DETECTION OF *Radopholus similis* IN ORGANS INFECTED BY *Radopholus multocida* B1-2 USING POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

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2007



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# DETECTION OF FIMBRIAL GENE IN ORGANS INFECTED BY *Pasteurella multocida* B: 2 USING POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

By

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***RESEARCH REPORT VERIFICATION***

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: DETECTION OF FIMBRIAL GENE IN ORGANS INFECTED BY *Pasteurella multocida* B: 2 USING POLYMERASE CHAIN REACTION (PCR) TECHNIQUE oleh Harny Chapi, no. matrik: UK10785 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi,Universiti Malaysia Terengganu.

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| | |
|---|---|
| bp | base pair |
| CFU | colony forming unit |
| $CO_2$ | carbon dioxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxynucleoside triphosphate |
| $ddH_2O$ | deionise distil water |
| $dH_2O$ | distil water |
| $H_2O_2$ | hydrogen peroxide |
| $H_2S$ | hydrogen sulphide |
| kDa | kilo Dalton |
| LPS | lipopolysaccharide |
| mer | Oligomer |
| mg | milligram |
| $MgCl_2$ | Magnesium chloride |
| mL | mililiter |
| OMP | outer membrane protein |
| OD | optical density |
| PCR | Polymerase Chain Reaction |
| rpm | rotation per minute |
| SDS | Sodium Dodcyl Sulphate |
| TE | Tris- EDTA |
| TAE | Tris-Acetate EDTA |
| TSI | Triple Sugar Ion |
| U | Unit(s) |
| V | Volt(s) |
| µg | microgram |
| µM | micromolar |
| µL | microliter |
| $^0C$ | Degree Celsius |

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# ABSTRACT

Rapid detection of *Pasteurella multocida* B: 2 are important due to the facts that these bacteria can cause serious outbreak that bring mortality to the affected animals. This study was conducted to detect the presence of fimbrial gene in rat's organs that infected with *P. multocida* B: 2 by using PCR technique. This technique was used to determine the ability of this technique to detect the presence of *P. multocida* B: 2 in the tissue organs. Three sets of fimbriae gene primers were used, Pair 1; EZ1(F) and EZ2(R), Pair 2; EZ3(F) and EZ4(R) and Pair 3; EZ5(F) and EZ6(R). This study has successfully identified the presence of fimbrial gene in the infected tissue organs; lung, liver and kidney which suggested that there was a presence of *P. multocida* B: 2 in the tissue organs. Results indicated that all 3 set of primers have generated fimbriae gene of approximately 400-500bp. The finding of this study has indicated that within 7 days of exposure, the bacteria has spread and colonize the tissue organs and it was suspected that the bacteria have spread through the blood stream of the infected animals. These studies have demonstrated that PCR technique using a fimbriae gene primers can be used as a diagnostic tool in detecting and identifying the presence of *P. multocida* B: 2 in the infected tissue organs.

**PENGESANAN GEN FIMBRIAL DALAM ORGAN YANG DIJANGKIT OLEH *Pasteurella multocida* B: 2 MENGGUNAKAN TEKNIK TINDAK BALAS RANTAI POLIMERASE (PCR)**

## ABSTRAK

Pengesanan *Pasteurella multocida* B: 2 yang cepat adalah penting berdasarkan kepada fakta bahawa bakteria ini boleh menyebabkan wabak yang serius dan membawa kematian kepada haiwan yang dijangkiti. Kajian ini telah dijalankan untuk mengenalpasti kehadiran gen fimbria dalam organ tikus yang dijangkiti oleh *Pasteurella multocida* B: 2 menggunakan teknik PCR. Teknik ini digunakan untuk mengesan kebolehan teknik ini untuk mengesan kehadiran *P. multocida* B: 2 dalam tisu organ tersebut. Tiga set primer gen fimbriae digunakan, P1; EZ1 (F) dan EZ2(R), P2; EZ3 (F) dan EZ4(R) dan P3; EZ5 (F) dan EZ6(R). Kajian ini telah berjaya mengenalpasti kehadiran gen fimbria dalam tisu organ yang dijangkiti; peparu, hati dan ginjal yang menunjukkan kehadiran *P. multocida* B: 2 dalam organ tersebut. Keputusan menunjukkan kesemua 3 set primer telah menghasilkan gen fimbria iaitu kira-kira 400-500bp. Hasil penemuan kajian ini telah menunjukkan bahawa dalam tempoh 7 hari selepas didedahkan, bakteria tersebut telah tersebar dan menghuni tisu organ, dan disyaki kemungkinan bakteria tersebut tersebar melalui saluran darah haiwan yang telah dijangkiti. Kajian ini telah menunjukkan bahawa teknik PCR menggunakan primer gen fimbriae boleh digunakkan sebagai alat diagnostik untuk mengesan dan mengenalpasti kehadiran *P. multocida* B: 2 dalam tisu organ yang dijangkiti.