 2011). Ademais, análises subsequentes sugerem que grande parte da diversificação antigênica ocorreu nas últimas décadas, coincidindo com a intensificação da produção suinícola e a ampliação das práticas de vacinação (Af et al., 2011; Vereecke et al., 2022). Essa rápida evolução indica que novas variantes do PPV1 podem surgir em intervalos de tempo relativamente curtos. Em termos de dinâmica populacional, uma alta taxa de substituição, combinada com grandes rebanhos de suínos, pode impulsionar significativamente a diversidade viral, conforme observado através do gráfico BSP (*Bayesian Skyline Plot*) e da nossa árvore filogenética.

No panorama vacinal brasileiro, desde a década de 1980 a grande maioria das granjas suinícolas utilizam vacinas monovalentes inativadas baseadas na cepa NADL-2, em razão de sua segurança e imunogenicidade estáveis (Moraes; Costa, 2007; Gava et al., 2009; Rocha, 2010). Porém, recentemente foi demonstrado que a vacina inativada (NADL-2) pode reduzir sinais clínicos, mas não previne infecção e replicação viral em fetos, mesmo contra variantes semelhantes à NADL-2, nas quais continham mutações nas posições 436 e 565 da VP2 (Foerster et al., 2016; Jóźwik et al., 2009; Serena et al., 2019). Embora alguns rebanhos possam apresentar elevadas taxas de anticorpos contra o PPV1 (Trindade et al., 2011), falhas na cobertura vacinal e a baixa imunidade podem favorecer a emergência de novas variantes virais. Por exemplo, na Europa foi observado um aumento de casos SMEDI positivos para PPV1, de 0.5% para 9.2% ao longo de dois anos, mesmo diante da adoção de programas de vacinação (Vereecke et al., 2022). Tendências semelhantes poderiam se manifestar em território nacional, caso surjam variantes capazes de driblar a resposta imunológica induzidas pelas vacinas atuais.

Do ponto de vista filogenético, a variabilidade do PPV1 no Brasil pode seguir padrões similares aos observados globalmente. Essa hipótese foi reforçada em um estudo anterior onde pelo menos duas linhagens virais co-circulantes em isolados de campo foram identificadas (Soares et al., 2003), nos quais foram detectados 26 sítios polimórficos no gene VP2 de 29 amostras de diferentes regiões do país, 22 deles situados na superfície externa do capsídeo, sugerindo diferenças antigênicas. Neste contexto, a existência de múltiplos clados, assim como o posicionamento da cepa brasileira (BRA-UEL/GO-1074/2018) dentro da linhagem PPV1b, indica que a imunidade adquirida por um único grupo genético (ex. NADL-2, pertencente a linhagem PPV1c), pode não conferir proteção contra variantes que pertencem aos demais clados, configurando escape vacinal. No entanto, ressaltamos que estudos adicionais são necessários para validar essa hipótese.

É importante salientar que nossa abordagem apresentou limitações significativas. A análise de 203 sequências completas do gene VP2 evidenciou um viés de amostragem, uma vez

que a maioria dos isolados são provenientes do continente europeu e asiático. Este viés interfere em quaisquer análises filogeográficas, razão pela qual optamos não a realizar. Partindo da premissa de que nosso banco de dados genômico é majoritariamente formado por amostras da Europa e Ásia, nossa inferência populacional tende a refletir em grande parte a realidade desses continentes. Ainda assim, considerando a natureza global da suinocultura, podemos prever que as características intrínsecas do PPV1 se mantêm independentemente do local de onde as amostras foram isoladas. Isso abre espaço para investigações direcionadas ao contexto da suinocultura brasileira, permitindo correlacionar nossos achados com as condições específicas do setor no país.

Com o objetivo de superar parte dos desafios relacionados à coleta e organização de um grande volume de dados genômicos, durante nossa pesquisa desenvolvemos o software SeqFinder v1.0. A ferramenta tem como objetivo automatizar a busca, download e a análise preliminar de sequências genômicas junto ao banco de dados do NCBI (National Center for Biotechnology Information), viabilizando o processamento sistemático e reproduzível de grandes volumes de dados. O SeqFinder permite filtrar sequências por organismo específico e exportar tanto as sequências (genomas completos e genes individuais no formato FASTA) quanto seus metadados (número de acesso, nome do isolado, nome do organismo, país de origem, data de coleta, genes disponíveis, fonte de isolamento, organismo hospedeiro e autoria) para uma planilha em Excel, organizando as informações para posterior análise.

Diante da rápida evolução viral, da pressão seletiva vacinal e da contínua circulação de múltiplas linhagens virais, é evidente a necessidade de reavaliar as estratégias de controle do PPV1. É fundamental a implementação de uma vigilância periódica das linhagens virais circulantes e o desenvolvimento de abordagens de vacinação atualizadas e mais abrangentes, já que programas de vacinação convencionais baseados em cepas antigas podem se tornar obsoletos diante da elevada variabilidade genética viral. Desta forma, estratégias vacinais de próxima geração, como o desenvolvimento de vacinas multi-epítopo, surgem como uma alternativa promissora para mitigar os riscos de falhas reprodutivas relacionadas à síndrome SMEDI. Diferentemente das vacinas convencionais baseadas em vírus atenuados ou inativados, que induzem uma resposta imune restrita à cepa vacinal, as vacinas multi-epítopo são projetadas *in silico* para incluir múltiplas regiões imunogênicas de epítopos B e T conservados e com alta afinidade por alelos do complexo de antígenos leucocitários suínos (SLA), promovendo uma resposta imune mais robusta e de amplo espectro (Chen; Li; Wang, 2024). Recentemente, o desenvolvimento de diversas vacinas multi-epítopo voltadas para doenças virais em suínos tem ganhado destaque (Bai et al., 2024; Chen; Li; Wang, 2024; Fagbohun et al., 2024; Fajardo et

al., 2025; Simbulan et al., 2024; Sira et al., 2025; Ullah et al., 2025; Venkateswaran et al., 2025; Zhu et al., 2024). Contudo, até o momento, não há registros do desenvolvimento de uma vacina que utilizam essa abordagem contra o PPV1. Neste contexto, os resultados de nossas pesquisas podem fornecer insumos para o desenvolvimento de uma vacina eficaz e inédita.

## 6. CONCLUSÃO

- A taxa evolutiva de $1{,}57 \times 10^{-5}$ substituições/sítio/ano destaca a persistência da alta capacidade de mutação do PPV1 e reforça a importância de atualizar regularmente os imunógenos para garantir proteção eficaz.
- A análise filogenética global do PPV1 revelou a existência de cinco linhagens genéticas distintas (PPV1a-PPV1e), evidenciando sua alta diversidade genética.
- A análise da dinâmica populacional viral exibiu três fases evolutivas (1863–1960, 1960–1968 e 1968–2021).
- Dois sítios da proteína VP2 (sítios 320 e 436) foram detectados sob forte pressão de seleção positiva, indicando que a variabilidade de aminoácidos nestas regiões está associada a mecanismos adaptativos.
- Modelagens estruturais revelaram epítopos promissores de alta afinidade aos alelos SLA-1*08:01, SLA-2*02:01 e SLA-3*04:01.

# 7. PERSPECTIVAS

O futuro do controle do PPV1 aponta para o constante monitoramento do patógeno e desenvolvimento de vacinas de nova geração, utilizando ferramentas de bioinformática. As vacinas multi-epitopo representam uma estratégia promissora para superar as limitações das vacinas atuais, oferecendo vantagens na segurança e potencial para induzir respostas imunes tanto humorais quanto celulares (Chen; Li; Wang, 2024).

Além disso, é importante ressaltar a importância da continuidade deste trabalho para confirmar os resultados obtidos até agora. As perspectivas futuras incluem:

- Detectar e sequenciar variantes circulantes em diferentes regiões do Brasil, com o objetivo de mapear a diversidade viral local e monitorar o surgimento de linhagens emergentes;
- Projetar, sintetizar e testar formulações vacinais contendo combinações de epítopos B e T identificados, incluindo o peptídeo $^{344}$FEYSNGGPF$^{352}$ em modelos pré-clínicos;
- Realização de testes *in vitro* (neutralização viral, ELISA) e *in vivo* (modelo suíno) da vacina projetada, buscando avaliar a eficácia da proteção imune oferecida;
- Construir modelos que simulem diferentes estratégias de vacinação (intervalos, cobertura e mistura de antígenos), avaliando o impacto sobre a dinâmica de variantes e construindo políticas de revacinação atualizadas;
- Patentear e promover parcerias com a indústria para produção e comercialização da vacina produzida.

# 8. REFERÊNCIAS


STRECK, A. F. *et al.* High rate of viral evolution in the capsid protein of porcine parvovirus. **The Journal of general virology**, v. 92, n. Pt 11, nov. 2011.

ALARCÓN, L. V.; ALLEPUZ, A.; MATEU, E. Biosecurity in pig farms: a review. **Porcine Health Management**, v. 7, n. 1, p. 5, 4 jan. 2021.

ÁLVAREZ-CARRETERO, S.; KAPLI, P.; YANG, Z. Beginner's Guide on the Use of PAML to Detect Positive Selection. **Molecular Biology and Evolution**, v. 40, n. 4, p. msad041, 25 abr. 2023.

ANISIMOVA, M.; BIELAWSKI, J. P.; YANG, Z. Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution. **Molecular Biology and Evolution**, v. 18, n. 8, p. 1585–1592, ago. 2001.

ANISIMOVA, M.; NIELSEN, R.; YANG, Z. Effect of Recombination on the Accuracy of the Likelihood Method for Detecting Positive Selection at Amino Acid Sites. **Genetics**, v. 164, n. 3, p. 1229–1236, 1 jul. 2003.

BAI, Y. *et al.* Designing a Candidate Multi-Epitope Vaccine against Transmissible Gastroenteritis Virus Based on Immunoinformatic and Molecular Dynamics. **International Journal of Molecular Sciences**, v. 25, n. 16, p. 8828, jan. 2024.

BARTHASSON, D. L. *et al.* Ocorrência de infecção por parvovírus suíno e gastrenterite transmissível em suínos, criados de forma extensiva, em Goiás. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 61, p. 1227–1229, out. 2009.

BELTRAN-ALCRUDO, D. *et al.* Transboundary spread of pig diseases: the role of international trade and travel. **BMC veterinary research**, v. 15, n. 1, p. 64, 22 fev. 2019.

BERGERON, J.; HÉBERT, B.; TIJSSEN, P. Genome organization of the Kresse strain of porcine parvovirus: identification of the allotropic determinant and comparison with those of NADL-2 and field isolates. **Journal of Virology**, v. 70, n. 4, p. 2508–2515, abr. 1996.

BERGERON, J.; MENEZES, J.; TIJSSEN, P. Genomic organization and mapping of transcription and translation products of the NADL-2 strain of porcine parvovirus. **Virology**, v. 197, n. 1, p. 86–98, nov. 1993.

BOLT, D. M. *et al.* Non-suppurative myocarditis in piglets associated with porcine parvovirus infection. **Journal of Comparative Pathology**, v. 117, n. 2, p. 107–118, ago. 1997.

BOTERO, O.; MARTINAT-BOTTÉ, F.; BARITEAU, F. Use of ultrasound scanning in swine for detection of pregnancy and some pathological conditions. **Theriogenology**, v. 26, n. 3, p. 267–278, 1 set. 1986.

BOUCKAERT, R. *et al.* BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. **PLOS Computational Biology**, v. 15, n. 4, p. e1006650, 8 abr. 2019.

BROWN, T. T.; WHITACRE, M. D.; ROBISON, O. W. Use of an inactivated vaccine for prevention of parvovirus-induced reproductive failure in gilts. **Journal of the American Veterinary Medical Association**, v. 190, n. 2, p. 179–181, 15 jan. 1987.

CABI. porcine parvovirus infection. **CABI Compendium**, v. CABI Compendium, p. 68583, 21 nov. 2019.

CADAR, D. *et al.* Phylogeny and evolutionary genetics of porcine parvovirus in wild boars. **Infection, Genetics and Evolution**, v. 12, n. 6, p. 1163–1171, 1 ago. 2012.

CARTWRIGHT, S. F.; LUCAS, M.; HUCK, R. A. A small haemagglutinating porcine DNA virus. I. Isolation and properties. **Journal of Comparative Pathology**, v. 79, n. 3, p. 371–377, jul. 1969.

CARTWRIGHT, S. F.; LUCAS, M.; HUCK, R. A. A small haemagglutinating porcine DNA virus: II. Biological and serological studies. **Journal of Comparative Pathology**, v. 81, n. 1, p. 145–155, 1 jan. 1971.

CETIN, I.; ALVINO, G. Intrauterine growth restriction: implications for placental metabolism and transport. A review. **Placenta**, v. 30 Suppl A, p. S77-82, mar. 2009.

CHAPMAN, M. S.; ROSSMANN, M. G. Structure, sequence, and function correlations among parvoviruses. **Virology**, v. 194, n. 2, p. 491–508, jun. 1993.

CHEN, H. *et al.* Rapid detection of porcine parvovirus DNA by sensitive loop-mediated isothermal amplification. **Journal of Virological Methods**, v. 158, n. 1–2, p. 100–103, jun. 2009.

CHEN, S. *et al.* SYNCRIP facilitates porcine parvovirus viral DNA replication through the alternative splicing of NS1 mRNA to promote NS2 mRNA formation. **Veterinary Research**, v. 52, p. 73, 2021a.

CHEN, S. *et al.* Coatomer protein COPԐ, a novel NS1-interacting protein, promotes the replication of Porcine Parvovirus via attenuation of the production of type I interferon. **Veterinary Microbiology**, v. 261, p. 109188, 1 out. 2021b.

CHEN, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. **Acta Crystallographica Section D: Biological Crystallography**, v. 66, n. 1, p. 12–21, 1 jan. 2010.

CHEN, X. S.; STEHLE, T.; HARRISON, S. C. Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. **The EMBO Journal**, v. 17, n. 12, p. 3233–3240, 15 jun. 1998.

CHEN, X.; LI, Y.; WANG, X.. Multi-epitope vaccines: a promising strategy against viral diseases in swine. **Frontiers in Cellular and Infection Microbiology**, v. 14, 20 dez. 2024.

CHO, K. *et al.* A Plant-Produced Porcine Parvovirus 1-82 VP2 Subunit Vaccine Protects Pregnant Sows against Challenge with a Genetically Heterologous PPV1 Strain. **Vaccines**, v. 11, n. 1, p. 54, 26 dez. 2022.

CHOI, C. S. *et al.* Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses. **Veterinary Microbiology**, v. 15, n. 1–2, p. 19–29, out. 1987.

CHOU, P. Y.; FASMAN, G. D. Prediction of the secondary structure of proteins from their amino acid sequence. **Advances in Enzymology and Related Areas of Molecular Biology**, v. 47, p. 45–148, 1978.

CHRISTIANSON, W. T. *et al.* Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. **Canadian Journal of Veterinary Research = Revue Canadienne De Recherche Veterinaire**, v. 57, n. 4, p. 262–268, out. 1993.

CHRISTIANSON, W. T. Stillbirths, Mummies, Abortions, and Early Embryonic Death. **Veterinary Clinics of North America: Food Animal Practice**, v. 8, n. 3, p. 623–639, 1 nov. 1992.

COTMORE, S. F.; TATTERSALL, P. Parvoviral Host Range and Cell Entry Mechanisms. *In*: **Advances in Virus Research**. *[S.l.]*: Academic Press, 2007. v. 70 p. 183–232.

CRUVINEL, W. M. *et al.* Immune system: Part I. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. **Revista Brasileira de Reumatologia**, v. 50, p. 434–447, ago. 2010.

DARBYSHIRE, J. H.; ROBERTS, D. H. Some respiratory virus and mycoplasma infections of animals. **Journal of Clinical Pathology. Supplement (College of Pathologists)**, v. 2, p. 61–92, 1968.

DARRIBA, D. *et al.* jModelTest 2: more models, new heuristics and parallel computing. **Nature Methods**, v. 9, n. 8, p. 772–772, ago. 2012.

DE MAIO, F. A. *et al.* Detection of porcine circovirus 2, porcine parvovirus 1, and torque teno sus virus k2a in wild boars from northeastern Patagonia, Argentina. **Archives of Virology**, v. 168, n. 8, p. 208, 18 jul. 2023.

DEE, S. *et al.* Further Evidence That Science-Based Biosecurity Provides Sustainable Prevention of Porcine Reproductive and Respiratory Syndrome Virus Infection and Improved Productivity in Swine Breeding Herds. **Animals**, v. 14, n. 17, p. 2530, jan. 2024.

DENG, H. *et al.* Isolation, characterization, and phylogenetic analysis of two new porcine parvovirus 1 isolates from Northern China. **Virus Research**, v. 339, p. 199247, 8 nov. 2023.

DEWULF, J. *et al.* Transmission of pig diseases and biosecurity in pig production. *In*: **Biosecurity in animal production and veterinary medicine: from principles to practice**. CABI Books. *[S.l.: S.n.]*. p. 295–328.

DIMITROV, I. *et al.* AllerTOP v.2--a server for in silico prediction of allergens. **Journal of Molecular Modeling**, v. 20, n. 6, p. 2278, jun. 2014.

DONEVA, N.; DIMITROV, Ivan. Viral Immunogenicity Prediction by Machine Learning Methods. **International Journal of Molecular Sciences**, v. 25, n. 5, p. 2949, jan. 2024.

DROLET, R. *et al.* Infectious agents identified in pigs with multifocal interstitial nephritis at slaughter. **Veterinary Record**, v. 150, n. 5, p. 139–143, 2002.

DRUMMOND, A. J. *et al.* Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. **Molecular Biology and Evolution**, v. 22, n. 5, p. 1185–1192, 1 maio 2005.

DRUMMOND, A. J. *et al.* Relaxed phylogenetics and dating with confidence. **PLoS biology**, v. 4, n. 5, p. e88, maio 2006.

DUFFY, S.; SHACKELTON, L. A.; HOLMES, E. C. Rates of evolutionary change in viruses: patterns and determinants. **Nature Reviews. Genetics**, v. 9, n. 4, p. 267–276, abr. 2008.

DUNNE, H. W. *et al.* Porcine reproductive failure associated with a newly identified "SMEDI" group of picorna viruses. **American Journal of Veterinary Research**, v. 26, n. 115, p. 1284–1297, nov. 1965.

EDDICKS, M. *et al.* Examination on the Occurrence of Coinfections in Diagnostic Transmittals in Cases of Stillbirth, Mummification, Embryonic Death, and Infertility (SMEDI) Syndrome in Germany. **Microorganisms**, v. 11, n. 7, p. 1675, 27 jun. 2023.

EMINI, E. A. *et al.* Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. **Journal of Virology**, v. 55, n. 3, p. 836–839, set. 1985.

FAGBOHUN, O. A. *et al.* Contriving a multi-epitope vaccine against African swine fever utilizing immunoinformatics. **Biomedical Research and Therapy**, v. 11, n. 8, p. 6642–6660, 31 ago. 2024.

FAJARDO, L. E. *et al.* Computational multi-epitope based design of a multivalent subunit vaccine against co-infecting African swine fever virus and porcine circovirus type 2 : https://doi.org/10.12982/VIS.2025.065. **Veterinary Integrative Sciences**, v. 23, n. 3, p. 1–30, 2025.

FAN, S. *et al.* Structural and Biochemical Analyses of Swine Major Histocompatibility Complex Class I Complexes and Prediction of the Epitope Map of Important Influenza A Virus Strains. **Journal of Virology**, v. 90, n. 15, p. 6625–6641, 1 ago. 2016.

FERNANDES, S.; BOISVERT, M.; TIJSSEN, P. Genetic Elements in the VP Region of Porcine Parvovirus Are Critical to Replication Efficiency in Cell Culture. **Journal of Virology**, v. 85, n. 6, p. 3025–3029, mar. 2011.

FILIPPITZI, M. E. *et al.* Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. **Transboundary and Emerging Diseases**, v. 65, n. 2, p. 381–398, 2018.

FOERSTER, T. *et al.* An inactivated whole-virus porcine parvovirus vaccine protects pigs against disease but does not prevent virus shedding even after homologous virus challenge. **Journal of General Virology**, v. 97, n. 6, p. 1408–1413, 2016.

FRANZO, G. *et al.* A Phylogeographic Analysis of Porcine Parvovirus 1 in Africa. **Viruses**, v. 15, n. 1, p. 207, jan. 2023.

FREDRIKSSON-AHOMAA, M. Wild Boar: A Reservoir of Foodborne Zoonoses. **Foodborne Pathogens and Disease**, v. 16, n. 3, p. 153–165, mar. 2019.

GAO, L. A. *et al.* Prokaryotic innate immunity through pattern recognition of conserved viral proteins. **Science**, v. 377, n. 6607, p. eabm4096, 12 ago. 2022.

GAVA, D. *et al.* Atualização sobre a parvovirose na suinocultura. **Acta Scientiae Veterinariae**, v. 37, n. 1, p. s105–s115, 2009.

GAVA, D. *et al.* Dynamics of vanishing of maternally derived antibodies of Ungulate protoparvovirus 1 suggests an optimal age for gilts vaccination. **Tropical Animal Health and Production**, v. 49, n. 5, p. 1085–1088, 1 jun. 2017.

GOUVEIA, A. M. G. Alterações reprodutivas e prevalência de anticorpos inibidores da hemoaglutinação para o parvovírus suino no estado de Minas Gerais. 23 abr. 1982.

HADDAD, J. J.; ABDEL-KARIM, N. E. NF-κB cellular and molecular regulatory mechanisms and pathways: therapeutic pattern or pseudoregulation? **Cellular Immunology**, v. 271, n. 1, p. 5–14, 2011.

HARBISON, C. E.; CHIORINI, J. A.; PARRISH, C. R. The parvovirus capsid odyssey: from the cell surface to the nucleus. **Trends in Microbiology**, v. 16, n. 5, p. 208–214, maio 2008.

HAYDEN, M. S.; GHOSH, S. NF-κB in immunobiology. **Cell Research**, v. 21, n. 2, p. 223–244, fev. 2011.

HELFRICH, P. *et al.* TreeAnnotator: Versatile Visual Annotation of Hierarchical Text Relations. *In*: CALZOLARI, Nicoletta *et al.* (orgs.). LREC 2018. **Proceedings of the Eleventh International Conference on Language Resources and Evaluation (LREC 2018)**. Miyazaki, Japan: European Language Resources Association (ELRA), maio 2018. Disponível em: <https://aclanthology.org/L18-1308>. Acesso em: 17 set. 2024

HELKE, K. L. *et al.* Chapter 16 - Biology and Diseases of Swine. *In*: FOX, James G. *et al.* (Orgs.). **Laboratory Animal Medicine (Third Edition)**. American College of Laboratory Animal Medicine. Boston: Academic Press, 2015. p. 695–769.

HEO, L; PARK, H.; SEOK, C. GalaxyRefine: protein structure refinement driven by side-chain repacking. **Nucleic Acids Research**, v. 41, n. Web Server issue, p. W384–W388, jul. 2013.

HERDT, G. *et al.* High prevalence of porcine circovirus 2, porcine parvovirus, and pathogenic leptospires in mummified swine fetuses in Southern Brazil. **Ciência Rural**, v. 49, p. e20180965, 11 abr. 2019.

JAKOV, N. *et al.* Genetic Analysis and Distribution of Porcine Parvoviruses Detected in the Organs of Wild Boars in Serbia. **Acta Veterinaria**, v. 71, n. 1, p. 32–46, 28 fev. 2021.

JESPERSEN, M. C. *et al.* BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. **Nucleic Acids Research**, v. 45, n. Web Server issue, p. W24–W29, 3 jul. 2017.

JIN, X. *et al.* Porcine parvovirus nonstructural protein NS1 activates NF-κB and it involves TLR2 signaling pathway. **Journal of Veterinary Science**, v. 21, n. 3, p. e50, maio 2020.

JOHNSON, R. H.; COLLINGS, D. F. Transplacental Infection of Piglets with a Porcine Parvovirus. **Research in Veterinary Science**, v. 12, n. 6, p. 570–572, 1 nov. 1971.

JOHNSON, R. H.; DONALDSON-WOOD, C.; ALLENDER, U. Observations on the epidemiology of porcine parvovirus. **Australian Veterinary Journal**, v. 52, n. 2, p. 80–84, fev. 1976.

JOO, H. S.; JOHNSON, R. H. Serological responses in pigs vaccinated with inactivated porcine parvovirus. **Australian Veterinary Journal**, v. 53, n. 11, p. 550–552, nov. 1977.

JOSEPH, A. P. *et al.* Atomic model validation using the CCP-EM software suite. **Acta Crystallographica. Section D, Structural Biology**, v. 78, n. Pt 2, p. 152–161, 1 fev. 2022.

JÓŹWIK, A. *et al.* Vaccination against porcine parvovirus protects against disease, but does not prevent infection and virus shedding after challenge infection with a heterologous virus strain. **The Journal of General Virology**, v. 90, n. Pt 10, p. 2437–2441, out. 2009.

JUSZKIEWICZ, M. *et al.* Effectiveness of Chemical Compounds Used against African Swine Fever Virus in Commercial Available Disinfectants. **Pathogens**, v. 9, n. 11, p. 878, 24 out. 2020.

KADEN, V. *et al.* Retrospective serological survey on selected viral pathogens in wild boar populations in Germany. **European Journal of Wildlife Research**, v. 55, n. 2, p. 153–159, 1 abr. 2009.

KARPLUS, P. A.; SCHULZ, G. E. Prediction of chain flexibility in proteins. **Naturwissenschaften**, v. 72, n. 4, p. 212–213, 1 abr. 1985.

KENNEDY, S. *et al.* Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. **Journal of Comparative Pathology**, v. 122, n. 1, p. 9–24, jan. 2000.

KIM, S. W. *et al.* - Invited Review - Current status of global pig production: an overview and research trends. **Animal Bioscience**, v. 37, n. 4, p. 719–729, abr. 2024.

KOLASKAR, A. S.; TONGAONKAR, P. C. A semi-empirical method for prediction of antigenic determinants on protein antigens. **FEBS letters**, v. 276, n. 1–2, p. 172–174, 10 dez. 1990.

KRESSE, J. I. *et al.* Parvovirus infection in pigs with necrotic and vesicle-like lesions. **Veterinary Microbiology**, v. 10, n. 6, p. 525–531, 1 dez. 1985.

LAGER, K. M.; MENGELING, W. L. Porcine parvovirus associated with cutaneous lesions in piglets. **Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc**, v. 6, n. 3, p. 357–359, jul. 1994.

LAMIABLE, A. *et al.* PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. **Nucleic Acids Research**, v. 44, n. Web Server issue, p. W449–W454, 8 jul. 2016.

LI, J. *et al.* A Systematic Investigation Unveils High Coinfection Status of Porcine Parvovirus Types 1 through 7 in China from 2016 to 2020. **Microbiology Spectrum**, v. 9, n. 3, p. e0129421, 22 dez. 2021.

LING, Z. *et al.* A Subunit Vaccine Based on the VP2 Protein of Porcine Parvovirus 1 Induces a Strong Protective Effect in Pregnant Gilts. **Vaccines**, v. 11, n. 11, p. 1692, 5 nov. 2023.

LIU, Q. *et al.* Performance of Akaike Information Criterion and Bayesian Information Criterion in Selecting Partition Models and Mixture Models. **Systematic Biology**, v. 72, n. 1, p. 92–105, 1 jan. 2023.

LÓPEZ-ASTACIO, R. A. *et al.* The Structures and Functions of Parvovirus Capsids and Missing Pieces: the Viral DNA and Its Packaging, Asymmetrical Features, Nonprotein Components, and Receptor or Antibody Binding and Interactions. **Journal of Virology**, v. 97, n. 7, p. e00161-23, 27 jun. 2023.

LÜ, J. *et al.* A slide latex agglutination test for the rapid detection of antibodies in serum against porcine parvovirus. **Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health**, v. 53, n. 2, p. 59–61, mar. 2006.

LUCAS, M. H.; CARTWRIGHT, S. F.; WRATHALL, A. E. Genital infection of pigs with porcine parvovirus. **Journal of Comparative Pathology**, v. 84, n. 3, p. 347–350, jul. 1974.

MA, X. *et al.* Ferulic Acid Protects against Porcine Parvovirus Infection-Induced Apoptosis by Suppressing the Nuclear Factor-κB Inflammasome Axis and Toll-Like Receptor 4 via Nonstructural Protein 1. **Evidence-Based Complementary and Alternative Medicine**, v. 2020, n. 1, p. 3943672, 2020.

MA, X. *et al.* Phytochemical Constituents of Propolis Flavonoid, Immunological Enhancement, and Anti-porcine Parvovirus Activities Isolated From Propolis. **Frontiers in Veterinary Science**, v. 9, 8 abr. 2022.

MACLACHLAN, N. James; DUBOVI, Edward J. **Fenners Veterinary Virology (pp. 269-297)**. Cambridge, USA: Academic Press Elsevier, , 2017.

MAES, D. G. D. *et al.* A critical reflection on intensive pork production with an emphasis on animal health and welfare. **Journal of Animal Science**, v. 98, n. Suppl 1, p. S15–S26, 18 ago. 2020.

MARTIN, D. P. *et al.* RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. **Virus Evolution**, v. 7, n. 1, p. veaa087, 12 abr. 2020.

MAYR, A.; MAHNEL, H. Cultivation of hog cholera virus in pig kidney cultures with cytopathogenic effect. **Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale**, v. 195, n. 2, p. 157–166, 1964.

MAYR, A.; MAHNEL, H. [Further studies on the cultivation of swine plague virus in cell cultures with a cytopathogenic effect]. **Zentralblatt Fur Bakteriologie, Parasitenkunde, Infektionskrankheiten Und Hygiene. 1. Abt. Medizinisch-Hygienische Bakteriologie, Virusforschung Und Parasitologie. Originale**, v. 199, n. 4, p. 399–407, abr. 1966.

MCKILLEN, J. *et al.* Molecular beacon real-time PCR detection of swine viruses. **Journal of Virological Methods**, v. 140, n. 1–2, p. 155–165, mar. 2007.

MENG, X. J.; LINDSAY, D. S.; SRIRANGANATHAN, N. Wild boars as sources for infectious diseases in livestock and humans. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 364, n. 1530, p. 2697–2707, 27 set. 2009.

MENGELING, W. L. Prenatal infection following maternal exposure to porcine parvovirus on either the seventh or fourteenth day of gestation. **Canadian Journal of Comparative Medicine: Revue Canadienne De Medecine Comparee**, v. 43, n. 1, p. 106–109, jan. 1979.

MENGELING, W. L. *et al.* Efficacy of an inactivated virus vaccine for prevention of porcine parvovirus-induced reproductive failure. **American Journal of Veterinary Research**, v. 40, n. 2, p. 204–207, fev. 1979.

MENGELING, W. L.; CUTLIP, R. C. Reproductive Disease Experimentally Induced by Exposing Pregnant Gilts to Porcine Parvovirus. 1 dez. 1976.

MENGELING, W. L.; LAGER, K. M.; VORWALD, A. C. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. **Animal Reproduction Science**, v. 60–61, p. 199–210, 2 jul. 2000.

MENGELING, W. L.; PAUL, P. S. Interepizootic survival of porcine parvovirus. 1 jun. 1986.

MENGELING, W. L.; PAUL, P. S.; BROWN, T. T. Transplacental infection and embryonic death following maternal exposure to porcine parvovirus near the time of conception. **Archives of Virology**, v. 65, n. 1, p. 55–62, 1 mar. 1980.

MENGELING, W. L.; RIDPATH, J. F.; VORWALD, A. C. Size and Antigenic Comparisons among the Structural Proteins of Selected Autonomous Parvoviruses. **Journal of General Virology**, v. 69, n. 4, p. 825–837, 1988.

MÉSZÁROS, I. *et al.* The SAT Protein of Porcine Parvovirus Accelerates Viral Spreading through Induction of Irreversible Endoplasmic Reticulum Stress. **Journal of Virology**, v. 91, n. 16, p. e00627-17, 15 ago. 2017a.

MÉSZÁROS, I. *et al.* Biology of Porcine Parvovirus (Ungulate parvovirus 1). **Viruses**, v. 9, n. 12, p. 393, dez. 2017b.

MONTAGNARO, S. *et al.* Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (Sus scrofa) in Campania Region, Italy. **Journal of Wildlife Diseases**, v. 46, n. 1, p. 316–319, jan. 2010.

MORÓN, G. *et al.* CD8alpha- CD11b+ dendritic cells present exogenous virus-like particles to CD8+ T cells and subsequently express CD8alpha and CD205 molecules. **The Journal of Experimental Medicine**, v. 195, n. 10, p. 1233–1245, 20 maio 2002.

MOROÑO, R.; ALEJANDRO, David. **Creation, refinement, and evaluation of conformational ensembles of proteins using the Torsional Network Model**. masterThesis—*[S.l.: S.n.]*.

MORRIS, G. M. *et al.* AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. **Journal of computational chemistry**, v. 30, n. 16, p. 2785–2791, dez. 2009.

NARITA, M. *et al.* Histopathological changes of the brain in swine fetuses naturally infected with procine parvovirus. **National Institute of Animal Health Quarterly**, v. 15, n. 1, p. 24–28, 1975.

NGUYEN, L. *et al.* IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. **Molecular Biology and Evolution**, v. 32, n. 1, p. 268–274, 1 jan. 2015.

NIESTEN, E.; RAYMAEKERS, J.; SEGERS, Y.. **Lekker dier!? Dierlijke productie en consumptie in de 19de en 20ste eeuw.** Leuven: CAG Centrum voor agrarische geschiedenis, 2003.

OH, W. *et al.* Perspectives on the Evolution of Porcine Parvovirus. **Viruses**, v. 9, n. 8, p. 196, ago. 2017.

ORAVEERAKUL, K.; CHOI, C. S.; MOLITOR, T. W. Restriction of porcine parvovirus replication in nonpermissive cells. **Journal of Virology**, v. 66, n. 2, p. 715–722, fev. 1992.

ORAVEERAKUL, K.; CHOI, C. S.; MOLITOR, T. W. Tissue tropisms of porcine parvovirus in swine. **Archives of Virology**, v. 130, n. 3, p. 377–389, 1 set. 1993.

OTAKE, S.; YOSHIDA, M.; DEE, S.. A Review of Swine Breeding Herd Biosecurity in the United States to Prevent Virus Entry Using Porcine Reproductive and Respiratory Syndrome Virus as a Model Pathogen. **Animals**, v. 14, n. 18, p. 2694, jan. 2024.

O'TOOLE, Á.; AZIZ, A.; MALONEY, D.. Publication-ready single nucleotide polymorphism visualization with snipit. **Bioinformatics (Oxford, England)**, v. 40, n. 8, p. btae510, 2 ago. 2024.

OUH, I. *et al.* Prevalence of Porcine Parvovirus 1–6 Detected in South Korean Domestic Pigs. **Archives of Microbiology & Immunology**, v. 8, n. 3, p. 397–409, 5 set. 2024.

PACINI, M. I. *et al.* Detection and Characterization of Viral Pathogens Associated with Reproductive Failure in Wild Boars in Central Italy. **Animals**, v. 11, n. 2, p. 304, fev. 2021.

PARK, G. *et al.* Genetic analysis of porcine parvoviruses detected in South Korean wild boars. **Archives of Virology**, v. 166, n. 8, p. 2249–2254, 1 ago. 2021.

PARKER, J. M.; GUO, D.; HODGES, R. S. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. **Biochemistry**, v. 25, n. 19, p. 5425–5432, 23 set. 1986.

PARTHIBAN, S. *et al.* Molecular detection of porcine parvovirus 1–associated reproductive failure in southern India. **Tropical Animal Health and Production**, v. 54, n. 3, p. 195, 2 jun. 2022.

PAUL, P. S.; MENGELING, W. L. Evaluation of a modified live-virus vaccine for the prevention of porcine parvovirus-induced reproductive disease in swine. **American Journal of Veterinary Research**, v. 41, n. 12, p. 2007–2011, dez. 1980.

PAUL, P. S.; MENGELING, W. L. Oronasal and intramuscular vaccination of swine with a modified live porcine parvovirus vaccine: multiplication and transmission of the vaccine virus. **American Journal of Veterinary Research**, v. 45, n. 12, p. 2481–2485, dez. 1984.

PAUL, P. S.; MENGELING, W. L.; BROWN, T. T. Replication of porcine parvovirus in peripheral blood lymphocytes, monocytes, and peritoneal macrophages. **Infection and Immunity**, v. 25, n. 3, p. 1003–1007, set. 1979.

PAUL, P. S.; MENGELING, W. L.; PIRTLE, E. C. Duration and biological half-life of passively acquired colostral antibodies to porcine parvovirus. **American Journal of Veterinary Research**, v. 43, n. 8, p. 1376–1379, ago. 1982.

PAUL, Prem S.; MENGELING, William L. Vaccination of swine with an inactivated porcine parvovirus vaccine in the presence of passive immunity. 15 fev. 1986.

PÉNZES, J. J. *et al.* Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. **Archives of Virology**, v. 165, n. 9, p. 2133–2146, set. 2020.

PESCADOR, C. A. *et al.* Co-infection by porcine circovirus type 2 and porcine parvovirus in aborted fetuses and stillborn piglets in southern Brazil. **Pesquisa Veterinária Brasileira**, v. 27, p. 425–429, out. 2007.

POTTERTON, L. *et al.* CCP4i2: the new graphical user interface to the CCP4 program suite. **Acta Crystallographica. Section D, Structural Biology**, v. 74, n. Pt 2, p. 68–84, 1 fev. 2018.

PRESSING, A. L. *et al.* Prostaglandin-induced abortion in swine: endocrine changes and influence on subsequent reproductive activity. **American Journal of Veterinary Research**, v. 48, n. 1, p. 45–50, jan. 1987.

RABENAU, H. F. *et al.* Evaluation of a Virucidal Quantitative Carrier Test for Surface Disinfectants. **PLoS ONE**, v. 9, n. 1, p. e86128, 27 jan. 2014.

RAMBAUT, A. *et al.* Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). **Virus Evolution**, v. 2, n. 1, p. vew007, 1 jan. 2016.

RAMBAUT, A. *et al.* Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. **Systematic Biology**, v. 67, n. 5, p. 901–904, set. 2018.

RATHORE, A. S. *et al.* ToxinPred 3.0: An improved method for predicting the toxicity of peptides. **Computers in Biology and Medicine**, v. 179, p. 108926, set. 2024.

REDMAN, D. R.; BOHL, E. H.; FERGUSON, L. C. Porcine parvovirus: natural and experimental infections of the porcine fetus and prevalence in mature swine. **Infection and Immunity**, v. 10, n. 4, p. 718–723, out. 1974.

RENAULT, V.; HUMBLET, M.; SAEGERMAN, C.. Biosecurity Concept: Origins, Evolution and Perspectives. **Animals: an open access journal from MDPI**, v. 12, n. 1, p. 63, 28 dez. 2021.

REYNISSON, B. *et al.* NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. **Nucleic Acids Research**, v. 48, n. W1, p. W449–W454, 2 jul. 2020.

RHEE, C. H. *et al.* Chemical stability of active ingredients in diluted veterinary disinfectant solutions under simulated storage conditions. **Frontiers in Chemistry**, v. 11, p. 1204477, 16 jun. 2023.

RIQUELME, G. Placental chloride channels: a review. **Placenta**, v. 30, n. 8, p. 659–669, ago. 2009.

ROCHA, D. L.; ALBERTON, G. C.; SANTOS, J. L. Identificação do circovírus suíno tipo 2 e do parvovírus suíno em fetos suínos natimortos e mumificados provenientes de granjas no Brasil. **Ciência Animal Brasileira / Brazilian Animal Science**, v. 11, n. 3, p. 600–606, 2 out. 2010.

ROCHIRA, W.; AGIRRE, J. Iris: Interactive all-in-one graphical validation of 3D protein model iterations. **Protein Science : A Publication of the Protein Society**, v. 30, n. 1, p. 93–107, jan. 2021.

RODRIGUEZ, C. A. R. *et al.* Soroprevalência de anticorpos anti-parvovírus suíno em suínos do município de Uruará, estado do Pará. **Arquivos do Instituto Biológico**, v. 70, n. 4, p. 501–503, 2003.

SABIR, J. S. M. *et al.* Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. **Science (New York, N.Y.)**, v. 351, n. 6268, p. 81–84, 1 jan. 2016.

SAEKHOW, P.; SRIPHANNAM, C.; YAMSAKUL, P. Molecular characterization of porcine parvovirus 1 based on partial VP2 gene in the ovaries of Thai pigs: https://doi.org/10.12982/VIS.2022.035. **Veterinary Integrative Sciences**, v. 20, n. 2, p. 475–488, 6 jun. 2022.

SERENA, M. S. *et al.* Detection and molecular characterization of porcine parvovirus in fetal tissues from sows without reproductive failure in Argentina. **Heliyon**, v. 5, n. 11, p. e02874, 22 nov. 2019.

SHACKELTON, L. A. *et al.* Comparative analysis reveals frequent recombination in the parvoviruses. **The Journal of General Virology**, v. 88, n. Pt 12, p. 3294–3301, dez. 2007.

SIEGL, G. Properties of Parvoviruses. *In*: SIEGL, Günter (Org.). **The Parvoviruses**. Vienna: Springer, 1976. p. 6–85.

SILVA, M. S. Sequenciamento de última geração aplicado à virologia veterinária. 2015.

SIMBULAN, A. M. *et al.* Immunoinformatics-guided approach for designing a pan-proteome multi-epitope subunit vaccine against African swine fever virus. **Scientific Reports**, v. 14, n. 1, p. 1354, 16 jan. 2024.

SIMPSON, A. A. *et al.* The structure of porcine parvovirus: comparison with related viruses1. **Journal of Molecular Biology**, v. 315, n. 5, p. 1189–1198, 1 fev. 2002.

SIRA, E. M. J. S. *et al.* In silico design of multi-epitope vaccine candidate based on structural proteins of porcine reproductive and respiratory syndrome virus. **Veterinary Immunology and Immunopathology**, v. 280, p. 110881, fev. 2025.

SOARES, R. M. *et al.* Genetic variability of porcine parvovirus isolates revealed by analysis of partial sequences of the structural coding gene VP2. **The Journal of General Virology**, v. 84, n. Pt 6, p. 1505–1515, jun. 2003.

SONG, C. *et al.* Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene. **Virology Journal**, v. 7, n. 1, p. 353, 2 dez. 2010.

STRECK, A. F. Detecção e caracterização de amostras de parvovírus suíno. 2009.

STRECK, A. F. *et al.* Population dynamics and in vitro antibody pressure of porcine parvovirus indicate a decrease in variability. **Journal of General Virology**, v. 94, n. 9, p. 2050–2055, 2013.

STRECK, A. F. *et al.* A TaqMan qPCR for quantitation of *Ungulate protoparvovirus 1* validated in several matrices. **Journal of Virological Methods**, v. 218, p. 46–50, 15 jun. 2015.

STRECK, A. F.; CANAL, C. W.; TRUYEN, U. Molecular epidemiology and evolution of porcine parvoviruses. **Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases**, v. 36, p. 300–306, dez. 2015.

STRECK, A. F.; CANAL, C. W.; TRUYEN, U. Viral Fitness and Antigenic Determinants of Porcine Parvovirus at the Amino Acid Level of the Capsid Protein. **Journal of Virology**, v. 96, n. 2, p. e01198-21, 26 jan. 2022.

SUN, J. *et al.* Identification of three PPV1 VP2 protein-specific B cell linear epitopes using monoclonal antibodies against baculovirus-expressed recombinant VP2 protein. **Applied Microbiology and Biotechnology**, v. 99, n. 21, p. 9025–9036, nov. 2015.

TAMURA, K.; STECHER, G.; KUMAR, S.. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. **Molecular Biology and Evolution**, v. 38, n. 7, p. 3022–3027, 1 jul. 2021.

TECHAKRIENGKRAI, N. *et al.* Diversity of the Swine Leukocyte Antigen Class I and II in Commercial Pig Populations. **Frontiers in Veterinary Science**, v. 8, p. 637682, 30 abr. 2021.

TOO, H. L. *et al.* Evaluation of a gel diffusion precipitin test for porcine parvovirus. **Australian Veterinary Journal**, v. 60, n. 6, p. 161–165, jun. 1983.

TRINDADE, I. M. S. *et al.* Pesquisa sorológica do parvovirus suíno em reprodutoras de rebanhos suínos da Bahia. **Pubvet**, v. 5, n. 25, 2011.

TROTT, O.; OLSON, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. **Journal of computational chemistry**, v. 31, n. 2, p. 455–461, 30 jan. 2010.

TRUYEN, U.; STRECK, A. F. Parvoviruses. *In*: **Diseases of Swine**. *[S.l.]*: John Wiley & Sons, Ltd, 2019. p. 611–621.

ULLAH, S. *et al.* In Silico Designed Multi-Epitope Vaccine Based on the Conserved Fragments in Viral Proteins for Broad-Spectrum Protection Against Porcine Reproductive and Respiratory Syndrome Virus. **Veterinary Sciences**, v. 12, n. 6, p. 577, jun. 2025.

VALLET, J. L.; FREKING, B. A. Differences in placental structure during gestation associated with large and small pig fetuses. **Journal of Animal Science**, v. 85, n. 12, p. 3267–3275, dez. 2007.

VALLET, J. L.; MILES, J. R.; FREKING, B. A. Development of the pig placenta. **Society of Reproduction and Fertility Supplement**, v. 66, p. 265–279, 2009.

VARGAS, A. J. *et al.* Que decisão tomar frente a matrizes que apresentam falhas reprodutivas: elas merecem uma nova chance? 2007.

VENKATESWARAN, D. *et al.* Designing a multi-epitope vaccine against African swine fever virus using immunoinformatics approach. **Scientific Reports**, v. 15, n. 1, p. 16044, 8 maio 2025.

VEREECKE, N. *et al.* Molecular epidemiology of Porcine Parvovirus Type 1 (PPV1) and the reactivity of vaccine-induced antisera against historical and current PPV1 strains. **Virus Evolution**, v. 8, n. 1, p. veac053, 1 jan. 2022.

WANG, L. *et al.* Proteomics analysis of PK-15 cells infected with porcine parvovirus and the effect of PCBP1 on PPV replication. **Microbiology Spectrum**, v. 12, n. 6, p. e03914-23, *[S.d.]*.

WHITAKER, H. K.; NEU, S. M.; PACE, L. W. Parvovirus infection in pigs with exudative skin disease. **Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc**, v. 2, n. 3, p. 244–246, jul. 1990.

WILHELM, S. *et al.* Tissue distribution of two field isolates and two vaccine strains of porcine parvovirus in foetal organs after experimental infection of pregnant sows as determined by real-time PCR. **Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health**, v. 52, n. 7–8, p. 323–326, 2005.

WILHELM, S. *et al.* Real-time PCR protocol for the detection of porcine parvovirus in field samples. **Journal of Virological Methods**, v. 134, n. 1–2, p. 257–260, jun. 2006.

WILLIAMS, C. J. *et al.* MolProbity: More and better reference data for improved all-atom structure validation. **Protein Science: A Publication of the Protein Society**, v. 27, n. 1, p. 293–315, jan. 2018.

WLODAWER, A. Stereochemistry and Validation of Macromolecular Structures. **Methods in molecular biology (Clifton, N.J.)**, v. 1607, p. 595–610, 2017.

WORD, J. M. *et al.* Visualizing and quantifying molecular goodness-of-fit: small-probe contact dots with explicit hydrogen atoms1. **Journal of Molecular Biology**, v. 285, n. 4, p. 1711–1733, 29 jan. 1999.

XIE, H. *et al.* The epitope of the VP1 protein of porcine parvovirus. **Virology Journal**, v. 7, n. 1, p. 161, 16 jul. 2010.

YANG, J.; ZHANG, Y.. I-TASSER server: new development for protein structure and function predictions. **Nucleic Acids Research**, v. 43, n. W1, p. W174-181, 1 jul. 2015.

YANG, Z.. PAML 4: Phylogenetic Analysis by Maximum Likelihood. **Molecular Biology and Evolution**, v. 24, n. 8, p. 1586–1591, 1 ago. 2007.

YASUHARA, H. *et al.* Characterization of a parvovirus isolated from the diarrheic feces of a pig. **Nihon Juigaku Zasshi. The Japanese Journal of Veterinary Science**, v. 51, n. 2, p. 337–344, abr. 1989.

ZÁDORI, Z.; SZELEI, J.; TIJSSEN, P. SAT: A late NS protein of porcine Parvovirus. **Journal of Virology**, v. 79, n. 20, p. 13129–13138, 2005.

ZEEUW, E. J. L. *et al.* Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. **The Journal of General Virology**, v. 88, n. Pt 2, p. 420–427, fev. 2007.

ZHANG, L. *et al.* ARFGAP1 binds to classical swine fever virus NS5A protein and enhances CSFV replication in PK-15 cells. **Veterinary Microbiology**, v. 255, p. 109034, 1 abr. 2021.

ZHANG, N. *et al.* Crystal structure of swine major histocompatibility complex class I SLA-1 0401 and identification of 2009 pandemic swine-origin influenza A H1N1 virus cytotoxic T lymphocyte epitope peptides. **Journal of Virology**, v. 85, n. 22, p. 11709–11724, nov. 2011.

ZHOU, H.; YAO, G.; CUI, S.. Production and purification of VP2 protein of porcine parvovirus expressed in an insect-baculovirus cell system. **Virology Journal**, v. 7, p. 366, 10 dez. 2010.

ZHOU, Y. *et al.* Porcine parvovirus infection activates inflammatory cytokine production through Toll-like receptor 9 and NF-κB signaling pathways in porcine kidney cells. **Veterinary Microbiology**, v. 207, p. 56–62, ago. 2017.

ZHU, X. *et al.* Design of multi-epitope vaccine against porcine rotavirus using computational biology and molecular dynamics simulation approaches. **Virology Journal**, v. 21, n. 1, p. 160, 22 jul. 2024.

ZIMMERMANN, P. *et al.* VP1 sequences of German porcine parvovirus isolates define two genetic lineages. **The Journal of General Virology**, v. 87, n. Pt 2, p. 295–301, fev. 2006.

MARTINS, R .M.; ROEHE, P. M.; GUIMARÃES, I. J.; BANGEL, E. V.; GUIZZAROI, I. I.; BRUCHMAN, H. A.; VIDOR, T. Sorologia de parvovírus suíno em granjas do estado de Santa Catarina e Rio Grande do Sul. In: I Congresso Nacional de Veterinários Especialistas em Suínos, Curitiba, 1984. **Anais...** Curitiba, 1984. p. 39.

LAWSON, J. R. Infectious infertility of swine. In: Meeting of the Expert Panel of Livestock Infertility, Rome 1961, **Reports…**, p. 67, Rome, 1961.

RASBECH, N. O. A Review of the Causes of Reproductive Failure in Swine *Presented at the first International Pig Veterinary Congress, Cambridge, July 1969. **British Veterinary Journal**, v. 125, n. 12, p. 599–616, 1 dez. 1969.

DUNNE, H. W. Abortion, stillbirth, fetal death, and infectious infertility. In: Dunne, H. W. **Disease of Swine.** 3.ed. Ames: Iowa States University Press, p.836-868, 1970.

GORDON, W. A. M. e LUKE, D. An outbreak of Aujeszky's disease in swine with heavy mortality in piglets, illness in sows and death in utero. **Vet. Rec.** v. 67 n. 32 p. 591-597, 1955.

BACHMANN, P. A. Vorkommen und verbreitung Von Picodna (Parvo) - virus bein chwein. **Zentralbl Veterinarmed B**, v. 16, p. 341, 1969.

GENOV, I.; SURTMADZHIEV, K.; FODOROV, V.; MERMERSKY, K. Parvoviruses isolated from pigs in Bulgaria. **Veterinaria Sbirka**, v. 68, n. 6, p. 3-6, 1971.

MORIMOTO, T.; KURIGI, H.; MIURA, Y.; SUGIMORI, T.; FUJISAKI, Y. Isolation of Japanese encephalitis virus and hemagglutinating DNA virus from the brain of still born piglets. **National Institute of Animal Health**, v. 12, p. 127-136, 1972.

COACKLEY, W.; SMITH, V. W. Porcine parvoviruses in Western Australia. **Australian Veterinary Journal**, v. 48, n. 9, p. 536-536, 1972.

MENGELING, W. L. Porcine parvovirus: properties and prevalence of a strain isolated in the United States. **American Journal of Veterinary Research**, v. 33, n. 11, p. 2239- 2248, 1972.

JOHNSON, R. H. Isolation of swine parvovirus in Queensland. **Australian Veterinary Journal**, v. 49, n. 3, p. 157-159, 1973.

GILLICK, J. C. AN OUTBREAK OF SWINE FOETAL MUMMIFICATION ASSOCIATED WITH PORCINE PARVOVIRUS. **Australian Veterinary Journal**, v. 53, n. 2, p. 105-106, 1977.

RIVERA, E.; CONCHA, C.; BRAGANÇA, M.; GUNNARSSON, A.; KARLSSON, K. A. Acute outbreak of porcine parvovirus infection in Mozambique. **Tropical Animal Health and Production**, v. 27, n. 4, p. 217-220, 1995.

SONG, C. et al. Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene. **Virology Journal**, v. 7, p. 353-353, 2010.

MOLINI, U. et al. Viral Co-Infections of Warthogs in Namibia with African Swine Fever Virus and Porcine Parvovirus 1. **Animals**, v. 12, n. 13, p. 1697, 2022.

YOUNG, G. A.; KITCHELL, R. L.; LUEDKE, A. J.; SAUTTER, J. H. The effect of viral and other infections of the dam on fetal development in swine. I. Modified live hog cholera viruses - immunological, virological, and gross pathological studies. **Journal of the American Veterinary Medical Association**, v. 126, p. 165-171, 1955.

HORWITZ, M. S. Parvoviridae: the viruses and their replication. In: FIELDS, B.N.; KNIPE, D.M.; HOWLEY, P.M. (Org.). **Fields Virology**. Philadelphia: Lippincott-Raven, 1996. p. 2197-2229.

MENGELING, W. L. Porcine Parvovirus. In: STRAW, B. E.; D'ALLAIRE, S.; MENGELING, W. L.; TAYLOR, D. J. (Org.). **Diseases of Swine**. 8.ed. Iowa State University Press: Ames, 1999. Cap. 8, p. 119-124.

FUJISAKI, Y. Haemagglutination-inhibition test for porcine parvovirus. **Journal of the Japan Veterinary Medical Association**, v. 28, n. 3, p. 135-138, 1975

JOO, H. S.; DONALDSON-WOOD, C.R.; JOHNSON, R. H. A microneutralization test for the assay of porcine parvovirus antibody. **Archives of Virology**, v. 47, n. 4, p. 337-341, 1975.

JOO, H. S.; DONALDSON-WOOD, C. R.; JOHNSON, R. H. A standardized heamagglutination inhibition test for porcine parvovirus antibody. **Australian Veterinary Journal**, v. 52, p. 422-424, 1976.

JOO, H. S.; DONALDSON-WOOD, C. R.; JOHNSON, R. H. Observations on the pathogenesis of porcine parvovirus infection. **Archives of Virology**, v. 51, p. 123–129, 1976.

JOO, H. S.; DONALDSON-WOOD, C. R.; JOHNSON, R. H. Rapid diagnostic techniques for detection of porcine parvovirus infection in mummified foetuses. **Australian Veterinary Journal**, v. 52, n. 1, p. 51-52, 1976.

ROEHE, P.; SOBESTIANSKY, J.; BARCELLOS, D. Parvovirose. In: SOBESTIANSKY, J. & BARCELLOS, D. (Org.). **Doenças dos Suínos**. Goiânia: Cânone editorial. 2007. p. 286-293.

MENGELING, W. L., 1999. Porcine Parvovirus. In: Straw, B.E., D'allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), **Diseases of Swine**, 8th ed. Iowa State University Press, Ames, IA, pp.119-124.

THOMSON, G. R.; PROZESKY, L. Porcine parvovirus infection. In: COETZER, J. A. W.; THOMSON, G. R.; TUSTIN, R. C. (Org.). **Infectious Diseases of Livestock**, South Africa: Oxford University Press, p. 888-894, 1994.

MORAES, M. P.; COSTA, P. R. S. Parvoviridae. In: FLORES E. (Org.). **Virologia Veterinária**. Santa Maria: UFSM. 2007. p. 275-296.

TIZARD, I. **Imunologia Veterinária**. Rio de Janeiro: Elsevier Brasil, 9 ed., 2014.

BERSANO, J. G. et al. Dados preliminares sobre a ocorrência de anticorpos para o parvovírus suíno no Estado de São Paulo. **REUNIÃO ANUAL DO INSTITUTO BIOLÓGICO**, v. 6, p. 17, 1993.

ALTHERR, B., IMMERMANN, P., ETSCHMANN, B., RITZMANN, M., HEINRITZI, M., SELBITZ, H. J., TRUYEN, U. Detection of porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) in aborted fetuses. **4th International Symposium on Emerging and Re-emerging Pig Disease**, Rome, p.218-219, 2003.

# Anexos

ANEXO 1 – Script completo do software SeqFinder.

```
import pandas as pd
from Bio import Entrez
from Bio import SeqIO

# Configuração da API do NCBI
Entrez.email = "pafspontes@ucs.br"  # Substitua pelo seu e-mail

# Função para buscar sequências no NCBI com base no organismo, limite e opção de
    sequências completas ou parciais
def search_sequences(organism, limit, complete_only=False):
    # Constrói a string de pesquisa para buscar sequências do organismo específico
    search_query = f"{organism}[Organism]"
    if complete_only:
        search_query += " AND complete"

    # Realiza a pesquisa no NCBI
    handle = Entrez.esearch(db="nucleotide", term=search_query, retmax=limit)
    record = Entrez.read(handle)
    handle.close()

    return record["IdList"]

# Função para buscar informações de autores
def fetch_authors(sequence):
    authors = ", ".join(ref.authors for ref in sequence.annotations.get("references", ["*"]))
    return authors

# Função para buscar informações de hospedeiro
def get_host(sequence):
    source_features = sequence.features[0].qualifiers
    host = source_features.get("host", ["*"])[0]
    return host

# Função para buscar informações de fonte de isolamento
def fetch_isolation_source(sequence):
    source_features = sequence.features[0].qualifiers
    isolation_source = source_features.get("isolation_source", ["*"])[0]
    return isolation_source

# Função para recuperar detalhes das sequências do NCBI
def fetch_sequence_details(sequence_ids):
    sequences = []
    for sequence_id in sequence_ids:
        handle = Entrez.efetch(db="nucleotide", id=sequence_id, rettype="gb", retmode="text")
        record = SeqIO.read(handle, "genbank")
        sequences.append(record)
        handle.close()
```

```
    return sequences

# Função para formatar o nome das sequências
def format_sequence_name(sequence, gene_name):
    source_features = sequence.features[0].qualifiers
    isolate = source_features.get("isolate", source_features.get("strain", "*"))[0].strip("[]")
    country = source_features.get("country", "*")[0].strip("[]")
    collection_date = source_features.get("collection_date", "*")[0].strip("[]")
    return f"{isolate}|{gene_name}|{country}|{collection_date}"

# Função para salvar as sequências em um arquivo FASTA
def save_complete_genome_to_fasta(sequences, output_file):
    with open(output_file, "w") as fasta_file:
        for sequence in sequences:
            source_features = sequence.features[0].qualifiers
            isolate = source_features.get("isolate", source_features.get("strain", "*"))[0].strip("[]")
            country = source_features.get("country", "*")[0].strip("[]")
            collection_date = source_features.get("collection_date", "*")[0].strip("[]")
            accession = sequence.annotations.get("accessions", ["*"])[0]
            seq_name = f"{accession}|{isolate}|Complete|{country}|{collection_date}"
            fasta_file.write(f"> {seq_name}\n")
            fasta_file.write(str(sequence.seq) + "\n")

# Função para salvar as sequências em um arquivo FASTA
def save_sequences_to_fasta(sequences, output_file):
    with open(output_file, "w") as fasta_file:
        for sequence in sequences:
            for feature in sequence.features:
                if feature.type == "CDS":
                    gene_name = feature.qualifiers.get("gene", feature.qualifiers.get("product",
    ["*"]))[0]
                    gene_seq = feature.location.extract(sequence).seq
                    source_features = sequence.features[0].qualifiers
                    isolate = source_features.get("isolate", source_features.get("strain",
    "*"))[0].strip("[]")
                    country = source_features.get("country", "*")[0].strip("[]")
                    collection_date = source_features.get("collection_date", "*")[0].strip("[]")
                    accession = sequence.annotations.get("accessions", ["*"])[0]
                    seq_name = f"{accession}|{isolate}|{gene_name}|{country}|{collection_date}"
                    fasta_file.write(f"> {seq_name}\n")
                    fasta_file.write(str(gene_seq) + "\n")


# Função para extrair as informações das sequências
def extract_sequence_info(sequence, organism_name):
    source_features = sequence.features[0].qualifiers
    accession = sequence.annotations.get("accessions", ["*"])[0]
    isolate = source_features.get("isolate", source_features.get("strain", "*"))[0].strip("[]")
    country = source_features.get("country", "*")[0].strip("[]")
    collection_date = source_features.get("collection_date", "*")[0].strip("[]")
```

```
    host = source_features.get("host", "*")[0].strip("[]")  # Adiciona a informação do host
    authors = sequence.annotations.get("references", ["*"])[0].authors  # Busca o nome dos
     autores
    isolation_source = source_features.get("isolation_source", "*")[0].strip("[]")  # Busca a
     fonte de isolamento
    genes = []
    for feature in sequence.features:
        if feature.type == "CDS":
            gene_name = feature.qualifiers.get("gene", feature.qualifiers.get("product", ["*"]))[0]
            gene_seq = feature.location.extract(sequence).seq
            if gene_seq:
                genes.append(gene_name)
    genes_str = ", ".join(genes)

    # Adiciona o nome do organismo ao nome da sequência
    seq_name = f"{isolate}|{organism_name}|{genes_str}|{country}|{collection_date}"

    return {
        "Número de Acesso NCBI": accession,
        "Nome do Isolado": isolate,
        "Nome do Organismo": organism_name,  # Adiciona o nome do organismo à tabela
        "País": country,
        "Data da Coleta": collection_date,
        "Genes Disponíveis": genes_str,
        "Autores": authors,  # Adiciona o nome dos autores à tabela
        "Isolation Source": isolation_source, # Adiciona a fonte de isolamento à tabela
        "Host": host,  # Adiciona a informação do host à tabela
        "Nome da Sequência": seq_name  # Adiciona o nome da sequência à tabela
    }


# Função para salvar as sequências em um DataFrame
def save_sequences_to_dataframe(sequences):
    data = []
    for sequence in sequences:
        sequence_info = extract_sequence_info(sequence,
     sequence.annotations.get("organism"))
        data.append(list(sequence_info.values()))

    df = pd.DataFrame(data, columns=list(sequence_info.keys()))
    return df

print("Desenvolvido por Pedro Augusto F. de Sá - LDMV - Universidade de Caxias do Sul |
     Favor não compartilhar o programa sem autorização")

# Função para buscar um organismo no NCBI
def search_organism(organism):
    handle = Entrez.esearch(db="taxonomy", term=organism)
    record = Entrez.read(handle)
    handle.close()
```

```
    return record["IdList"]

# Função para obter sugestões de nomes de organismos semelhantes
def get_organism_suggestions(organism):
    handle = Entrez.espell(db="taxonomy", term=organism)
    record = Entrez.read(handle)
    handle.close()
    return record["Query"]

# Solicita ao usuário o organismo a ser buscado
while True:
    organism_to_search = input("Digite o organismo a ser buscado: ")
    organism_ids = search_organism(organism_to_search)
    if organism_ids:
        print("Organismo encontrado")
        break
    else:
        suggestions = get_organism_suggestions(organism_to_search)
        print("Organismo não encontrado. Aqui estão algumas sugestões:")
        for suggestion in suggestions[:1]:
            print(suggestion)

# Solicita ao usuário o limite de sequências a serem buscadas
limit = int(input("Digite o limite de sequências a serem buscadas: "))

# Solicita ao usuário se deseja buscar apenas sequências completas
complete_only_input = input("Deseja buscar apenas sequências completas? (S/N): ")
complete_only = complete_only_input.upper() == "S"
print("Aguarde enquanto o script busca as sequências...")

# Busca as sequências no NCBI
sequence_ids = search_sequences(organism_to_search, limit, complete_only)

# Recupera os detalhes das sequências
sequences = fetch_sequence_details(sequence_ids)

# Converta as sequências em um DataFrame
df = save_sequences_to_dataframe(sequences)

# Verifica se o usuário deseja salvar apenas uma sequência com o genoma completo ou
    separá-las por genes
output_choice = input("Deseja salvar em formato de genoma completo (C) ou separar o
    genoma em genes(G)? (C/G): ")
if output_choice.upper() == "C":
    output_file = f"{organism_to_search}_complete_genome.fasta"
    save_complete_genome_to_fasta(sequences, output_file)
    print("Genoma completo salvo com sucesso.")
else:
    output_file = f"{organism_to_search}_genes.fasta"
    save_sequences_to_fasta(sequences, output_file)
```

```
    print("Sequências de genes salvas com sucesso.")

# Verifica se o usuário deseja salvar o DataFrame em um arquivo Excel
save_excel_choice = input("Deseja salvar os dados em um arquivo Excel? (S/N): ")
if save_excel_choice.upper() == "S":
    output_excel_file = f"{organism_to_search}_sequences.xlsx"
    df.to_excel(output_excel_file, index=False)
    print(f"Os dados foram salvos com sucesso no arquivo {output_excel_file}.")
```

ANEXO 2 – Artigo configurado nas originais da revista Viruses.

*viruses*

MDPI

*Article*

# *In Silico* Insights into the Dynamic Evolution of Porcine Parvovirus Lineages and Vaccine Efficacy Evaluation

**Pedro Augusto Freire de Sá Pontes[1,*1], Nicole Amoedo Luvison[1], Rafael Sartori Flores[1], Vagner Ricardo Lunge[1], Scheila de Avila e Silva[1], André Felipe Streck[1,*2].**

[1] Laboratório de Diagnóstico em Medicina Veterinária (LDMV), Universidade de Caxias do Sul, R. Francisco Getúlio Vargas, 1130, Brazil.
[*1] Correspondence: pafspontes@ucs.br
[*2] Correspondence: afstreck@ucs.br

**Abstract:** Porcine parvovirus type 1 (PPV1) is globally recognized as the etiological agent responsible for one of the primary diseases causing reproductive failures in swine herds, known by the acronym SMEDI. Although it has been identified since the mid-1960s, PPV1 continues to be isolated worldwide, leading to significant financial losses. Vaccination remains the primary preventive measure against the virus. However, the emergence of new variants globally raises concerns regarding the efficacy of currently commercialized vaccines. This study aimed to elucidate the evolutionary dynamics of porcine parvovirus type 1 (PPV1) through a detailed bioinformatics analysis of 200 complete sequences of the gene encoding the VP2 protein, the primary structural component of the viral capsid spanning the years 1963 to 2021. The methodology involved data collection, genetic sequence analysis, followed by phylogenetic tree construction, simulation of different VP2 proteins, and prediction of positive selection sites and B and T cell epitope regions. Phylogenetic analysis revealed a total of five lineages. Two of those groups did not contain vaccine reference variants. The first group is predominantly composed of strains isolated in Europe, and the second is mainly divided between Asian and European strains. Predictions and simulations confirmed structural differences at various protein positions among the five groups identified in the phylogenetic tree and their three reference vaccine variants (NADL-2, 27a, and PPV014), which were classified as epitope regions and positive selection sites, suggesting that the strains used as vaccine references are possibly not providing protection against the new variants.

**Keywords:** PPV1; Ungulate protoparvovirus1; SMEDI; epidemiology; epitope prediction;

## 1. Introduction

Porcine parvovirus 1 (PPV1) is considered one of the most important infectious agents related to reproductive disorders in sows. It was first identified in Germany in 1965 as a contaminant of porcine primary cell cultures used for classical swine fever virus propagation **[1]**, PPV clinically manifests predominantly as SMEDI syndrome, which is an acronym for stillbirth, fetal mummification, embryonic death, and infertility. Beyond reproductive issues, PPV has been linked to cases of diarrhea, skin lesions, and joint inflammation in swine **[2]**. Although the virus has been identified since the mid-sixties, it continues to be isolated worldwide. In recent years, infection has spread widely, and the virus spreads quickly, which further aggravates its impact on the reproductive performance of sows, severely affecting the swine industry and causing huge economic losses worldwide **[3–5]**.

PPV1, currently named *Ungulate protoparvovirus 1* (https://global/taxonomy, accessed on May 21, 2024), is icosahedral-shaped, having a single-stranded, linear DNA, non-enveloped, approximately 5 kb in length **[6]**. Its genome contains two main coding regions, designated ORF1 and ORF2. ORF1 is located in the 5' region and is responsible for encoding three non-structural proteins: NS1, NS2, and NS3. ORF2, located in the 3' region, encodes three structural