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Comparison of DNA analysis of 'Pasteurella multocida' serotype B in cattle and buffalo in Terengganu using RAPD-PCR technique / Lee Sok Leng.

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COMPARISON OF DNA ANALYSIS OF *Pasteurella multocida*
SEROTYPE B IN CATTLE AND BUFFALO IN TERENGGANU
USING RAPD-PCR TECHNIQUE

BY

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This project is submitted in partial Fulfilment of the requirement
for the Degree of Bachelor of Applied Science
(Biodiversity Conservation and Management)

Department of Biological Sciences
Faculty of Science and Technology
Kolej Universiti Sains dan Teknologi Malaysia
KUSTEM
2003

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This project report should be cited as:

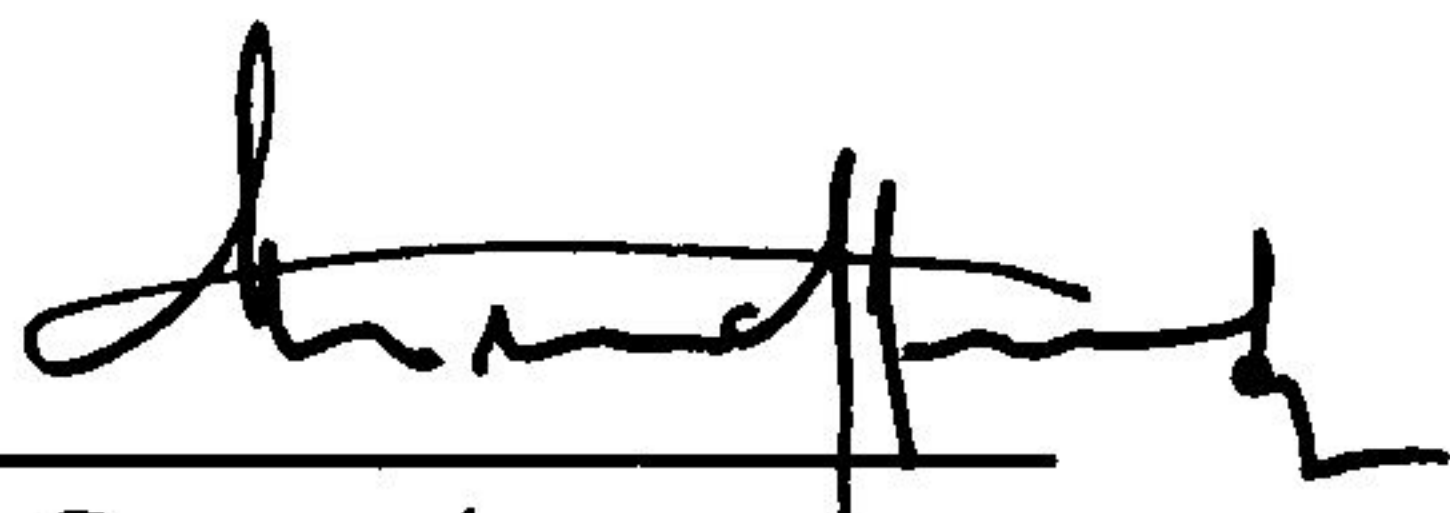
Lee, S.L. 2003. Comparison of DNA Analysis of *Pasteurella multocida* serotype B in cattle and buffalo in Terengganu using RAPD-PCR technique. Report of Final Year Academic Project, Bachelor of Applied Science (Biodiversity Conservation and Management), Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia. 51p.

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APPROVAL AND CERTIFICATION FORM

I certify that the report of this final year project entitled 'Comparison of DNA Analysis of *Pasteurella multocida* Serotype B in Cattle and Buffalo in Terengganu Using RAPD-PCR Technique' by LEE SOK LENG, matric no. UK4106 have been read and all the alteration and correction recommended by examiners has been done. This thesis submitted to Department of Biological Sciences as fulfillment of requirement for degree of Sarjana Muda Applied Science in Management and Conservation of Biodiversity, Faculty Science and Technology, University College Science and Technology Malaysia.

Approved by:



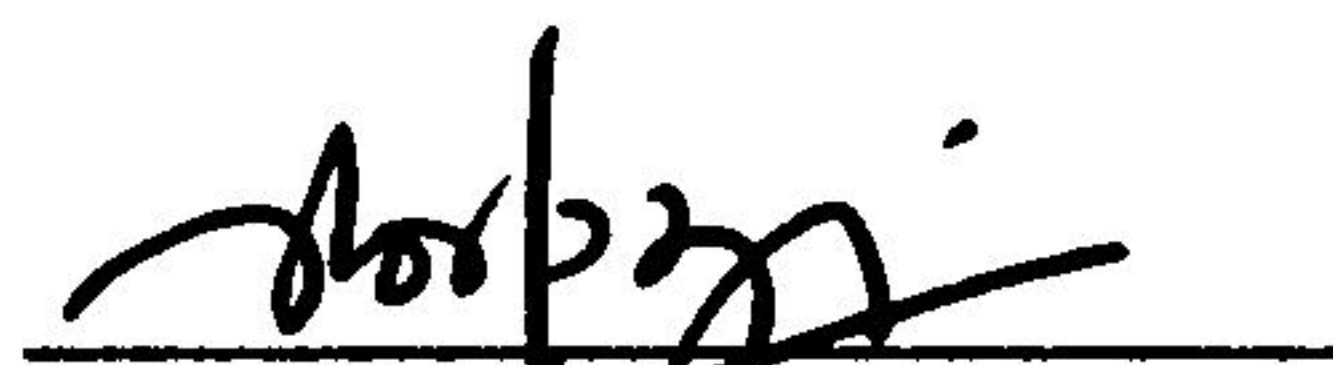
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ACKNOWLEDGEMENTS

My most sincere thanks go to my supervisor, Dr. Mohd. Effendy Hj. Abdul Wahid for his endless patience, trust, guidance, and his willingness to teach me everything in my project. Without him, I would not have completed it.

My deepest gratitude is also expressed to Miss Noraznawati Ismail as my second supervisor for her warmth – a load of funs, directness and advice in my project.

Special thanks to Dr. Sabri, Mr. Hajaraih and Kak Ernie from University Putra Malaysia whose were kind enough to welcome me, help and teach me despite their busy schedules during our one month in UPM. Thanks also due to Dr. Faisur, Mr. Shukri and Mr. Zaki from Veterinary Service Department, Terengganu for their help in my project.

To my family, Mom and grandma... it has been 23 years of hard work, love, courage and support that you gave me and I have finally made it here! This is for you... and I love you guys!

To Su Aun, my mentor + my best friend + my beloved one. Your time, energy, loves, understanding and infectious creative spirits that you gave me have made differences in my life. You always have been there for me... I am truly forever grateful.

In addition, for their kindness too numerous to recount, thanks to Dr. Muthafar, Dr. Ahmad Shamsuddin, Puan Kartini, Kak Wan, all the lab assistants in KUSTEM, my course mates, friends and my old friends who have helped and supported me from far. Thank you so much.

Moreover, my most humble thanks to GOD, who has blessed me with so many gifts.

ABSTRAK

Hawar berdarah merupakan penyakit kronik yang menyerang lembu dan kerbau disebabkan oleh bakteria *Pasteurella multocida* serotaip B:2. Satu kajian telah dibuat untuk memencil dan mengenalpasti *P. multocida* serotaip B daripada lembu serta membuat perbandingan analisis DNA di antara lembu dan kerbau dengan menggunakan teknik RAPD-PCR. Sampel bakteria telah dipencilkan daripada 30 ekor lembu di tiga lokasi yang berlainan di Terengganu. Sebanyak lima sampel didapati terdiri daripada *P. multocida* serotaip B melalui ujian biokimia dan pengesahan serotaip oleh Institut Perubatan Veterinar. Sampel yang dipencilkan dari kerbau yang dijangkiti hawar berdarah dijadikan sebagai sampel kawalan. Genomik DNA dari enam sampel diekstrakkan menggunakan Wizard® Genomic DNA Purification Kit (Promega). Ketulenan DNA sampel adalah berjulat dari 1.686 – 1.846 manakala kepekatan DNA adalah berjulat dari 800 ng/μl – 1282.5 ng/μl. Primer OPA09, OPA16 dan OPA19 telah dipilih dalam amplifikasi RAPD dan ketiga-tiga primer menghasilkan corak DNA yang berlainan serta kehadiran jalur-jalur DNA yang sama pada setiap primer. Primer OPA09 menghasilkan jalur DNA yang sama pada 700Kb dalam semua sampel. Primer OPA16 menghasilkan jalur DNA yang sama pada 760Kb dan 1180Kb dalam semua sampel. Primer OPA19 menghasilkan jalur DNA yang sama pada 540Kb dalam semua sampel. Kehadiran jalur DNA yang sama menunjukkan *P. multocida* daripada lembu dan kerbau adalah sama dan berpotensi menyebabkan wabak HS. Jarak genetik *P. multocida* serotaip B adalah berjulat dari 0.133 – 0.462 dan indeks similariti adalah berjulat dari 0.320 – 0.867. Aplikasi teknik RAPD-PCR dalam penghasilan corak DNA telah dibuktikan keberkesanannya dalam mengenalpastian bakteria dan malah dapat membantu dalam pengecaman *P. multocida* serotaip B seterusnya dalam analisis DNA ataupun keunikan bakteria tersebut.

ABSTRACT

Haemorrhagic septicaemia (HS) is an acute and septicaemic disease of cattle and buffaloes caused by *Pasteurella multocida* serotype B:2. A study was done to isolate and identify *P. multocida* serotype B from cattle and comparison study on DNA analysis between cattle and buffalo using RAPD-PCR technique. Bacteria samples were isolated from 30 cattle in three separate farms in Terengganu. Five cattle were found to have *P. multocida* serotype B through biochemical test and were serotyped by Veterinary Research Institute. Isolates from buffalo infected with HS were used as a control sample. Genomic DNA of six samples were extracted by using Wizard® Genomic DNA Purification Kit (Promega). The DNA purity of six samples ranged from 1.686 – 1.846. The final concentration of DNA samples ranged from 800 ng/μl – 1282.5 ng/μl. Three primers (OPA09, OPA16, and OPA19) were selected for RAPD amplification. Study revealed that primer OPA09, OPA16 and OPA19 generated different DNA banding pattern and presences of identical DNA bands in each primer. In primer OPA09, identical DNA bands were present in all samples at 700Kb. In primer OPA16, identical DNA bands were present in all samples at 760Kb and 1180Kb. In primer OPA 19, identical DNA bands were present in all samples at 540Kb. The presence of identical bands indicates cattle and buffalo shared the same strains of *P. multocida* and has the potential to trigger an outbreak of HS. Genetic distance of *P. multocida* serotype B ranged from 0.133 – 0.462 meanwhile similarity index ranged from 0.320 – 0.867. Potential application of RAPD-PCR in producing identical banding pattern for strain differentiation has proved effective and may help further studies of *P. multocida* serotype B in DNA analysis or the unique organism itself.