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Research Article

Development of Brucellosis Vaccine Based on Determinant Antigenic of Outer Membrane Protein (OMP) 36 kDa From *Brucella abortus* Local Isolate

Aulanni'am Aulanni'am[1*], Wiwiek Tyasningsih[2], Dyah Kinasih Wuragil[1], Fedik Abdul Rantam[2]

[1]*Faculty of Veterinary Medicine, Brawijaya University, INDONESIA*
[2]*Faculty of Veterinary Medicine, Airlangga University, INDONESIA*

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ABSTRACT

Brucellosis is a disease that can be prevented through vaccination. Yet, the effectiveness of the vaccination to fight this disease is considered weak. Fortunately, attempts to modify brucellosis vaccine is still keep going. Some brucellosis vaccines have been found and developed in the past time such as the vaccine *B.abortus* strain 19-BA and 104M which was made from weakened microbes which had been widely used in Uni Soviet and China. The other brucellosis vaccine that were used in the past were the phenolinsoluble peptidoglycan vaccine which was made in France and polysaccharide-protein vaccine which was used in Russia. This research attempted to see the determinant of antigenic Outer Membrane Protein (OM) 36 kDa *Brucella abortus* local isolation which has immunogenic character to be developed as an advanced brucellosis vaccine. The method used in this research was the *Omp2* gene *of Brucella abortus* of local isolate employed the PCR technique. The result of the PCR was then sequenced to analyze the determinant antigenic and the bounding prediction of either the T cell or the B cell which were responsible for immune response. The result of this study showed that the gen *Omp2* which encoded the OMP 36 kDa *Brucella abortus* of local isolation with primary JPF 5' GCG CTC AGG CTG CCG ACG CAA 3' and JPR 5' CAT TGC GGT CGG TAC CGG AG 3' targeted the gene 162 bp, was then translated into amino acids to be later undergo the *in silico* test using Kolaskar & Tongaonkar Antigenicity Prediction method. The epitope prediction resulted were MSRVCDAYGAGYFYI and TETCLRVHGYVRYD. The result of the epitope prediction of MSRVCDAYGAGYFYI showed that there was a bond with MHC I in YGAGYFYI of the 8th amino acid series to the 15th series, while the epitope prediction of TETCLRVHGYVRYD showed that there was a bond to the ETCLRVHGY of the series of amino acids number 2 to 10. Bond with MHC II existed in the amino acid series of MSRVCDAYGAGYFYI, while the bond with the B cells existed in BCSAYGA and CLRVHG amino acid series. This research has been successful in predicting the epitope of the OMP 36 kDa *Brucella abortus* of local isolate which had immunogenic characteristic for its ability to bond with the MHC I, MHC II and B cells.

Keywords: determinant antigenic, OMP 36 kDa, *Brucella abortus.*

INTRODUCTION

Brucellosis is a disease that is caused by *Brucella sp* which is a bacteria with negative Gram in the form of coccobacilli and intra-cell facultative[1,2]. Brucellosis can be spread through either direct contact or indirect contact with infected animals as well as the product of the animals. This disease can also be spread by consuming milk or dairy products without being pasteurized from infected animals and it can also spread from body fluids or tissues of infected animals that contact the non-intact skin[2,3].

Brucellosis in animals is often called infectious Bang's Disease which is caused by the bacteria of the Brucella genus which have intracellular facultative characteristic that makes the medication toward animals that suffer from this disease become ineffective[4]. There are many Brucella species from the natural hosts of the *Brucella abortus* (B. *abortus)* found in cows, B. *melitensis* found in goats, and B. *suis* found in pigs which are zoonotic. Whereas, in human, this disease is called Undulant Fever or Malta Fever. Brucellosis is a hazardous disease for cattle that can cause huge economic loss such as abortion to pregnant animals (*gravid),* decreasing amount of milk production up to temporary or permanent nuisances on reproduction system[5,6], stated that Brucellosis caused financial loss of around 385 trillion rupiahs per year for it caused abortion, young mortality, sterility, infertility and decreased milk production.

In Indonesia, Brucellosis was found for the first time in 1935 which serologically found in cows in Grati, Pasuruan district, East Java[7]. At present time, this disease has spreaded to 26 provinces in Indonesia causing major economic and social loss. The case of the Brucellosis tends to be increasing seen from its number of case (level of prevalency) as well as its number of distribution. This

**Author for Correspondence: aulani@ub.ac.id*

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          10        20        30        40        50        60
    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
GGCTGCCACC CAAACTTGAT ATGTCCCGCG TTTGCGACGC TTACGGCGCT GGCTACTTCT

          70        80        90       100       110       120
    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ACATTCCGGG CACCGAAACC TGCCTGCGCG TCCATGGTTA CGTCCGTTAC GACGTAAAGG

         130       140       150       160
    ....|....| ....|....| ....|....| ....|.. 
GCGGCGATGA CGTTTACTCC GGTACCGACC GCAATGATTT TT
```

Table 1: Amino Acid Series from Sequensing Process of *Omp2 Brucella abortus* Gene.

Types	Amino Acid Series
Local *Brucella abortus*	XLPPKLD**MSRVCDAYGAGYFYIP**G**TETCLRVHGYVRYD**VKGGDDVYSGTDRNDX

Notes : Letters in bold show epitope prediction of amino acid series.

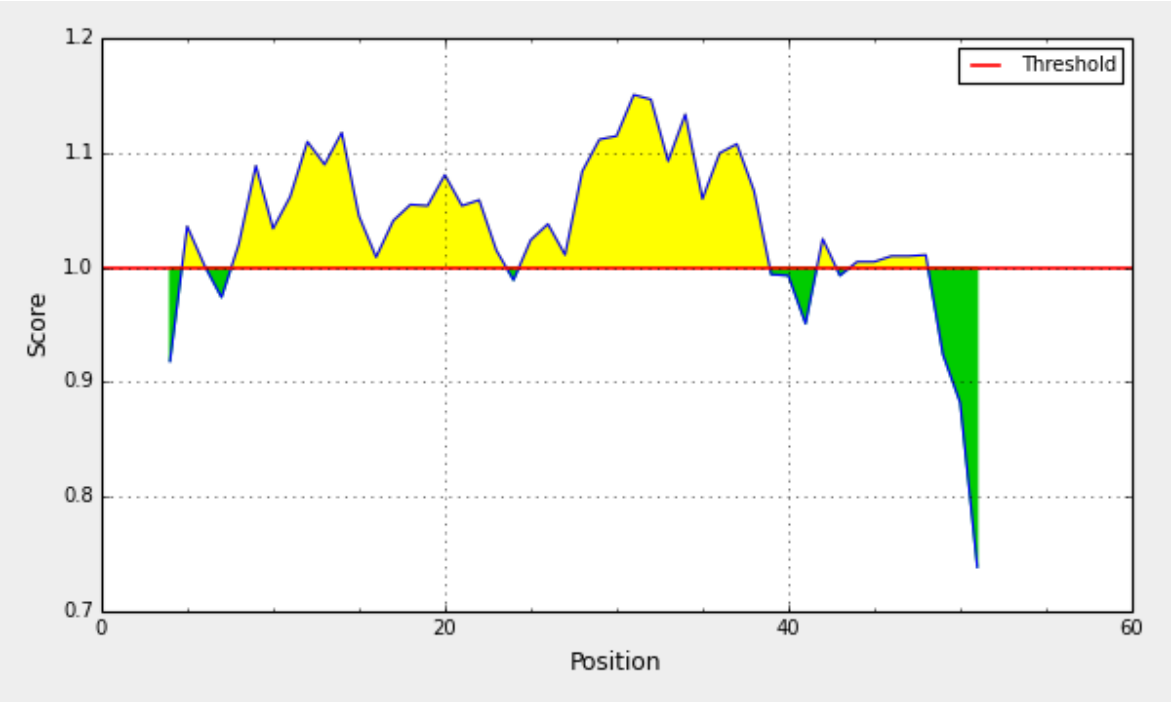

Figure 1: Epitope Prediction of OMP 36 kDa *Brucella abortus* Local Isolate (Using Kolaskar & Tongaonkar Antigenicity Prediction). Protective epitope is shown in yellow mark.

problem is caused by the trasnfer of the cattle from an area to another area which appears to be huge threats to the development of cattle especially the milking cows.

The use of *Brucella abortus* S19 vaccine has not yet achieved an optimum result in preventing the case fo Brucellosis in milking cows[6]. It was assumed that the protective level of the vaccine was still around 65% - 75% which was still far from the expectation. The data from the field evaluation showed that there were still found high number of Brucellosis case in milking cow farming in Indonesia.

Bacterial outer membrane protein (OMP) is a potential antigen that directly induce the humoral immune respose which quicky triggers the creation of antibody[8,9]. *Brucella abortus's* OMP which consists of complex amino acids are the peptide chain which function as epitope or antigenic determinant that can be potential to be developed as advanced vaccine of Brucellosis disease. OMP *Brucella abortus* has been successfully identified and classified into three groups which were the group 1 of the protein which molecule weight is around 88 – 94 kDa, group 2 which molecule weight is around 36 - 38 kDa, and the group 3 which protein weight is around 25 – 27 kDa[10]. Meanwhile, the gene with OMP codes of 36 to 38 kDa is the *Omp2* while the gene of *Omp 25* holds the codes of OMP 25 to 27 kDa and the *Omp 31* is for the codes of OMP 31 to 34 kDa[11,12].

Brucella abortus local isolate has the virulence factor in the form of lipopolysaccharide (LPS) and outer membrane protein (OMP). OMP is a potential antigenic which is immunogenic that is able to induct the immune response[9]. OMP from the gram negative bacteria is immunogenic that

Table 2: The Prediction of the Epitope OMP 36 kDa Local Isolate *Brucella abortus* Bond to the T Cells MHC I (Immune Epitope Data Base).

Peptide Prediction	TETCLRVHGYVRYD					
B. abortus	#	Start	End	Length	Peptide	Method	
	1	2	10	9	ETCLRVHGY	smm	6
Peptide Prediction	MSRVCDAYGAGYFYIP					
	1	8	15	8	YGAGYFYI	netmhcpan	0,3

Table 3: The Prediction of the Epitope OMP 36 kDa Local Isolate *Brucella abortus* Bond to the T Cells MHC II (Immune Epitope Data Base).

Peptide Prediction	MSRVCDAYGAGYFYIP				
	Start	End	Peptide	Method	
B. abortus	1	15	MSRVCDAYGAGYFYI	Smm/nn/stumiolo	5.03

Table 4: Bond Prediction of Local Isolate Epitop OMP 36 kDa *B. abortus* to the B Cell (Kolaskar & Tongaonkar Antigenicity Prediction).

Brucella abortus Local Isolate					
Peptide Prediction	MSRVCDAYGAGYFYIP				
Position	Residue	Start	End	Peptide	
7	A	4	10	VCDAYGA	1.118
Peptide Prediction	TETCLRVHGYVRYD				
7	V	4	10	CLRVHG	1.151

it inducts the immune response which can be used as the component of subunit vaccine development[13].This research attempted to investigate the antigenic determinant molecule or the epitope of OMP which can be seen from *in silico* test using the sequenced OMP2 gene that can be used as the vaccine candidate to fight the local isolate Brucellosis. This research was done due to the fact that the use of the *Brucella abortus* S19 has not yet reaching its optimum result in preventing and fighting the Brucellosis disease found in cattle in Indonesia.

Presently, research and development is still on progress to create vaccine from the *Brucella abortus* bacteria of the local isolate to be used as the vaccine seeds or diagnostic elements that will decrease the dependence on the imported elements. OMP 36 kDa from local isolate *Brucella abortus* has immunogenic and protective functions[14].Thus, based on the explanation above, it was necessary to conduct a research that attempted to see the epitope prediction which is the main virulence factor of the OMP 36 kDa *Brucella abortus* of local isolate.

METHOD

Isolated bacteria used in this study were obtained from the *Veterinary Research Center of Maros*, South Sulawesi, INDONESIA, through several research steps including the analysis of the *Omp 2 Brucella abortus* local isolate gene, sequencing the PCR product, analysis of the antigenic determinant and prediction on bounding of the T cells and B cells that were responsible for inducing immune responses.

Analysis of the Omp2 Brucella abortus Local-Isolate Gene with the Polymerase Chains Reaction (PCR)

Preparation of *Brucella abortus* DNA

DNA was isolated by salting out methode[14,15]. Quality and Quantity DNA were measured by using Nano Drop Spectrophometer and 1 % Agarose.

DNA Amplification

DNA of Brucella abortus was amplified by JFR dan JPR Primers:

JPF 5' GCG CTC AGG CTG CCG ACG CAA 3'

JPR 5' CAT TGC GGT CGG TAC CGG AG 3'

PCR Program : Hot start 94ºC for 1 minute, denaturation 95ºC for 30 seconds, annealing 66ºC for 60 seconds, extension 72ºC (35 cycles) and then post extension 72ºC for 7 minutes. PCR products qualitatively analyzed using 2 % agarose gel elactrophoresis. PCR product were sequenced by same primer to identified OMP 36.

The software analysis on the result of the sequencing process using Bioedit and BLAST (Basic Local Aligment Search Tool)

The analysis of the result of the Bioedit was done using the Sequence Aligment Editor program which analyzed the bioinformatics toward the sequence of DNA, RNA and protein. One of the processes was the sequence aligment procedure to analyze the sequence of a DNA fragment which was interpreted in two-way method (forward-reverse).

The result of the sequences were in the form of a series of nucleotide base which was then interpreted into amino acid series and were then analyzed in oder to see the prediction of the antigenic determinant or epitope in OMP 36 kDa of the *B. abortus* S19 and local isolate using the Kolastar and Tongaonkar Antigenicity Prediction Program. After the prediction of the epitope has been found, the prediction of the bond of each epitope with the T cells and the B cells which were responsible for creating immune response.

RESULTS AND DISCUSSIONS

The result of the sequencing process of *Omp2* local isolate *Brucella abortus* gene produced a PCR prodct of 224 bp[14], in this following base series:

Based on the result of the nucleotide sequencing process of the PCR product, prediction of the OMP2 epitope of the local isolate *Brucella abortus* was administered. The PCR process employed a pair of the JPF and JPR primary which resulted a series of amino acid as shown in Table 1.

Epitope prediction using Kolaskar and Tongaonkar Antigenicity Prediction method showed result as shown in Figure 1. The prediction result showed that there was a peptide chain in a part of the series of OMP2 peptide which functioned as epotipe (Figure 1, the yellow area).

The prediction result of epitope OMP 36 kDa local isolate *Brucella abortus* was MSRVCDAYGAGYFYIP which was located in the amino acid series number 8 to 23 and TETCLRVHGYVRYD in amino acid series number 25 to 38. The prediction of the epitope was then analyzed using the *Immune Epitop Data Base* (IEDB) to see the bond between the epitope and the T cells which were the MHC 1 and MHC II as well as its bond to the B cells using *Kolaskar & Tongaonkar Antigenicity Prediction.*

Table 2 showed the result that the amino acids that constructed the epitope OMP 36 kDa *Brucella abortus* of local isolate had strong bond to the T cell molecule of MHC I in the peptide of amino acid series ETCLRVHGY and YGAGYFYI which consisted of 9 and 8 amino acids. The result of the prediction using IEDB showed that the epitope OMP 36 kDa *Brucell abortus* of local isolate bounded with the T cell molecule of MHC II in the peptide series in the amino acid series of MSRVCDAYGAGYFYI. The result of the prediction showed that the epitope OMP 36 kDa *Brucella abortus* of local isolate bounded with the B cell of the peptide which was included in the amino acid series of VCDAYGA and CLRVHG. The ability of this epitope in bounding the lymphocyte of the T cell and the B cell would determine the immune response produced by an antigenic.

CONCLUSIONS

The antigenic determinant of the local isolate e OMP 36 kDa *Brucella abortus* had immunogenic characteristic since it had the ability to bond with the T cell MHC I through peptide chain of MSRVCDAYGAGYFYI and bond with the MHC II as well as the B cell through the peptide chain of VCDAYGA and CLRVHG using the *Kolastar and Tongaonkar Antigenicity Prediction*.

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