Computational Evaluation of the B-Cell Epitope of 37-kDa Outer Membrane Protein H Pasteurella multocida Type B from Nusa Tenggara Timur, Indonesia

Firdausy Kurnia Maulana, Didik Handijatno

A B-cell epitope prediction study was conducted to evaluate the epitope of the 37-kDa OmpH derived from the amino acid sequence of Pasteurella multocida from the NTT and Katha strains and compared the epitopes of the two strains. Amino acid sequences were obtained from NCBI and analyzed for multiple sequence alignment, and homology was analyzed using the BLASTp program at NCBI. Epitope prediction was performed using the IEDB B-cell epitope and ABCPred prediction tools. The VaxiJen v.2 online platform was used for antigenicity analysis, and IEDB was used for epitope conservancy analysis. The results of the homology analysis revealed that local NTT isolates had a high (>95%) identity with the Katha strain and isolates from China, India, Iran, Japan, and the USA. The epitope predictions from both methods were cross-checked, overlapping epitopes were shortlisted, and only five epitopes were predicted. Among the five, one epitope, ALEVGLN, appeared to be antigenic to both NTT and Katha strains. The antigenic sequence of 37 kDa OmpH can be used for peptide-based vaccine development and immunotherapeutic design.

**Keywords**
Haemorrhagic Septicemia, P. multocida, Outer membrane protein H (OmpH), Epitope, Antigenicity

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>HS</td>
<td>Haemorrhagic Septicemia</td>
</tr>
<tr>
<td>OmpH</td>
<td>Outer membrane protein H</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
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<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
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**Number of Figures:** 2  
**Number of Tables:** 6  
**Number of References:** 32  
**Number of Pages:** 8

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Introduction

HS, also known as snoring disease in Indonesia, is caused by Pasteurella multocida, a gram-negative bacterium that commonly infects cattle and buffalo [1].

HS has a wide distribution, especially in tropical regions, such as Middle-East, Central Africa, North-East Africa, South Africa, South Asia, and South-East Asia [2]. In Indonesia, 12,058 cases of HS were reported between July 2007 and December 2019, with cases in buffaloes (4,047), cattle (5,809), pigs (2,108), goats/sheep (64), and Equidae (30) [3].

The disease causes huge losses due to livestock mortality, as well as losses in the meat- and dairy-related industries [4]. The economic loss of the livestock industry is estimated at 792 million USD per year [5], and the mortality rate of HS is up to 100% [6]. Considering its socioeconomic impact, HS is classified as one of 22 strategic infectious diseases in Indonesia, where control measures are coordinated at the central level [7].

The disease remains endemic in Indonesia, and several regions continue to report outbreaks [8, 9, 10] despite vaccination efforts. Currently, the disease is present in multiple areas of Indonesia, including Bali, Bengkulu, DKI Jakarta, Java, Aceh, West Nusa Tenggara, East Nusa Tenggara, North Sumatra, South Kalimantan, South Sumatra, Jambi, Riau, Central Sulawesi, West Sumatra, East Kalimantan, and Central Kalimantan [3].

The HS vaccines in Indonesia are administered once annually in the form of either an oil adjuvants vaccine or an aluminum precipitate vaccine, both of which are developed using the Katha strain from Burma [7]. The most effective option in the market is the oil adjuvant vaccine, which can provide immunity for up to one year. However, this vaccine has limitations, including the high viscosity of the solution, which makes injection challenging and can lead to swelling and abscesses at the injection site [2, 11]. Furthermore, this type of vaccine has a short storage time because it is susceptible to damage caused by temperature fluctuations [12].

The OmpH is a prominent protein in the purified envelope of P. multocida envelope and was identified as an immunodominant porin. Ongoing research has explored the feasibility of utilizing OmpH as a subunit vaccine in both native and recombinant forms to combat avian cholera, bovine respiratory illness, and HS [4, 13-16]. Vaccination with the recombinant form of OmpH, which has a molecular weight of 37 kDa, can induce both antibody- and cell-mediated immune responses in dairy calves and swamp buffaloes, protecting HS [4, 17]. Research conducted by Maulana et al. (2018) demonstrated that 37-kDa OmpH from a local strain of P. multocida isolated from NTT and Katha strains exhibit similar B-cell epitopes [18].

Immunoinformatics is a valuable technique for identifying new antigenic epitopes that can be used to design new vaccines against a variety of infectious diseases, including peptide-based vaccines. Given the advancement of immunoinformatics technologies, more accurate prediction of B-cell epitopes can now be made. Compared to strict laboratory studies, employing immunoinformatics methods to anticipate epitopes and develop peptide-based vaccines minimizes costs and saves time, whilst also elevating precision [19, 20].

This study was conducted to computationally evaluate the B-cell epitope of the 37-kDa OmpH derived from the amino acid sequence of P. multocida from NTT and Katha strains, and compare the epitopes of the two strains. The results can be used for further evaluation in the development of a peptide-based vaccine candidate.

Results

Multiple Sequence Alignment and Homology Analysis

Conserved and varied regions of the 37 kDa OmpH from NTT and other sources were evaluated through homology analysis and multiple sequence alignment as depicted in Figure 1. Specifically, Clustal W was employed to assess seven different sequences of the OmpH gene from NTT-Indonesia, China, Japan, India, Iran, the USA, and Katha strains. Most of the alterations were point mutations, whereas there were identified insertions at positions 66-71, and deletions at positions 60-61, 189, and 196-197. The Entropy (Hx) Plot in Figure 2 indicates high entropy, revealing a significant variation between each sequence. The highest entropy values of 0.95570 were was recorded at positions 66-71, and deletions at positions 60-61, 189, and 196-197. The homology analysis results showed that the 37 kDa OmpH asam amino sequence in local NTT isolates was significantly similar (>95%) to the Katha strain and isolates from China, India, Iran, Japan, and the USA (Table 1).

Prediction of the B-Cell Epitope of the OmpH P. multocida isolates of NTT and Vaccines

Kolaskar and Tangoankar method was employed to predict both the NTT isolates and Katha strain, possessing 11 potential B-cell epitopes within the 37 kDa OmpH gene that met the specified threshold value. In addition, the ABCpred prediction server was utilized in this study for predicting linear B-cell epitope regions in an antigen sequence through an artificial neural network. The results of both methods

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Figure 1.
Multiple alignment of amino acid sequences of 37 KDa gene OmpH P. multocida NTT isolates compared with and isolates from GenBank

Figure 2.
Entropy (Hx) Plot graphic of multiple sequence alignment of all amino acid sequence of 37 KDa gene OmpH P. multocida NTT isolates with vaccines and isolates frim GenBank

Table 1.
Homological value of the nucleotide sequences of OmpH P. multocida NTT isolate and vaccine

<table>
<thead>
<tr>
<th>Comparison isolate (Data GenBank)</th>
<th>NTT Isolate QDC35621 (%)</th>
<th>Katha Strain QDF60493.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer membrane protein [Pasteurella multocida]. Accession Number ABR24803.1 - China</td>
<td>99.30%</td>
<td>98.95%</td>
</tr>
<tr>
<td>Adhesive protein, partial [Pasteurella multocida]. Accession Number ABX58059.1 - India</td>
<td>98.95%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Outer membrane protein, partial [Pasteurella multocida]. Accession Number AAC02257.1 - USA</td>
<td>96.92%</td>
<td>95.24%</td>
</tr>
<tr>
<td>OmpH, partial [Pasteurella multocida]. Accession Number AQM74565.1 - Iran</td>
<td>96.54%</td>
<td>95.53%</td>
</tr>
<tr>
<td>Outer membrane protein [Pasteurella multocida]. Accession Number ABD94067.1 - Japan</td>
<td>95.85%</td>
<td>94.85%</td>
</tr>
<tr>
<td>Katha Strain Accession Number QDC35621</td>
<td>98.95%</td>
<td>100%</td>
</tr>
</tbody>
</table>

χ²(3)=1532, p =< 0.001

were cross-checked, and overlapped epitopes were shortlisted. Next, only five epitopes were ultimately selected and listed in Tables 2 and 3.
### Antigenicity Analysis

Antigenicity analysis was conducted using the Vaxijen test with a threshold value of 0.5 (tables 4 and 5). Out of the five epitopes, one appeared to be antigenic in both NTT and Katha strains with the same peptide sequence ALEVGLN and a score of 1.3471.

### Conservancy Analysis

Conservation analysis was performed on 100 sequences retrieved from the NCBI database via the BLASTp tool against the NTT isolate. Only ALEVGLN was deemed as a possible antigen when it came to antigenicity, while both GFVVAGL and ALEVGLN showed relatively high conservation levels (93%) (Table 6).

### Table 4.
Prediction of B-cell epitope antigenicity from outer membrane protein H (OmpH) amino acid sequences of P. multocida local isolates NTT

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Score</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVGVSDFYFLGG</td>
<td>0.2146</td>
<td>Probable Non-Antigen</td>
</tr>
<tr>
<td>GAYVFLSA</td>
<td>-0.1360</td>
<td>Probable Non-Antigen</td>
</tr>
<tr>
<td>GFVVAGL</td>
<td>-0.1978</td>
<td>Probable Non-Antigen</td>
</tr>
<tr>
<td>SQKYVTKVA</td>
<td>0.2844</td>
<td>Probable Non-Antigen</td>
</tr>
<tr>
<td>ALEVGLN</td>
<td>1.3471</td>
<td>Probable ANTIGEN</td>
</tr>
</tbody>
</table>

*threshold score 0.5

### Table 5.
Prediction of B-cell epitope antigenicity from outer membrane protein H (OmpH) amino acid sequences of Katha vaccine strain

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Score</th>
<th>Probability</th>
</tr>
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<tbody>
<tr>
<td>DVGVSDFYFLGG</td>
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<td>ALEVGLN</td>
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</tr>
</tbody>
</table>

*threshold score 0.5

### Table 6.
Conservation analysis of selected peptides from P. multocida NTT isolate and Katha strain through the IEDB epitope conservancy analysis website

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>%Protein Sequence Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVGVSDFYFLGG</td>
<td>84.00% (84/100)</td>
</tr>
<tr>
<td>GAYVFLSA</td>
<td>74.00% (74/100)</td>
</tr>
<tr>
<td>GFVVAGL</td>
<td>93.00% (93/100)</td>
</tr>
<tr>
<td>SQKYVTKVA</td>
<td>28.00% (28/100)</td>
</tr>
<tr>
<td>ALEVGLN</td>
<td>93.00% (93/100)</td>
</tr>
</tbody>
</table>

Antigenicity analysis was conducted using the Vaxijen test with a threshold value of 0.5 (tables 4 and 5). Out of the five epitopes, one appeared to be antigenic in both NTT and Katha strains with the same peptide sequence ALEVGLN and a score of 1.3471.

Conservation analysis was performed on 100 sequences retrieved from the NCBI database via the BLASTp tool against the NTT isolate. Only ALEVGLN was deemed as a possible antigen when it came to antigenicity, while both GFVVAGL and ALEVGLN showed relatively high conservation levels (93%) (Table 6).
Discussion

The OmpH is an antigenic surface protein in the envelope of Pasteurella multocida. It has been identified in all bovine isolates and is being considered a vaccine candidate by some researchers [2]. Conserved and varied regions of the 37 kDa OmpH protein from NTT and other sources were assessed through multiple sequence alignment. The entropy (Hx) plot graphs were utilized to illustrate the variations between sequences. The entropy values had a range of 0-1. The more varied a sequence, the higher the entropy value. The positions with the highest entropy were 61 and 299. Sequences resulting in high entropy alignment are unlikely to make good vaccine candidates. An entropy value of 0 was observed in regions 1-7, 14-27, 29-59, 78-188 191-195, 198-235, 240-248, 254-265, and 267-298. In the low-entropy alignment, it is crucial to consider regions that correspond to very low entropy when designing peptide vaccines. This highly conserved region across antigens results in the formation of antibodies by the peptide, which protects against pathogens of different serotypes. Furthermore, this region demonstrates several critical functions due to its high level of conservation, making it resistant to mutation under positive selection pressure. In addition, the binding of antibodies to this region deactivates essential functions [21]. The 37 kDa amino acid sequence of OmpH local isolates in NTT showed high identity (>95%) with the Katha strain and isolates from China, India, Iran, Japan, and the USA, suggesting that the nucleotide sequences of NTT, vaccines, and other reference isolates may share a common ancestor [22].

A B-cell epitope refers to a defined region on the surface of an antigen that binds to an antibody. There are two categories of epitopes, comprising continuous (linear or sequential) and discontinuous (non-linear or conformational) epitopes [23]. Linear B-cell epitopes are made up of peptides that can be readily used in immunization and antibody production as substitutes for antigens [24]. The Kolaskar and Tongaonkar methods revealed that both the NTT isolates and Katha strain possess 11 potential B-cell epitopes in the 37 kDa OmpH gene that surpass the specified threshold value in this study. The Kolaskar and Tongaonkar antigenicity techniques evaluate the antigenicity dependent on the physiochemical properties of amino acids as well as the number of experimentally confirmed epitopes [25].

Another prediction method for B-cell epitopes utilized in this study was the ABCpred prediction server. This server implements an artificial neural network to predict the linear regions of B-cell epitopes in an antigen sequence. The methodology for this server is developed on a recurrent neural network (machine-based technique), which relies on fixed-length patterns. The accuracy of this prediction method is 65.93%.

ABCpred was utilized to identify potential B-cell epitopes from 37 kDa OmpH P. multocida NTT isolate and Katha strain. The results from both methods were cross-checked, narrowed down, shortlisted to overlapping epitopes, and only five were selected. The combination of both methods can enhance the results of the prediction accuracy of B-cell epitope [26].

The peptides in the epitope prediction were chosen using the Kolaskar and Tongaonkar antigenicity methods and subsequently verified with the ABCpred method. They were then tested for antigenicity using the VaxiJen test, with a minimum threshold value of 0.5. VaxiJen is a pioneering server for the alignment-independent prediction of protective antigens [27]. Out of the five epitopes, one appeared to be antigenic in both NTT and Katha strains with the same peptide sequence ALEVGLN, scoring 1.3471. The high antigenicity value of this peptide sequence indicates its potential as an ideal vaccine candidate. A higher antigenicity value translates to a greater ability to induce the production of specific antibodies by B-cells [28].

The 37 kDa OmpH peptides with fairly high conservation (93%) comprise GFVVAGL and ALEVGLN, although only ALEVGLN is considered a probable antigen. In a study conducted by Bui et al. [29], conservation refers to the part of a protein sequence that contains an epitope that is considered to be at or above a certain level of identity. Regions with lower variability in the parts of the protein sequence that contain epitopes indicate greater epitope uniqueness. This indicates the degree of variability or uniqueness of the epitope. Therefore, these regions often serve as good targets for the development of epitope-based vaccines since the targeted epitopes are present in various strains of specific pathogens.

Conclusion

According to the findings of our bioinformatics studies, there was only one epitope of 37 kDa OmpH P. multocida both from NTT isolate and Katha strain that met the selected criteria of antigenicity and conservancy analysis. The epitope “ALEVGLN” is hypothesized to be the most potential candidate for a seed epitope/peptide vaccine within the 37 kDa OmpH. Further studies are needed to investigate the conserved regions of OmpH and assess their efficacy as vaccines against P. multocida infection.
Materials & Methods

Data Retrieval

The amino acid sequences of the 37 kDa OmpH P. multocida isolate retrieved from NCBI with accession numbers QDC35621 and QDF60493, as well as other reference isolates with accession numbers ABR24803.1, ABX58059.1, AAC02257.1, AOM74565.1, and ABD94067.1 were analyzed [31].

Multiple Sequence Alignment

Amino acid sequences obtained from NCBI were analyzed for multiple sequence alignment by employing the CrustalW method in the BioEdit program. Amino acid sequences of seven different strains were included in this analysis to identify the conserved regions of 37 kDa OmpH [32].

Homology Analysis

Homology analysis was performed using the BLASTp program at NCBI [22]. Specifically, the amino acid sequence of the 37 kDa OmpH of the NTT local isolate was compared to the Katha strain and five other isolates from different countries retrieved from GenBank, NCBI.

Epitope Prediction

Epitope prediction was performed using the IEDB B-Cell epitope prediction tool (http://tools.iedb.org/bcel/) with default thresholds. The Kolaskar and Tongaonkar antigenicity method was employed, which can predict antigenic determinants with approximately 75% accuracy. Moreover, ABCpred (http://crdd.osdd.net/raghava/abcpred/) was utilized, which can achieve an accuracy of up to 65.93% using recurrent neural networks with a 0.5 threshold [24]. Predicted peptides were acquired and analyzed further for their antigenicity using the VaxiJen v 2.0 tools [27].

Antigenicity Analysis

The antigenicity analysis was completed using VaxiJen v 2.0 online platform (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The targeted proteins in VaxiJen v2.0 were predicted using the auto-cross covariance method with a threshold of 0.5 [30].

Conservancy Analysis

The epitope conservancy analysis tool used in this study was epitope conservancy analysis (http://tools.iedb.org/conservancy/) at the IEDB (Immune Epitope Database) webserver with sequence identity threshold set at 100%. Selected peptide sequences from the previous step were assessed for their conservation among 100 homologous sequences of P. multocida retrieved from the NCBI database where BLASTp was performed against the NTT isolate [29].

Authors’ Contributions

F.K.M and D.H conceived and planned the experiments. F.K.M carried out the experiments. F.K.M and D.H. contributed to the interpretation of the results. F.K.M took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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