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KEPUTUSAN
DEKAN FAKULTAS KEDOKTERAN HEWAN
UNIVERSITAS AIRLANGGA
NOMOR 101/UN3.1.6/2022

Tentang

PENGANGKATAN DOSEN PENGUJI UJIAN TERTUTUP DISERTASI MAHASISWA
PROGRAM STUDI S3 SAINS VETERINER
FAKULTAS KEDOKTERAN HEWAN
UNIVERSITAS AIRLANGGA
APRIL 2022

DEKAN FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA,

Menimbang :

a. Bahwa dalam rangka melaksanakan Tri Dharma Perguruan Tinggi dipandang perlu mengangkat Dosen Penguji Ujian Tertutup Disertasi Mahasiswa Program Studi S3 Sains Veteriner Fakultas Kedokteran Hewan Universitas Airlangga April 2022;
b. Sehubungan dengan butir (a) tersebut di atas, dipandang perlu menerbitkan Keputusan Dekan Fakultas Kedokteran Hewan Universitas Airlangga.

Mengingat :

1. Undang-Undang Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional (Lembaran Negara Republik Indonesia Tahun 2003 Nomor 78, Tambahan Lembaran Negara Republik Indonesia Nomor 4301;
2. Undang-Undang Nomor 12 Tahun 2012 tentang Pendidikan Tinggi (Lembaran Negara Republik Indonesia Tahun 2012 Nomor 158, Tambahan Lembaran Negara Nomor 5336);
3. Peraturan Pemerintah Nomor 57 Tahun 1954 tentang Penetapan Universitas Airlangga di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah Nomor 3 Tahun 1955 tentang Pengubahan Peraturan Pemerintah Nomor 57 Tahun 1954 (Lembaran Negara RI Tahun 1954 Nomor 99, Tambahan Lembaran Negara Republik Indonesia Nomor 695 juncto Lembaran Negara RI Tahun 1955 Nomor 748);
4. Peraturan Pemerintah Nomor 4 Tahun 2014 tentang Penyelenggaraan Pendidikan Tinggi dan Pengelolaan Perguruan Tinggi (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 16, Tambahan Lembaran Negara Nomor 5500);
5. Peraturan Pemerintah Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 100, Tambahan Negara Nomor 5535;
6. Peraturan Pemerintah Nomor 8 Tahun 2020 tentang Perubahan Atas Peraturan Pemerintah Nomor 26 Tahun 2015 tentang Bentuk dan Mekanisme Pendanaan Perguruan Tinggi Negeri Badan Hukum (Lembaran Negara Republik Indonesia Tahun 2020 Nomor 28, Tambahan Lembaran Negara Republik Indonesia Nomor 6461;
7. Surat Keputusan Menteri Pendidikan dan Kebudayaan Republik Indonesia Nomor: 055/O/1972 tanggal 25 Maret 1972 tentang Pendirian Fakultas Kedokteran Hewan Universitas Airlangga;
8. Keputusan Menteri Pendidikan Nasional Republik Indonesia Nomor:232/U/2000 tentang Pedoman Penyusunan Kurikulum Pendidikan Tinggi dan Penilaian Hasil Belajar Mahasiswa;
9. Peraturan Rektor Nomor 39 Tahun 2017 tentang Perubahan atas Peraturan Rektor Nomor 42 Tahun 2016 tentang Organisasi dan Tata Kerja Universitas Airlangga;

10. Keputusan Rektor Universitas Airlangga Nomor : 2158/H3/KR/2011 tanggal 7 Nopember 2011 tentang Izin Penyelenggaraan Program Studi Sains Veteriner Jenjang S-3 Pada Program Pascasarjana Universitas Airlangga;
11. Keputusan Rektor Universitas Airlangga Nomor : 762/UN3/2020 tanggal 30 September 2020 tentang Pengangkatan Dekan Fakultas dan Direktur Sekolah Pascasarjana Periode 2020-2025 di lingkungan Universitas Airlangga.Airlangga.

Memperhatikan : Surat keputusan Rektor Nomor 698/UN3/2019 tentang Perpanjangan Izin Penyelenggaraan Program Studi di Lingkungan Universitas Airlangga.

MEMUTUSKAN:

Menetapkan : **PENGANGKATAN DOSEN PENGUJI UJIAN TERTUTUP DISERTASI MAHASISWA PROGRAM STUDI S3 SAINS VETERINER FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA APRIL 2022**

PERTAMA : Mengangkat para Dosen Penguji Ujian Tertutup Mahasiswa Program Studi S3 Sains Veteriner Fakultas Kedokteran Hewan Universitas Airlangga Bulan April 2022 seperti tercantum dalam daftar lampiran Keputusan ini ;

KEDUA : Dosen Penguji Ujian Tertutup Mahasiswa Program Studi S3 Sains Veteriner dalam melaksanakan tugasnya berpedoman pada peraturan dan ketentuan yang berlaku dan mempertanggung jawabkan tugasnya kepada Dekan Fakultas Kedokteran Hewan Universitas Airlangga;

KETIGA : Keputusan ini berlaku sejak tanggal ditetapkan.

Ditetapkan di Surabaya
Pada tanggal 1 April 2022

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NIP. 196201161992032001

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DOSEN PENGUJI UJIAN TERTUTUP DISERTASI MAHASISWA
PROGRAM STUDI S3 SAINS VETERINER
FAKULTAS KEDOKTERAN HEWAN
UNIVERSITAS AIRLANGGA
APRIL 2022

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1.	Era Hari Mudji/ 061517117301	Senin/ 25 April 2022	Model Hubungan Sumber Daya Manusia, Morfometri, Berat Hidup Dan Karkas Terhadap Efisiensi Usaha Ternak Sapi Potong	Prof. Dr. Sri Pantja Madyawati, drh., M. Si	(K e t u a)
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2.	Aamir Shehzad/ 061817117310	Selasa/ 26 April 2022	*Molecular Characterization Of SARS-CoV-2 Isolates Of Surabaya And Development Of Candidate Vaccine*	Prof. Dr. Bambang Sektiari Lukiswanto, drh., DEA	(K e t u a)
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MOLECULAR CHARACTERIZATION OF SARS-COV-2 ISOLATES OF SURABAYA AND DEVELOPMENT OF CANDIDATE VACCINE.

DISSERTATION

By

AAMIR SHEHZAD

NIM. 061817117310

DOCTORAL PROGRAM VETERINARY SCIENCE

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF AIRLANGGA

SURABAYA, INDONESIA

(2022)

MOLECULAR CHARACTERIZATION OF SARS-COV-2 ISOLATES OF SURABAYA AND DEVELOPMENT OF CANDIDATE VACCINE.

DISSERTATION

DOCTORAL PROGRAM (Ph.D.)

VETERINARY SCIENCE

FACULTY OF VETERINARY MEDICINE UNIVERSITY OF AIRLANGGA SURABAYA INDONESIA

By

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(26. April.2022)

APPROVAL OF DISSERTATION

(26. April .2022)

By

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Exam Date 26.April 2022

DECLARATION STATEMENT

I Aamir Shehzad NIM: 061817117310 hereby declare that in this dissertation entitled: "Molecular Characterization of SARS-CoV-2 Isolates of Surabaya and Development of Candidate Vaccine" no work has ever been submitted for a doctorate in a college and as far as I know there is also no work or opinions that have been written or published by others, except those in writing referred to in this manuscript and mentioned in the bibliography.

Dated. 26. April 2022

Aamir Shehzad

NIM. 061817117310

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SUMMARY

Background: At the end of December 2019, a few people from Wuhan, China, were reported to have pneumonia symptoms. Upon examination, the SARS-CoV-2 virus was revealed as the causative agent of the infection. The novel coronavirus disease (COVID-19) pandemic was designated a public health emergency by the World Health Organization (WHO) in January 2020. It has been estimated that since 30 December 2019, over 214.27 million people have been infected with COVID-19. Moreover, about 4.27 million people have died. It is not only a significant threat to the rest of the world but also to Indonesia, where it has caused the deaths of 129293 people and infected a total of 4.027 people.

Researchers worldwide are studying this novel virus (SARS-CoV-2) and seeking to find successful interventions for disease control and prevention. Due to the high demand for safe and effective therapies against SARS-CoV-2. Undoubtedly, the vaccine-based measures could be highly beneficial in the event of outbreaks or seasonal re-emergence, largely dependent on long-term protective volution. In an outbreak crisis, traditional vaccination techniques based on laboratory trials could not address the immediate needs. Therefore, traditional vaccine development techniques, combined with bioinformatic approaches, can accelerate vaccine development.

Aim: The current study aimed to molecularly characterize the Surabaya SARS-CoV-2 isolates and develop a candidate vaccine.

Material & Methods: The whole-genome sequences (WGSs) of SARS-CoV-2 Surabaya isolates from RCVTD-ITD with accession numbers 1366503, 1366505,

and 1366509, as well as whole-genome sequences of Wuhan/WH04 as a reference and 15 sequences from other countries: Bangladesh, India, Pakistan, South Africa, Brazil, Egypt, France, Italy, USA, UK, Japan, South Korea, Malaysia, Singapore, and UAE, were carefully selected with the same linage of B.1.

The required editing of WGSs of SARS-CoV-2 was completed using Bio-edit 7.2 software. Moreover, the alignment and phylogenetic analysis were performed in Mega-II Software using the CLUSTAL-W and Neighbor-Joining methods, respectively. Finally, the Surabaya isolates WGS were converted into amino acids (candidate or primary structure proteins) through the ExPASy translator web tool. In addition to this, the selected segments of proteins (N, S, M, E) sequences were confirmed through the NCBI protein BLAST webserver.

Furthermore, the NCBI webserver was used to understand the transformations in the Nucleotides and amino acids of SARS-CoV-2 Surabaya Isolates. The selected proteins (ORF1ab, N, M, and S) were fed to the IEDB webserver to predict the linear B-cell and T-cell (MHC-1 and MHC-II) epitopes, The predicted epitopes' antigenicity, non-toxicity, and non-allergenicity were tested using the VaxiJen, ToxinPred," and AllerTOP web tools.

The candidate vaccine sequence was constructed by linking the potential epitopes of B-cells to the MHC Class-I and MHC Class-1 with MHC Class-II through the EAAAK, GPGPG, and AAY linkers, respectively. Moreover, the 50S ribosomal protein L7/L12 and cholera toxin B subunit adjuvants were linked by EAAAK to B-cell epitopes of the candidate vaccine to enhance immunogenic effects. The physicochemical properties and solubility rate of developed candidate

vaccines were evaluated using the ExPASY ProtParam and SOLpro webservers, respectively. In addition, the Raptor X and SOPMA tools were used to analyze the secondary structures of the developed candidate vaccine.

Thus, the tertiary structure of the developed candidate vaccines was generated using the PHYRE2 and Swiss-Model web tools. The Galaxy Refine tool was used to refine the tertiary structures, and the RAMPAGE web tool was used to validate the refined tertiary structures. The Cluspro, iMODS, and C-ImmSim web servers were used for molecular docking of candidate vaccine constructs with TLRs (3 &4), molecular dynamics simulation, and immune stimulation respectively. Furthermore, the PDBsum web tool was used to assess the interaction of the candidate vaccine constructs with TLRs. The Java Codon Adaptation Tool (J-Cat) was used to optimize candidate vaccine sequences, and Snap-Gene software was used to perform cloning of candidate constructs in E. coli pET-38a (+) and pET-23a (+) expression vectors.

The % SDS-PAGE and Western blotting techniques were used to characterize the molecular weights and immunogen proteins of SARS-CoV-2 Surabaya isolate No. 33, respectively. Furthermore, we used an indirect ELISA to test the immunoreactivity of SARS-CoV-2 Surabaya Isolate No.33 with different series of dilution folds of rabbit serum immunized with the whole virion inactivated vaccine. Elisa's results were examined using the one-way ANOVA paired sample test of IBM-SPSS 25 software.

Results: The Surabaya isolates, RSDS-RCVTD-UNAIR-49-A (A-Id;1366505), 54-A (A-Id;1366503), and 42-A (A-Id;1366509), had 10, 20, and 16 mutations in

nucleotides and depicted a phylogenetically close relationship to isolates of Egypt, Pakistan, and Bangladesh, respectively. The epitopic analysis of Surabaya isolates predicted 71 sequential ORF1ab B-cell epitopes, with only three peptides being antigenic, non-allergenic, and non-toxic. These epitopes were linked with the EAAAK linker to develop a 3D refined and validated structure. This construct was docked with TLR-3 receptor by the Cluspro webserver and found a high affinity of ORF1ab+TLR3 due to 15 hydrogen bonds.

The construct demonstrated good humoral and cellular immune responses in the C-ImmSim server, and cloning in the expression vector pET28a (+) yielded a colon of 846bp. The epitopic analysis of spike glycoprotein conserved in SARS-CoV-2 Surabaya isolates accession Nos.1366503 and 1366509 revealed 2-B-cell and 15-T-cell (8-MHC-I and 7MHC-II) and 5-B-cell and 14-T-cell (5-MHC-I and 9MHC-II) potential antigenic, nontoxic and non-allergic epitopes respectively. A multi-epitopic vaccine of isolate;1366503, containing potential B-cell and T-cell was developed using 392 amino acids with a molecular weight of 40825.59 Da. While a poly-epitopic vaccine of isolate; 1366503, containing B-cell and T-cell was constructed with 374 amino-acids. having a molecular weight of 39492.08 Da.

The both spike proteins based constructed vaccines sequences of isolates accession Nos.1366503 and 1366509 revealed satisfactory physiochemical analysis results and were converted into 3D structures. The refinement and validation of these structures was performed. The molecular docking of both 3D vaccine structures of isolates No.1366503 and 1366509 with TLR-3 revealed strong interactions between vaccine structures and TLR-3 with energy scores of-728.2 and

693.1 Kcal/mol, respectively. Immune-simulation analysis of both spike proteins-based vaccines revealed high levels of IgM, IgG1+IgG2, IgM+IgG, IgG1, and IgG2, as well as a significant increase in IFN-g, TGF-b, IL-4, L-10, and L12.

The optimization process of spike protein-based vaccines sequences of both isolates: 1366503 and 1366509 revealed CAI-values of 0.89 and 0.95 as well as the GC Content values of 57.23 and 51.51 respectively. The optimized vaccine sequences of isolates 1388503 and 1366509 were successfully cloned in E. coli pET-28a (+) expression vectors by inserting between the restriction sites HpaI & SmaI and HincII & AccI, yielding cloned products of 2724 bp and 1632 bp, respectively.

The structural proteins-based analysis of Surabaya isolate accession No.1366505 yielded a total of 5 B-cell (N=2, and S=3) and 10 T-cell [MHC-I= 7(M=3, S=1 and N=3) and MHC-II = 10(M =2, S =8)] potential epitopes. A multi-epitope subunit vaccination was developed by linking the 50S ribosomal protein L7/L12 an adjuvant with EAAAK linkers to the B-cell epitopes. In contrast, GPGPG and AAY linkers were used to link the B-cell to the MHC-I and MHC-II epitopes, respectively. The constructed multi-epitope subunit sequence had a molecular weight of 59974.20 Da based on 563 amino-acids. The developed vaccine showed all physiochemical parameters: theoretical pI, instability index (II), aliphatic index, and GRAVY.

A refine and validated tertiary (3D) structure was developed and docked with the TLR-4. The docking complex showed lowest docking energy of 753.3 kcal/mol and 92 cluster members. Our developed vaccine linked TLR-4 potential residues

through 62 hydrogen bonds and 18 salt bridges. The vaccine +TLR-4 normal mood analysis revealed easier deformability, with the lowest eigenvalue of 4.816138 e-06. The vaccine sequence optimization was done and insert successfully into the E. coli pET-28a (+) expression vector and found the cloned product of 4843 bp. The Immune stimulation of the designed vaccine showed the increase in the levels of IgM, IgG1 +IgG2, IgM, and IgG + IgM. Moreover, the TGF-, IFN-, and IL-2 were also identified in significant concentrations.

The molecular weight characterization of structural proteins of the SARS-CoV-2 Surabaya isolate indicated that the S protein has a molecular weight of 200KDa, and its subunits S1 and S2 have molecular weights of 75 and 65KDa, respectively. Furthermore, the N, M, and E proteins M.W were discovered to be 50KDa, 22KDa, and 12KDa, respectively. The western blotting of Surabaya isolate depicted a strong immunogenicity of S and N protein while the M and E also detected as lesser immunogen. Moreover, the immunoreactivity was reconfirmed through the I-ELISA and found statistically significant overall relationship among the OD values and the serum dilution factor.

Conclusion: The current study revealed that the Surabaya isolates accession Nos. 1366505, 1366503, and 1366509 had 10, 20, and 16 transformations in nucleotides have close relationship to the isolates of Egypt, Pakistan, and Bangladesh, respectively. The Molecular weight characterization revealed that the S, N, M, and E proteins in the SARS-CoV-2 Surabaya isolate have molecular weights of 200 kDa, 50 kDa, 22 kDa, and 12 kDa, respectively in which, spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 Surabaya isolates were found to be strong

immunogenic. Moreover, the developed candidate vaccines by SARS-CoV-2 Surabaya isolates elicited robust immunological responses by inducing IgG1 + IgG2, IgM, and IgG + IgM levels, as well as TGF-b, IFN-g, L-12, IL-4, and IL-2. In addition, the novel immune responses generated by ORF1ab polyprotein B-cell epitopes imply that incorporating ORF1ab into the development of subunit vaccines against SARS-CoV-2 might result in substantial and long-lasting immunological protection.

ABSTRACT

The COVID-19 outbreak has infected millions of people worldwide, but no vaccine has been discovered to combat it efficiently. The purpose of this work was to molecularly characterize the SARS-CoV-2 Surabaya isolates and design an immunogenic candidate vaccine utilizing bioinformatics and in vitro techniques. For this study, we used three different Surabaya isolates (Accession IDs:1366503, 1366505, and 1366509) of RCVTD-ITD. These isolates' phylogenetic analysis, transformations assessment, prediction of antigenic, non-allergic and non-toxic B-cell and T-cell epitopes, engineering of multiepitope vaccines, and evaluation of immunogenicity parameters of vaccine contract were, all carried out through the bioinformatics tools: Mega-XI software, NCBI webserver, VaxiJen-v2.0 webserver, AllerTOP-v.2.0 webserver, ToxinPred webserver, IEBD webserver, C-ImmSim webserver and Snap Gene v3.2.1 software respectively. Furthermore, these SARS-CoV-2 Surabaya isolates were molecularly characterized based on their masses and immunoreactivity using the SDS-Page, western blot methods and I-Elisa.

The Surabaya isolates, RSDS-RCVTD-UNAIR-49-A, 54-A, and 42-A, had 10, 20, and 16 mutations in nucleotides and depicted a phylogenetically close relationship to isolates of Egypt, Pakistan, and Bangladesh, respectively. The predicted epitopes: ORF1ab, and other structural protein's-based vaccines showed strong docking and immunogenicity that met all established parameters for constructing a quality vaccine. Furthermore, the masses of structural proteins discovered in Surabaya isolates are 200, 75, 65, 50, 22, and 12KDa's for S, S1, S2, N, M, and E, respectively. Furthermore, S and

N proteins were revealed to have strong immunogenicity. Importantly, a significant immunoreactivity between our antigen and different dilutions of antibody serum of SARS-Cov-2 immunized rabbit were noticed in I-Elisa.

The Surabaya isolates predicted robust cellular and humeral immunogenic responses. Especially strong immunogenicity findings of ORF1ab, S and N proteins imply that using these proteins in a vaccine can vigorously protect the public from the Covid-19.

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