



## Molecular analysis of ompA gene *Pasteurella multocida* Indonesia local isolates

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### Abstract

The aim of this research was to analyze ompA molecular gene of *Pasteurella multocida* buffalo isolate and bovine isolate from Nusa Tenggara Timur, Indonesia and Katha strain isolate from hemorrhagic septicemia vaccine. Determinant of *P. multocida* local isolates ompA gene amplification sequencing PCR then conducted to see the sequence of nucleotide sequences of ompA gene. The results of PCR amplification showed an amplicon of 559 bp of all isolates. The homology analysis result of the isolates ranged from 93 - 100% with 13 *P. multocida* isolates from GenBank, and phylogenetic tree analysis shows that buffalo isolate was closely related to Katha strain, Iran, India and China isolate. Whereas bovine isolate far enough with buffalo and Katha strain isolate. Nucleotide sequences were compared to amino acids then by the method of Kolaskar and Tongaonkar antigenicity predicted antigens in *P. multocida*. B cell epitope predictions from local isolates and Katha strain were found in five peptides QVSPVFAG, IPELALRVEYQ, QGSVYVPEVVSKT, LKSASVAVAG, and ANYLVAKG.

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### Introduction

Haemorrhagic Septicaemia (HS) or snoring disease is an infectious disease caused by acute to fatal bacteria in ruminants including cattle and buffaloes in tropical countries, including Asia and the African continent. The causes of this disease are *Pasteurella multocida* type B:2 in Southeast Asia and E:2 in Africa (1). Haemorrhagic Septicaemia has spread in all regions of the world that have high rainfall, including Indonesia, Malaysia, the Philippines and Thailand (2). The losses caused by HS disease reach 100,000 dead each year in several Asian regions (3). The control of HS in Indonesia uses the Katha vaccine, the inactive vaccine of *P. multocida* strain Katha from Burma. The Katha vaccine provides immunity for five months and is injected twice a year (2). Recently

recombinant vaccines have been widely studied manufacture vaccines and recombinant DNA sequencing technology have created shortly knew raft in vaccines epitope-based, which is able to stimulate specific immune responses, has been identified and used to achieve advanced vaccine formulation, which can replace the overall formulation pathogens (4). Epitope-based vaccines are specific, capable of avoiding unwanted, producing immune responses long immunity, and the price is cheaper (5). The outer membrane protein encoding genes have been widely studied and some of them are used as the basis for making vaccines which heterologically can protect against infection with several strains of *P. multocida* (6), one of which is ompA can be used as a vaccine because it is immunogenic (7,8). *P. multocida* ompA gene consists of 1047 to 1077 nucleotides that encode proteins of 349 to 360 amino acids. Some studies state that

ompA is an immunogenic, surface-exposed, and expressed antigen in vivo (9), and has been shown to cause antibodies to *P. multocida* in cattle (10,11) and mice (9). The OMP with a molecular weight of 37 kDa was an immunodominant protein in *P. multocida* isolates that caused HS (12). The specific part of the *P. multocida* encoding gene with a molecular weight of 37 kDa can be cloned and expressed that its recombinant proteins act as specific antigen for development diagnostic test (13). 37 kDa ompA from *P. multocida* isolates were obtained by heating 100°C for 5 minutes by SDS-PAGE (14).

The aim of this research was to analyze ompA molecular gene of *Pasteurella multocida* buffalo isolate and bovine isolate from Nusa Tenggara Timur, Indonesia and Katha strain isolate from hemorrhagic septicemia vaccine.

## Materials and methods

### Preparation of bacterial isolates

*Pasteurella multocida* local isolates from buffalo and bovine were obtained from the Bali Veterinary Center in Denpasar and *P. multocida* Katha strain isolate from the hemorrhagic septicaemia vaccine. Local isolates are cultured in blood agar. The reconfirm tests are biochemical test: Triple Sugar Iron Agar, Sulfide Indole Motility, Simmon's Citrate Agar, and urea; gram staining; and growth on MacConkey Agar.

### PCR amplification

*Pasteurella multocida* local isolates and Katha strain isolate were determined by polymerase chain reaction amplification with specific primers, which is modification of research (15,16). Forward: 5'CGCATAGCACTCAAGTTTCTCC 3' and Reverse: 5'CGATCGTCAGCTAAACATGC 3' with 559 bp amplicon. Then the genomic DNA of *P. multocida* isolates are obtained by DNA extraction using genomic DNA purification kit GeneJET of Thermo Fisher Scientific. The PCR product amounts to 25µl which consists of 5µl DNA templates, 1µl each primer, 12.5µl master mix, and 0.5µl aquades are then inserted into the microtube. Initial denaturation at 94°C for five minutes, denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, elongation at 72°C for 30 seconds, and final elongation of 72°C for five minutes with 35 cycles.

### Nucleotide Sequences Analysis

Nucleotide sequencing of *P. multocida* local isolates and Katha strain isolate PCR product at CV Biotek Prima Indoplus, Sidoarjo. The nucleotide sequence homology analysis using Nucleotide BLAST program at <http://www.ncbi.nlm.nih.gov>, as well as phylogenetic tree analysis by Mega- X using Neighboring joining method and bootstrapped to ruminant isolates from several countries in the world from NCBI GenBank (CP017961.1, JQ859919.1,

AY903603.1, HQ829458.1, AY643794.1, AY643797.1, KU342614.1, KU342613.1, KU342627.1, JQ230325.1, KU342629.1, JX473017.1, and KU342624.1) and predictions using the Ellipro Epitope Program: Epitope Prediction Antibody on <https://www.iedb.org/> with the Kolaskar and Tongaonkar antigenicity methods.

## Results

*Pasteurella multocida* local isolates colonies look gray, has a distinctive smell and no hemolytic the media of Blood Agar which has been incubated at 37°C for 24 h. Biochemical tests showed the properties of *P. multocida* bacteria and Gram staining appeared to be red colony, coccobacilli, and encapsulated. The results of PCR amplification of local isolates and Katha strain isolate using forward and reverse primers showed 559 bp which were in accordance with the number of amplicon targets used in the manufacture of ompA encoding gene primers (Figure 1).

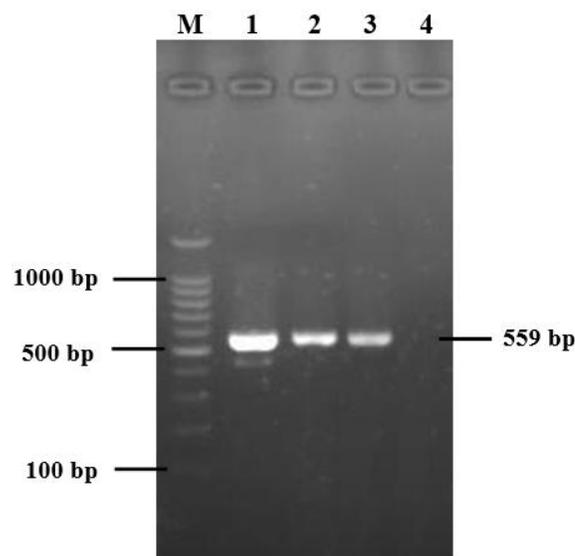


Figure 1: PCR amplification ompA gene of *P. multocida* isolates with amplicon 559 bp. Lane M: DNA ladder 100 bp Vivantis, 1: buffalo isolate, 2: bovine isolate, 3: Katha strain, 4: negative control.

The ompA gene local isolates and Katha strain isolate represent nucleotide sequence length of 559 bp encoding 186 amino acids. The results of homology analysis of local isolates and Katha strain isolates with GenBank data using the nucleotide BLAST program at NCBI ranged from 93 - 100%. The highest results in the analysis of nucleotide sequence homology between buffalo isolates and Katha strains with ompA gene of *P. multocida* isolates from eight ruminants: Iran (bovine, CP017961.1), India (bovine, AY903603.1), India (bovine, HQ829458.1), India (cattle,

KU342613.1), India (calf, KU342627.1), China (yak, JQ230325.1), and India (sheep, KU342624.1). While the analysis of nucleotide sequence homology of bovine isolate with comparative ruminants isolates did not reach 100% but only reached 96% with eight ruminant isolates: India (bovine, AY903603.1), India (bovine, HQ829458.1), USA (bovine, AY643797.1), India (buffalo, KU342614.1), India (cattle, KU342613.1), India (calf, KU342627.1), China (yak, JQ230325.1) and India (sheep, KU342624.1). Phylogenetic tree analysis show that buffalo isolate and strains Katha isolate are related to bovine from India (AY903603.1,

HQ829458.1), and Iran (CP017961.1); buffalo from India (KU342614.1); yak from China (JQ230325.1); calf from India (KU342627.1); and cattle from India (KU342613.1), while bovine isolate were in different clusters with buffalo isolate and strains of Katha isolate (Figure 2).

B cell epitope prediction in all isolates were found five peptides. There have the same position, length, and peptide sequence with score range 1,031 - 1,118. The peptides were QVSPVFAG, IPELALRVEYQ, GQSVYVPEVVSKT, LKSASVAVAG, and ANYLVAKG (Figure 3, Table 2).

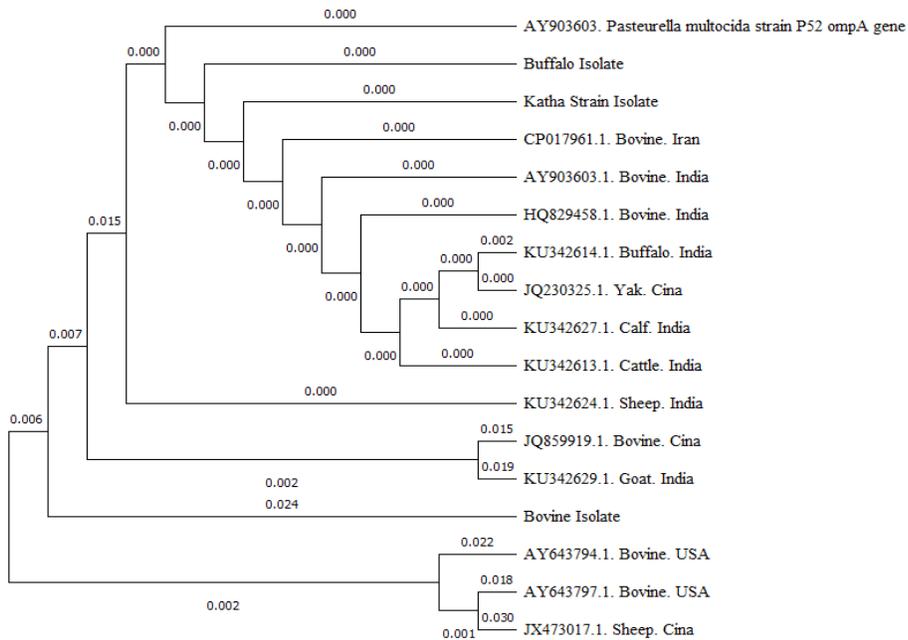


Figure 2: Phylogenetic tree neighbor joining method analysis nucleotide sequence of ompA gene *P. multocida* local isolates with 13 *P. multocida* ruminant isolates from GenBank.

Table 1: The nucleotide homology analysis of *P. multocida* ompA gene from buffalo isolate, bovine isolate, and Katha strain against comparable country ruminants isolates from GenBank

No.	Ruminant Isolates (GenBank)	Buffalo Isolate	Bovine Isolate	Katha Strain
1.	CP017961.1. Bovine. Iran.	100%	96%	100%
2.	JQ859919.1. Bovine. Cina.	97%	95%	97%
3.	AY903603.1. Bovine.India.	100%	96%	100%
4.	HQ829458.1. Bovine. India.	100%	96%	100%
5.	AY643794.1. Bovine. USA	93%	93%	93%
6.	AY643797.1. Bovine. USA.	95%	96%	95%
7.	KU342614.1. Buffalo. India.	99%	96%	99%
8.	KU42613.1. Cattle. India.	100%	96%	100%
9.	KU342627.1. Calf. India.	100%	96%	100%
10.	JQ230325.1. Yak. Cina.	100%	96%	100%
11.	KU342629.1 Goat. India.	97%	95%	97%
12.	JX473017.1. Sheep. Cina.	94%	94%	94%
13.	KU342624.1. Sheep. India.	100%	96%	100%

Table 2: Epitopes prediction of ompA gene *P.multocida* from buffalo isolate, bovine isolate, and Katha strain

No.	Position	Peptida	Long	Log score		
				Buffalo isolate	Bovine isolate	Katha strain
1.	4 - 11	QVSPVFAG	8	1,095	1,095	1,095
2.	18 - 28	IPELALRVEYQ	11	1,081	1,084	1,081
3.	62 - 74	GQSVYVPEVVSKT	13	1,032	1,118	1,118
4.	108 - 117	LKSASVAVAG	10	1,099	1,099	1,099
5.	140 - 147	ANYLVAKG	8	1,11	1,11	1,11

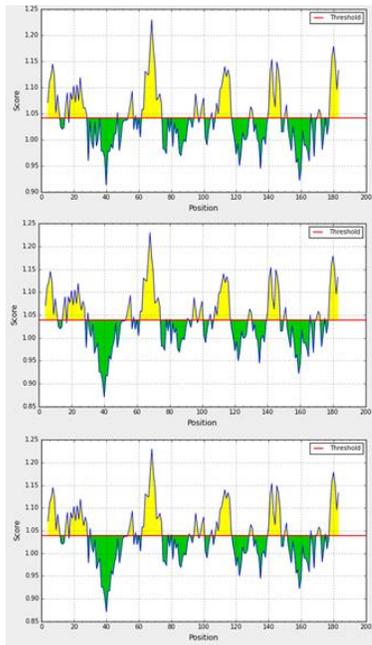


Figure 3: Graphical representation epitope prediction of ompA gene *P. multocida*. Buffalo isolate (a), bovine isolate (b), and strain Katha (c).

**Discussion**

This study used three sample of *P.multocida* isolate from buffalo and bovine from Nusa Tenggara Timur obtained from the Denpasar, Bali Veterinary Center and also *P.multocida* strain Katha isolate from HS vaccine. The study was conducted to see the presence of an ompA encoding gene in local isolates and Katha strain isolates, nucleotide sequences and amino acid sequences obtained from local isolates and Katha strain isolates were homologized with sequences of ruminant isolates from several countries in the world, and prediction of B cell epitopes from amino acid sequences of *P. multocida* local isolates.

The *P. multocida* local isolates were reconfirmed bacteria tests, then local isolates and Katha strain isolate determinant an ompA gene using the PCR amplification technique showing a result of 559 bp using modified primers with amplicon 201 bp (15) and 965 bp (16). Therefore in this

study using the primers design of two different studies so that the results of the amplification obtained were 559 bp.

The results of the analysis of nucleotide sequences of buffalo isolate and Katha strain isolate showed the highest number reached 100% while bovine isolate only reached 96% with ruminant isolates from several countries in the world from GenBank. There was homogeneity of antigens in the protein profile of six isolates of *P.multocida* type B from cattle and buffalo (17). High homology can be used as a basic reference for candidates to use vaccines and diagnostic kits. This is due to *P.multocida* from isolates in Indonesia probably originating from these countries or vice versa through export and import activities from buffalo and cattle between countries (18). Low levels of diversity in OMP profiles can be attributed to genetic diversity is lacking in *P.multocida* isolates causes of HS (19,20).

Based on the phylogenetic tree analysis results, it was found that among the ompA gene of *P.multocida* buffalo and strain isolate Katha had close relationships with bovine from India (AY903603.1, HQ829458.1), and Iran (CP017961.1); buffalo from India (KU342614.1); yak from China (JQ230325.1); calf from India (KU342627.1); and cattle from India (KU342613.1), while bovine isolate had a long relationship with buffalo isolate and Katha strain isolate. The smaller the scale on the phylogenetic tree, the closer the relationship. While the large scale on the phylogenetic tree, the kinship is further away. In addition, the results of homology and phylogenetic analysis in a study with a composition of 600 bp will have different results with a longer size of nucleotides. The results of homology and phylogenetic tree analysis will be clearer and more valid with longer nucleotide sizes, because of the broader scope of nucleotide data. According to previous research (6) that molecular evolution of ompA proteins can be used to classify *P.multocida* isolates into various types of capsules, host types, and levels of pathogenicity. The ompA is involved in adhesion and experiences strong selective pressure, further research is needed to clarify whether polymorphisms affect protein function (16).

B cell epitope prediction of Kolaskar and Tongaonkar antigenicity method based on a single-parameter with semi-empirical method which judiciously makes use of physicochemical properties of amino acid residues and experimental data, is developed to predict antigenic

determinants, and its accuracy has been tested by application to a large number of proteins with about 75% accuracy (21). 186 amino acids from all isolates found five peptides predicted as epitope / antigenic protein with log score above 1, the highest score is 1.118 and the lowest score is 1.084. Interpretation of output graph (Figure 3) and table (Table 2) which is higher score have a probability as epitope that antigenic potential shown in the yellow graph with value above 1 (22). Epitop is very attractive for clinical and basic biomedical researchers because epitopes have great potential for vaccine design, disease prevention, diagnosis and treatment. Using rDNA technology, specific epitopes can replace overall pathogen in the vaccine (4). OMP plays an immunoprotective role and has strong potential for the development of candidate subunits vaccine against HS (23). Exploring the molecular ompA gene of *P. multocida* local isolates provided information about understanding the spread of *P. multocida* bacteria and control strategies using epitope-based vaccines.

## Conclusion

Nucleotide sequences were compared to amino acids then by the method of Kolaskar and Tongaonkar antigenicity predicted antigens in *P. multocida*. B cell epitope predictions from local isolates and Katha strain were found in five peptides QVSPVFAG, IPELALRVEYQ, GQSVYVPEVVSKT, LKSASVAVAG, and ANYLVAKG.

## Conflict of interest

No conflict.

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تم تضخيم العزلة المحلية لجرثومة *P. multocida* المحددة بواسطة تفاعل البلمرة المتسلسل ثم أجري تسلسل القواعد النيتروجينية لجين ompA. وأظهرت نتائج التضخيم للقطعة المراد تضخيمها ودراساتها بوزن جزيئي ٥٥٩ زوج قاعدي في جميع العزلات. تراوحت نتائج تحليل التشابه لتسلسل ١٣ عزلة من العزلات مع تسلسل العزلات المثبتة في بنك الجينات وبمدى ٩٣-١٠٠٪، وتحليل شجرة المظهرية الجينية لعزلات الجاموس كانت ذات علاقة وثيقة مع عزلات كاثا وإيران والهند والصين. في حين كانت عزلات الأبقار بعيدة كل البعد عن عزلات الجاموس وكاثا. تم مقارنة تسلسل القواعد النيتروجينية النيوكليوتيدات مع الأحماض الأمينية ثم عن طريق طريقة كولاسكار وتونغونكار المستضدية المتوقعة في مستضدات *P. multocida*. تم العثور على تنبؤات الخلية B من العزل المحلية وسلالة كاثا في خمس البيبتيدات، QGSVYVPEVVSKT، IPELALRVEYQ، QVSPVFAQ و LKSASVAVAG و ANYLVAKG.

## التحليل الجزيئي لجين ompA للعزلات المحلية لجرثومة *Pasteurella multocida* في إندونيسيا

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### الخلاصة

الهدف من هذا البحث هو التحليل الجزيئي لجين ompA لجرثومة *Pasteurella multocida* المعزولة من الجاموس والأبقار في نوسا تي نغارا تيمور، إندونيسيا وسلالة كاثا المعزولة من لقاح تسمم الدم النزفي.