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In-Silico Study to Design Foot and Mouth Disease Vaccine Candidates

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Abstract: Foot and Mouth Disease (FMD) is a viral disease that affects livestock and can cause significant economic losses. Vaccination has been recognized as the primary strategy for FMD prevention, but vaccine development, especially conventional vaccine production, has time, cost, and effectiveness limitations. Using software-based immunoinformatics methods has cost and time efficiency for simulation development and calculations in vaccine development research. This study aimed to design FMD vaccine candidates using an epitope-based in-silico approach, focusing on identifying potential epitopes of the pathogen that causes FMD. The in silico approach was used to analyze FMD virus genome sequences from UniProtKB (https://www.ebi.ac.uk/Tools/msa/clustalo) and the National Library of Medicine application (https://www.ncbi.nlm.nih.gov). Furthermore, the prediction of FMD virus epitopes was analyzed using the Immuno-Epitope database, and the prediction of peptide antigenicity levels using VaxiJen 2.0 software. The analysis identified eight potential epitope candidates, such as CSIQKR, TEFGFHPNA, EIRPMEKVRA, YEGVEL, SFARRGT, APGLPWALQGKRRGALIDFESGTV, MASLEDKGKPF, and TLPTSFNYGAI with antigen binding affinities of 2. 9424nM, 1.65nM, 1.4702nM, 1.2966nM, 1.2643nM, 1.1967nM, 1.1921nM and 1.0143nm respectively. These epitopes are expected to form the basis for developing more selective and safe peptide vaccines. By focusing on epitopes, the resulting vaccine can improve the effectiveness and safety of vaccination and accelerate the development of new vaccines for FMD prevention.

Keywords: Epitopes, vaccine, foot and mouth disease, in-silico, bioinformatic.

Introduction

Foot and Mouth Disease (FMD) is a highly contagious viral disease that significantly impacts the health of farm animals, particularly cattle, sheep, goats, and pigs, with a high morbidity rate of almost 100% (Sobrino *et al*., 2020; Sudarsono, 2022). The disease is caused by the Foot and Mouth Disease Virus (FMDV), which belongs to the genus Aphthovirus of the Picornaviridae family. In Indonesia, FMD poses a severe threat to the livestock sector, especially after a re-occurring outbreak in 2022 after more than 30 years of freedom from the disease (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2023).

Foot and Mouth Disease causes sores in the mouth and hooves of infected animals, eventually resulting in weight loss, decreased milk production, infertility, and death in severe cases (Zamroni *et al*., 2022). The disease causes substantial economic losses, especially in the livestock sector (Afriani *et al*., 2022; Rohma *et al*., 2022). An FMD outbreak in the UK in 2001 was estimated to cost more than $8 billion, including direct and indirect costs (Sumption *et al*., 2020). These losses came from the costs of outbreak control, reduced production, international trade restrictions, and culling infected and high-risk animals (Auty *et al*., 2019; Sumption *et al*., 2020). The losses caused by FMD in Indonesia are enormous. In 2022,

thousands of livestock were infected, leading to a 50 percent reduction in milk production in some areas and economic losses estimated at billions of rupiah (Purwadi & Prasetyo, 2024; Wulandari, 2024). In addition, restrictions on interprovincial trade and livestock exports have disrupted the food supply chain and reduced the income of smallholder farmers.

The economic losses caused by FMD are not limited to developed countries. Developing countries that rely heavily on the livestock sector are also significantly affected (Yadav *et al.*, 2020; Van Andel *et al*., 2021). Foot and Mouth Disease often hampers livestock development in Asia and Africa due to periodic outbreaks, reducing farmers' income and compromising food security (Gortázar *et al*., 2022; Aslam & Alkheraije, 2023). This reinforces the urgency of developing an effective and long-lasting vaccine to control the spread of FMD (Belsham, 2020; Wong *et al*., 2020).

One of the biggest challenges in FMD control is the significant antigenic variation among different FMDV strains. The virus has seven major serotypes (O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3), each with different subtypes (Mahapatra & Parida, 2018; Alhaji *et al*., 2020; Ahmar & Boj, 2021). This variation makes developing a vaccine that provides comprehensive protection against all strains difficult. In addition, current vaccines are often ineffective against new strains, requiring regular vaccine updates (Kamel & Castañeda, 2019). In Indonesia, serotypes O and A are the most common. Antigenic variation among these serotypes makes it challenging to develop a universal vaccine that can protect against all strains (Seeyo & Rukkwamsuk, 2019).

Immunoinformatics approaches, which utilize bioinformatics technology and computer simulations to predict immune responses to antigens, have emerged as a promising solution in modern vaccine development (Ribeiro *et al*., 2024). In silico studies allow researchers to identify potential epitopes on FMDV antigens that can trigger strong immune responses without conducting expensive and time-consuming laboratory experiments (Sid Ahmed *et al.*, 2022; Bhutta *et al*., 2024). This approach speeds up the vaccine development process and enables the design of more specific and efficient vaccines.

This study aims to design FMD vaccine candidates using an immunoinformatics approach to identify conservative and immunogenic epitopes among various FMDV strains, especially serotypes O and A circulating in Indonesia. This approach is expected to accelerate the development of specific and effective vaccines for the circulating virus strains to help overcome the challenges in FMD control. The results of this study can also contribute to Indonesia's readiness to deal with future FMD outbreaks and support national food security through increased livestock productivity.

Materials and Methods

The study was conducted in-silico, using computer simulations and bioinformatics data analysis. The steps in this study include:

a. Use of Specific Software and Website

The first step in this study was to obtain the protein sequences of the FMD virus from the UniProtKB database. UniProtKB (https://www.ebi.ac.uk/Tools/msa/clustalo/) provides information on various proteins from different organisms. The protein sequences of the FMD virus can be screened and downloaded from the UniProtKB URL. After the viral protein sequences were downloaded, the next step was to search the FMD virus genome through the NIH (National Library of Medicine) application to obtain complete genomic data. The complete genome data of the FMD virus can then be downloaded by accessing the NIH GenBank URL (https://www.ncbi.nlm.nih.gov/).

b. Determination of Peptide Antigenicity Level

After obtaining the FMD virus protein sequence, the next step is to determine the antigenicity level of the peptide using VaxiJen 2.0 software. VaxiJen 2.0 is a server-based antigen prediction tool that allows the determination of antigen potential from protein sequences without using three-dimensional structures (Martinelli, 2022). The steps taken were to access VaxiJen 2.0, upload the protein sequence of the FMD virus, and analyze and determine the antigenicity value of the resulting peptide.

c. Identification of Epitope Candidates

Specific steps taken to identify candidate epitopes include using bioinformatics software for epitope mapping on FMD virus antigens, evaluating the affinity of candidate epitopes using computer simulations, and determining the most potential candidate epitope based on the

resulting affinity value. The candidate epitope with an affinity value > 1.000 has the potential for peptide vaccine development.

Results and Discussion

Obtaining the protein sequences of the FMD virus from the database

The in-silico design of peptide vaccine candidates to produce antibodies against FMD virus is a computer-based approach to developing vaccines without initial laboratory experiments. In this study, the amino acid sequence of FMD virus taken from UniProtKB (https://www.unitprot.org) consists of 2332 amino acids with a molecular weight of 258.9KDa. The data search results using the UniProtKB Protein Bank application found 51 identifiers for FMD viruses in cattle serotype O that spread in the Asian region. Of the 51 identifiers, 37 viral protein domains are potential targets in vaccine development. Understanding the structure and function of these domains helps design effective vaccines that can induce a protective immune response and potentially stop the spread of the FMD virus.

In this study, the in-silico design of peptide vaccine candidates has identified 37 viral protein domains as potential targets for vaccine development. This finding aligns with previous research emphasizing the importance of targeting conserved regions of the FMD virus genome to develop broad-spectrum vaccines. For instance, Mahapatra and Parida (2018) highlighted that focusing on conserved epitopes among different serotypes could lead to more effective vaccines that offer protection across multiple FMDV strains. However, using in-silico methods to predict epitopes has also sparked debate. Some researchers argue that while in-silico predictions are efficient and cost-effective, they might sometimes translate into successful in-vivo results. When tested in animal models, Bhutta *et al.* (2024) found that specific epitopes predicted to be highly immunogenic in silico did not induce a robust immune response. This discrepancy underscores the necessity of validating in-silico findings with experimental data.

On the other hand, Ribeiro *et al.* (2024) reported success in using immunoinformatics to design a peptide vaccine for another virus, demonstrating the potential of this approach. Their study utilized similar bioinformatics tools and identified epitopes that triggered a robust immune response in silico and in subsequent laboratory tests. This successful translation from computational predictions to experimental validation supports the relevance of the findings in the current study.

Additionally, there is ongoing controversy regarding the selection of epitopes based solely on in-silico methods. Gortázar *et al.* (2022) assert that these methods might overlook critical structural aspects of epitopes, such as their three-dimensional conformation, which is crucial for effective antigen-antibody interactions. This criticism implies that integrating structural bioinformatics with epitope prediction could enhance the accuracy of vaccine candidate identification.

In this study, identifying 37 viral protein domains as potential targets is a promising step toward developing an effective FMD vaccine. However, as highlighted by the studies above, further validation through in-vitro and in-vivo experiments is essential to confirm the efficacy of these in-silico predictions. The success of the approach used in this study could contribute to a more refined strategy for developing vaccines for FMD and other viral diseases that pose significant threats to livestock. Table 1 presents five types of viral proteins with catalytic activity, allowing the FMD virus in cattle to replicate rapidly and produce many virus particles quickly.

Table 1. FMD Virus Proteins in Cattle from UniProtKB data

No	UnitProtKB Identifier	Protein name	Gen name	Organisme	length
1	AOA5QAQH4	Genom polyprotein	-	Foot and mouth disease virus O	2,322AA
2	A0A0K0QSS3	Genom polyprotein	-	Foot and mouth disease virus O	2,332 AA
3	A0A481MSG3	Genom polyprotein	-	Foot and mouth disease virus O	131 AA
4	A0A1P8SN25	Genom polyprotein	-	Foot and mouth disease virus O	177 AA

5	A0A1L6UUR9	Genom polyprotein	-	Foot and mouth disease virus O	240 AA

Alignment of two protein sequences from FMD virus serotype O with viral proteins AOA5QAQH4 and A0A0K0QSS3 using Clustal Omega showed that both protein sequences from FMD virus showed a similarity value of 96.78% (Figure 1). The 96.78% similarity value indicates that the two sequences are identical in amino acid composition, meaning no mutations or variations were detected at the amino acid level. This has several important implications in terms of structural consistency and targets for vaccines. The total similarity in amino acid sequences suggests that the protein structures of these two FMD viruses are very likely identical.

Figure 1. Alignment of polyprotein sequences of FMD virus serotype O

Consistent structure is critical for biological function and antigenic interactions, which affect the effectiveness of the immune response. Furthermore, identity in protein sequences enables the development of more focused vaccine targets. As there is no variation, vaccines designed to target these proteins can be expected to be effective against both FMD virus sequences tested. Experiments using predicted peptides could lead to the development of region-tailored DNA vaccines (Jean *et al*., 2021). Similarity scores make it easier to develop diagnostic markers to detect infections accurately. Since there is no variation in the target protein, protein-based diagnostic tests will be more reliable. In vaccine development, these results support using the analyzed proteins as candidate epitopes for peptide-based vaccines. Epitope-based vaccines targeting highly similar proteins have great potential to trigger effective immune responses, as the adaptive immune system recognizes the epitopes without variations that reduce their effectiveness.

Foot and Mouth disease vaccine development began in the late 19th century and was one of the first animal vaccines developed. Vaccination aims to immunize animals through exposure to infectious viruses (Stenfeldt, 2020). Vaccination has subsequently become the primary tool for controlling FMD globally, although current vaccines do not provide cross-protection against all serotypes or even some virus subtypes. Current vaccines consist of purified and chemically inactivated whole virus formulations due to concerns regarding the use of attenuated viruses that could restore virulence in vaccinated animals. Vaccine production requires expensive facilities and sophisticated equipment to adapt vaccine strains continuously. Other challenges include the thermolability of FMD virus particles and reliance on the cold chain to maintain stability and immunogenicity, increasing production costs (Stenfeldt, 2016).

The attenuated FMD vaccine meets World Animal Health Organization standards and effectively controls FMD in some regions. However, they provide only short-term protection (4-6 months), require revaccination at least every six months, and increase the likelihood of contamination resulting in false positive results (Casey, 2018; Mansilla, 2020). In addition, conventional FMD vaccines do not

confer sterile immunity, so vaccinated animals remain vulnerable if exposed to infectious viruses, posing a severe threat to the international trade in livestock.

Although most vaccines licensed in the veterinary field use attenuated or inactivated whole pathogens, the adaptive immune system cells do not recognize them as a whole. Still, their molecular parts are known as epitopes (Dekker *et al.*, 2020). Most pathogenic proteins are not required to achieve a complete protective response, and some of them can cause unwanted side effects such as allergy, autoimmunity, and off-target responses. These and other safety concerns are the basis for a new generation of vaccines, called "epitope-based," which consist of a minimal subset of immunodominant regions responsible for inducing positive and desirable B- and T-cell-mediated immune responses.

B lymphocytes recognize pathogenic parts (such as FMD virus particles) to produce specific antibodies, mainly preventing FMD virus from attaching to host cells (Li, 2021; Xiao *et al.*, 2021). In cattle and pigs, effective activation of B cells and antibody production depends on proper stimulation of components of the adaptive mechanism, including Th lymphocytes (also known as T helper cells or CD4+ T lymphocyte cells) (Zhi *et al.*, 2021). In this context, it is clear that epitope recognition by T cells following the antigen presentation process via MHC class II molecules stimulates Th lymphocytes to produce cytokines such as IFN-γ, which are essential in the differentiation and interaction required with B cells to develop an adaptive immune response to FMD (Liu *et al*., 2020; Miqnaqui, 2020).

Determination of peptide antigenicity level and identification of epitope candidates

Figure 2 shows the results of sequence alignment analysis using the Immuno-Epitope database (IEDB). This analysis aims to identify potential epitopes for peptide-based vaccine development. The distribution and position of epitopes on the graph identified in the FMD virus protein sequence and the colors and symbols in the figure indicate the level of antigenicity and potential of each epitope for vaccine development. Prediction of Emini surface accessibility of the proposed epitopes with a minimum propensity score of 1 and maximum of 2500. The sequence position and surface probability are directed by the X and Y axes, respectively. The area with yellow color indicates the antigenic level.

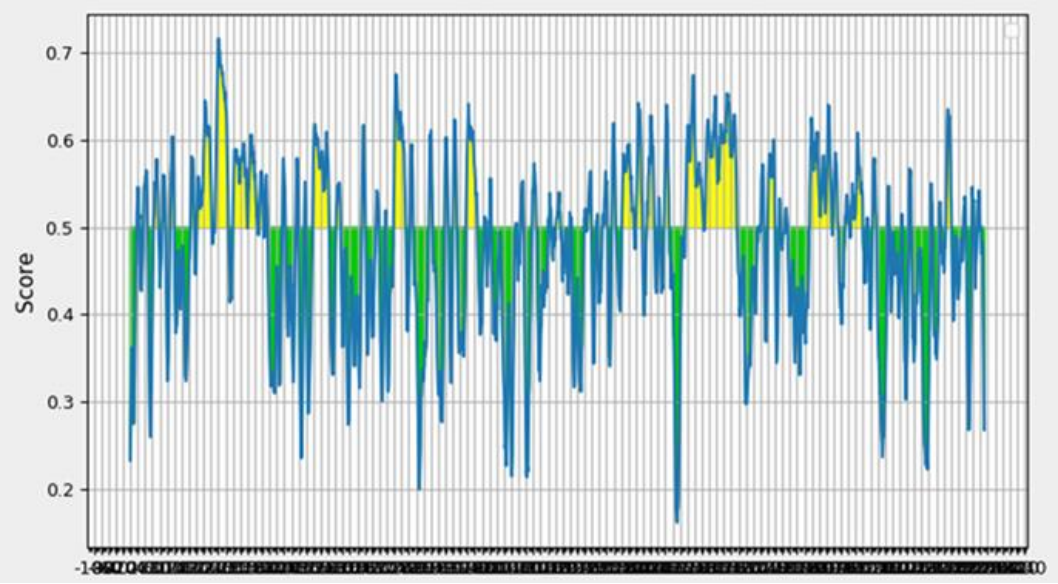

Figure 2. Epitope prediction analysis of FMD virus using IEDB

To explain the level of bond strength of the selected peptides based on the results of the IEDB graph, the antigenic potential of the peptides was evaluated using VaxiJen 2.0 software based on their antigenicity score, and vaccine candidates were then designed by selecting the antigens most likely to trigger a robust immune response. The highest score is >1, indicating that the protein or peptide is highly expected to be recognized as an antigen by the immune system (Jalalvand *et al.*, 2023).

The results of the analysis using VaxiJen 2.0 software in Table 2 show that there are eight peptides namely CSIQKR, TEFGFHPNA, EIRPMEKVRA, YEGVEL, SFARRGT, APGLPWALQGKRRGALIDFESGTV, MASLEDKGKPF, and TLPTSFNYGAI with antigen binding affinities of 2. 9424nM, 1.65nM, 1.4702nM, 1.2966nM, 1.2643nM, 1.1967nM, 1.1921nM and 1.0143nm which are potential vaccine candidates for FMD.

Table 2. Prediction results of the antigenicity level of FMD virus using VaxiJen 2.0 software

Peptide Sequence	Affinity (nM)	Probability
TLFLSRTQ	0.7726	Non-antigen
EKKTFYSRPNNH	0.2912	Non-antigen
EPFFDWVYESPENL	0.476	Non-antigen
EELTGLELHEG	0.8474	Non-antigen
LLHTGIGTASRP	0.9373	Non-antigen
DFYPWTPDP	0.7329	Non-antigen
QEPLNGGWKTKVQKRLKGAGQSS	0.6281	Non-antigen
PATGSQNQSGNTG		
QQYQNSMDTQLGDNAISGGSNEGSTDT	0.6281	Non-antigen
TSTHTTNTQNNDW		
GALLADKKTEETTLLEDRILTTRNGHTISTTQS	0.5108	Non-antigen
EDFVSGPNTSGLETRVVQAERFFKTHLFDWV	0.2076	Non-antigen
ELPTDHKG	-0.1666	Non-antigen
CSIQKR	2.9424*	Antigen
YDQYKV	0.2321	Non-antigen
FPSKEGIFPVACSDGYGGLVTTDPKTADP	0.5055	Non-antigen
AYGKVFNPPRNMLPGR		
GDVPYVTTKTDSD	0.5757	Non-antigen
GMKPPKTPEA	0.4350	Non-antigen
AYTASDA	0.4207	Non-antigen
ARTQTTSTGESADPVTTTVENYGGETQ	0.6353	Non-antigen
VQRRQHT		
VIPKDQIN	-0.1666	Non-antigen
APEAALDNT	0.1427	Non-antigen
KYGKGAVTNVR	0.7908	Non-antigen
TLPTSFNYGAI	1.0143*	Antigen
AIHPEQARHKQKIVAPVKQLLNFDLLKLA	0.5416	Non-antigen
GDVESNPGPFFFSDVRSNFS		
ETINQMQEDMSTKHGPDFNRLVS	0.1584	Non-antigen
DEAKPW	-0.2015	Non-antigen
RSTPEDLERAEKQLKA	0.7379	Non-antigen
EEKFVTMTD	0.5100	Non-antigen
EKQRDLNDPSKYKEAKEWLDN	0.0172	Non-antigen
PPDPDHFDGYN	0.3834	Non-antigen
MASLEDKGKPF	1.1921*	Antigen
YSGFTPRTMVCP	0.8981	Non-antigen
KDGYKINNKLDIIKALEDTHTNPVAMFQYDC	0.4263	Non-antigen
MKRMQQDMFKPQPPLQ	0.0248	Non-antigen
VELHEKVSSHPIFKQISIPSQKS	0.763	Non-antigen
KGQHEAAIE	0.7196	Non-antigen
VHDSIKEELRPLIQQ	0.1848	Non-antigen
QQMVDNAVNEYIEKANITTDDKTLDEAEKNPL		
ETSGASTVGFRERTLPGHKASGDVNSEPAKPVE	0.3325	
EQPQAEGPYAGPLERQKPLKVRAKLPQQEGPYA		Non-antigen
GPMERQKPLKVKAKAPVVKEGPYEGPVKKPVAL		
LIVTESGAPPTDLQKMVMG	0.2751	Non-antigen
KGQDMLS	0.807	Non-antigen
VRDITKHFRDVAKMKKG	0.3906	Non-antigen
DPEPHHEGLIVDTRDVEERVHVMRKTKL	0.6081	Non-antigen
TVAHGVFNPEF	0.6952	Non-antigen
LSNKDPRLNEGVVLDEVIFSKHKG	0.6566	Non-antigen

DTKMTEEDKK		
LGTANAPLSIYEAIK	0.2753	Non-antigen
VDGLDAMEPD	0.3504	Non-antigen
APGLPWALQGKRRGALIDFESGTV	1.1967*	Antigen
ELMEKR	0.5206	Non-antigen
EIRPMEKVRA	1.4702*	Antigen
NNGPQIGS	0.5695	Non-antigen
TEFGFHPNA	1.65*	Non-antigen
YEGVEL	1.2966*	Antigen
PADKSDKGFVLGHSI	0.5022	Non-antigen
SFARRGT	1.2643*	Antigen
EIPSYRSLYLR	0.2149	Non-antigen

***** *peptides that have an affinity value > 1*

Peptide-based vaccines have shown promise in triggering an immune response, but a single peptide may not always be enough to generate a strong and lasting immune response. Subunit vaccines consist of non-genetic material, such as peptides or proteins. They are considered safe as they have fewer side effects. However, they have low immunogenicity if used alone (Yu-Gyeong *et al*., 2020). Moreover, multiple epitopes derived from tumor-associated antigens or pathogenic proteins have been investigated to induce more robust immune responses than a single antigen (Naveed *et al*., 2022). Therefore, combining multiple peptides or epitopes in vaccine construction could be necessary for achieving robust and sustained immune responses against various pathogens, not only reducing costs, including vaccine materials, labor, and logistics, but also enabling rapid vaccination in emergencies that require efficient, economical, and flexible responses to viruses. Moreover, given the prevalence patterns and diversity of FMD across continents, it is essential to implement policies and develop research to produce strain-specific vaccines (Aslam & Alkheraije, 2023).

The application of peptide vaccines in Indonesia has not been widely practiced, especially in the veterinary field. Hence, production and development are still widely open, considering Indonesia has abundant bioactive peptide sources. Multidisciplinary collaboration is needed to get the expected results. Since pure peptide production is still expensive, especially for livestock production, the use of peptide hydrolysates without purification can be an option (Kusumaningtias, 2018)

Conclusion

The study successfully identified potential epitope candidates for developing an FMD vaccine using an epitope-based in-silico approach. By leveraging immunoinformatics methods, the research efficiently predicted epitopes from the FMD virus genome sequences obtained from UniProtKB and the National Library of Medicine application. The epitopes identified, such as CSIQKR, TEFGFHPNA, EIRPMEKVRA, YEGVEL, APGLPWALQGKRRGALIDFESGTV, SFARRGT, MASLEDKGKPF, and TLPTSFNYGAI, exhibited high antigen binding affinity, indicating their potential to form the basis for developing more selective and safe peptide vaccines. This in-silico approach demonstrates a cost-effective and time-efficient method for vaccine development, potentially improving the effectiveness and safety of FMD vaccination and accelerating the creation of new vaccines for FMD prevention.

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References

Afriani, T., Farez, F., Albadri, A., Wahyuni, S. A., Simbolon, R. A., & Fajri, M. H. (2022). Pemberdayaan masyarakat Nagari Palangki dalam mengatasi penyakit mulut dan kuku (PMK). Warta Pengabdian Andalas: *Jurnal Ilmiah Pengembangan dan Penerapan Ipteks*, 29(4), 377-381.

Ahmar, A. S., & Boj, E. (2021). Application of neural network time series (Nnar) and ARIMA to forecast infection fatality rate

(IFR) of COVID-19 in Brazil. *JOIV: International Journal on Informatics Visualization*, 5(1), 8-10. https://dx.doi.org/10.30630/joiv.5.1.372

Alhaji, N. B., Amin, J., Aliyu, M. B., Mohammad, B., Babalobi, O. O., Wungak, Y., & Odetokun, I. A. (2020). Economic impact assessment of foot-and-mouth disease burden and control in pastoral local dairy cattle production systems in northern Nigeria: A cross-sectional survey. *Preventive Veterinary Medicine*, 177, 104974. https://doi.org/10.1016/j.prevetmed.2020.104974

Aslam, M., & Alkheraije, K. A. (2023). The prevalence of foot-and-mouth disease in Asia. *Frontiers in Veterinary Science*, 10, 1201578. https://doi.org/10.3389/fvets.2023.1201578

Auty, H., Mellor, D., Gunn, G., & Boden, L. A. (2019). The risk of foot and mouth disease transmission posed by public access to the countryside during an outbreak. *Frontiers in Veterinary Science*, 6, 381. https://doi.org/10.3389/fvets.2019.00381

Belsham, G. J. (2020). Fundamentals of the molecular biology of picornaviruses: Implications for foot-and-mouth disease. *Frontiers in Microbiology*, 11, 294. https://doi.org/10.3389/fmicb.2020.00294

Bhutta, M. S., Awais, M., Sadaqat, S., Zanchi, F. B., Shahid, N., & Qayyum Rao, A. (2024). A novel immunoinformatics approach for developing a poly-epitope vaccine targeting foot and mouth disease virus, exploiting structural VP proteins. *Journal of Biomolecular Structure and Dynamics*, 1-17. https://doi.org/10.1080/07391102.2024.2328735

Casey-Bryars, M., Reeve, R., Bastola, U., Knowles, N. J., Auty, H., Bachanek-Bankowska, K., ... & Lembo, T. (2018). Waves of endemic foot-and-mouth disease in eastern Africa suggest feasibility of proactive vaccination approaches. *Nature Ecology & Evolution*, 2(9), 1449-1457. https://doi.org/10.1038/s41559-018-0636-x

Dekker, A., Sanz-Bernardo, B., Singanallur, N. B., Ludi, A. B., Horsington, J., Eblé, P. L., ... & Vosloo, W. (2020). Cross-protection induced by a A/MAY/97 emergency vaccine against intra-serotype heterologous challenge with a foot-and-mouth disease virus from the A/ASIA/G-VII lineage. *Vaccines*, *8*(1), 24. https://doi.org/10.3390/vaccines8010024

Direktorat Jenderal Peternakan dan Kesehatan Hewan. (2023). Statistik Peternakan dan Kesehatan Hewan. Jakarta: Direktorat Jenderal Peternakan dan Kesehatan Hewan. https://ditjenpkh.pertanian.go.id/storage/photos/shares/konten/publikasi/files/Buku%20Statistik%202023%20ISSN.pdf

Jalalvand, Alireza., Fotouhi, Fatemah., Bahramali, Golnaz., Bambai, Bijan., & Farahmand, Behrok. 2023. In silico design of a trivalent multi-epitope global-coverage vaccine-candidate protein against influenza viruses: evaluation by molecular dynamics and immune system simulation. *Journal of Biomolecular Structure & Dynamics.* https://doi.org/10.1080/07391102.2023.2292293

Jean, C, Udahemuka., Lunayo, Accadius., George, Obiero., Gabriel, Aboge., & Phiyani, Lebea. 2021. In Siliko Prediction and VP1 Modelling for Foot-and- Mouth Serotype SAT 2 for Vaccine design in East Africa. bioRxiv preprint doi: https://doi.org/10.1101/2021.09.13.460008

Kamel, M., El-Sayed, A., & Castañeda Vazquez, H. (2019). Foot-and-mouth disease vaccines: recent updates and future perspectives. *Archives of virology*, 164, 1501-1513. https://doi.org/10.1007/s00705-019-04216-x.

Kusumaningtyas, E. (2018). Aplikasi peptida untuk meningkatkan kesehatan dan produktivitas ternak. Bogor, Balai Besar Penelitian Veteriner, 28(2). DOI:10.14334/wartazoa.v28i2.1783

Li, L. (2021). "The Role of B Cells in the Immunity Against Foot-and-Mouth Disease Virus. *Immunology Letters*, 233, 54-62. DOI: 10.1016/j.imlet.2021.04.012.

Liu, X., Qi, L., Lv, J., Zhang, Z., Zhou, P., Ma, Z., ... & Pan, L. (2020). The immune response to a recombinant Lactococcus lactis oral vaccine against foot-and-mouth disease virus in mice. *Biotechnology Letters*, *42*, 1907-1917. doi: 10.1007/s10529-020-02900-6

Mahapatra, M., & Parida, S. (2018). "Foot and Mouth Disease: Global Status and Future Road Map. *Viruses*, 10(12), 729. DOI: 10.3390/v10120729.

Mansilla, F. C., Turco, C. S., Miraglia, M. C., Bessone, F. A., Franco, R., Pérez-Filgueira, M., ... & Capozzo, A. V. (2020). The role of viral particle integrity in the serological assessment of foot-and-mouth disease virus vaccine-induced immunity in swine. *PLoS One*, *15*(5), e0232782.

Martinelli, D. D. (2022). In silico vaccine design: A tutorial in immunoinformatics. *Healthcare Analytics*, *2*, 100044. https://doi.org/10.1016/j.health.2022.100044

Miqnaqui, M. (2020). B Cell Responses to Viral Infections: Mechanisms and Implications for Immunity and Vaccine Development. *Viral Immunology*, 33(6), 427-439. DOI: 10.1089/vim.2020.0056.

Naveed, M., Sheraz, M., Amin, A., Waseem, M., Aziz, T., Khan, A. A., Ghani, M., Shahzad, M., …. & Alhomrani, M. (2022). Designing a novel peptide-based multi-epitope vaccine to evoke a robust immune response against pathogenic multidrug-resistant Providencia heimbachae. *Vaccines*, *10*(8), 1300. https://doi.org/10.3390/vaccines10081300

Purwadi, P., & Prasetyo, A. B. (2024). Dampak Wabah Penyakit Mulut dan Kuku (PMK) terhadap Produksi Susu dan Pendapatan Peternak Sapi Perah Rakyat di Boyolali. *Ilmu Hewan Tropis* , 6 (1), 55-59.

Ribeiro, B. F. S., Rodrigues, D. L. N., Ariute, J. C., Dias, M. V., Nogueira, W. G., Ramos, R. T., ... & Aburjaile, F. F. (2024). Bioinformatics approach to design peptide vaccines for viruses. In *Reverse Vaccinology* (pp. 265-287). Academic Press.

Rohma, M. R., Zamzami, A., Utami, H. P., Karsyam, H. A., & Widianingrum, D. C. (2022). Kasus penyakit mulut dan kuku di Indonesia: epidemiologi, diagnosis penyakit, angka kejadian, dampak penyakit, dan pengendalian. In *Conference of Applied Animal Science Proceeding Series* (Vol. 3, pp. 15-22). https://doi.org/10.25047/animpro.2022.331

Seeyo, K. B., & Rukkwamsuk, T. (2019). A comprehensive review of antigenic variation in foot and mouth disease virus. *Veterinary Research Communications*, 43(4), 237-251.

Sid Ahmed, R. M., Almofti, Y. A., & Abd-Elrahman, K. A. (2022). Analysis of Foot and Mouth Disease Virus Polyprotein for Multi Peptides Vaccine Design: An In silico Strategy. *Journal of Pure & Applied Microbiology*, 16(3).

Sobrino, F., Sáiz, M., Jiménez-Clavero, M.Á., & Domingo, E. (2020). "Foot-and-Mouth Disease Virus: A Long Known Virus, but a Current Threat. *Viruses*, 12(10), 1183. DOI: 10.3390/v12101183.

Stenfeldt, C., Diaz-San Segundo, F., De Los Santos, T., Rodriguez, L. L., & Arzt, J. (2016). The pathogenesis of foot-and-mouth disease in pigs. *Frontiers in veterinary science*, *3*, 41. https://doi.org/10.3389/fvets.2016.00041

Stenfeldt, C., & Arzt, J. (2020). The carrier conundrum; a review of recent advances and persistent gaps regarding the carrier state of foot-and-mouth disease virus. *Pathogens*, *9*(3), 167. https://doi.org/10.3390/pathogens9030167

Sudarsono, R. P. E. (2022). Kajian Epidemiologi Kejadian Diduga Penyakit Mulut dan Kuku di Kabupaten Lamongan Epidemiological Study of Suspected Occurrence of Foot and Mouth Disease in Lamongan Regency. *Journal of Basic Medical Veterinary*, 11(1), 56-63. https://ejournal.unair.ac.id/JBMV http://dx.doi.org/10.36465/jop.v5i1.880

Sumption, K., Knight-Jones, T. J., McLaws, M., & Paton, D. J. (2020). Parallels, differences and lessons: a comparison of the management of foot-and-mouth disease and COVID-19 using UK 2001/2020 as points of reference. Proceedings of the Royal Society B, 287(1938), 20200906.

Van Andel, M., Tildesley, M. J., & Gates, M. C. (2021). Challenges and opportunities for using national animal datasets to support foot-and-mouth disease control. Transboundary and Emerging Diseases, 68(4), 1800-1813. https://doi.org/10.1111/tbed.13858.

Wong, C. L., Yong, C. Y., Ong, H. K., Ho, K. L., & Tan, W. S. (2020). Advances in the diagnosis of foot-and-mouth disease. *Frontiers in veterinary science*, *7*,

477. https://doi.org/10.3389/fvets.2020.00477

Wulandari, A. A. (2024). Kerjasama Bilateral Indonesia dan Australia Terhadap Penanganan Wabah Penyakit Mulut dan Kuku (PMK) dan Lumpy Skin Disease (LSD) pada tahun 2022-2024 (Bio-security) (Doctoral dissertation, Universitas Pendidikan Muhammadiyah Sorong).

Xiao, Y., Zhang, S., Yan, H., Geng, X., Wang, Y., Xu, X., & Tian, K. (2021). The high immunity induced by the virus-like particles of foot-and-mouth disease virus serotype O. *Frontiers in veterinary science*, *8*, 633706. https://doi.org/10.3389/fvets.2021.633706

Yadav, M. P., Singh, R. K., & Malik, Y. S. (2020). Emerging and transboundary animal viral diseases: Perspectives and preparedness. *Emerging and Transboundary Animal Viruses*, 1-25.

Yu-Gyeong, Kim., Yunsu, Lee., Joo Hee Kim., Sun-Young, Chang., Jong-Wha, Jung., Woo-Jae, Chung & Hyo-Eon, Jin. 2020. Self-Assembled Multi-Epitope Peptide Amphiphiles Enhance the Immune Response against Enterovirus 71. *Nanomaterials*. 10 (12). https://doi.org/10.3390/nano10122342

Zamroni, Rosidi, Moh., Qabilah Cita K. N. S., & Wahyudi, Agung Try. (2022). Sistem pakar diagnosa penyakit sapi sebagai upaya pencegahan penyebaran wabah pmk di lamongan JIF: *Jurnal Imiah Informatika* Vol. 10, Iss: 02, pp 145-152

Zhi, Y., Ji, H. J., Guo, H., Lim, J. H., Byun, E. B., Kim, W. S., & Seo, H. S. (2021). Salmonella vaccine vector system for foot-and-mouth disease virus and evaluation of its efficacy with virus-like particles. *Vaccines*, *9*(1), 22.