DETECTION OF *Burkholderia pseudomallei* USING POLYMERASE CHAIN REACTION (PCR) IN SHEEP (*Ovis aries*) IN NUEVA ECIJA

DENNIS OSOTEO SAYCO JR.

An Undergraduate Thesis Submitted to the Faculty of the College of Veterinary Sciences and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF VETERINARY MEDICINE

JUNE 2019

ACCEPTANCE SHEET

This undergraduate thesis entitled **"DETECTION OF *Burkholderia pseudomallei* USING POLYMERASE CHAIN REACTION (PCR) IN SHEEP (*Ovis aries*) IN NUEVA ECIJA"** prepared and submitted by **DENNIS O. SAYCO JR**, in partial fulfillment of the requirements for the degree of **DOCTOR OF VETERINARY MEDICINE**, is hereby accepted:

JAYLORD M. PIOQUINTO, DVM
Adviser

JUNE 10, 2019
Date Signed

CLARO N. MINGALA, DVSM, Ph.D.
Co - Adviser

Date Signed

ZETH ALLEN B. SANTIAGO, DVM
Critic

06/10/19
Date Signed

Accepted as partial fulfillment of the requirements for the degree of **DOCTOR OF VETERINARY MEDICINE**:

FREDELON B. SISON, DVM, MVPH
Chair, Department of Pathobiology

6-10-19
Date Signed

FREDELON B. SISON, DVM, MVPH
College Research Coordinator

6-10-19
Date Signed

VIRGINIA M. VENTURINA, DVM, Ph.D.
Dean, College of Veterinary Science and Medicine

Date Signed

BIOGRAPHICAL SKETCH

Dennis Osoteo Sayco Jr was born on May 19 1997 at Science City of Muñoz, Nueva Ecija. He lives at Purok Parang, Brgy. Villa Nati, Science City of Muñoz, Nueva Ecija. His parents are Dennis B. Sayco Sr. and Vivianita O. Sayco. He finished his primary education at Dep-Ed CLSU Elementary (Lab) School at CLSU, Science City of Munoz on 2009 and took his secondary education to San Sebastian School at Science City of Munoz on 2013 and later on finished his degree in bachelor in Animal Husbandry at Central Luzon State University, Science City of Munoz on 2017 and currently pursuing his degree in Doctor of Veterinary Medicine in the same institution. His research interests are in molecular biology and livestock industry. While taking his college degree, he was engaged in leadership and served as the CVSM Councilor for two years in the University Supreme Student Council, the highest governing student body in the University. He is also the current President of the Senior Clinicians Club of the Department of Veterinary Clinical Sciences and representative for the Veterinary Student Government. Lastly, he is also an affiliate of the Veterinary Student Achievers Society, a college based recognized organization for veterinary students.

ACKNOWLEDGMENT

The researcher would like to express his utmost gratitude to the people who guided him and worked with him during the conduct until the completion of the study.

To his adviser, Dr. Jaylord M. Pioquinto for his advice, patience, and understanding during the writing of this piece of work;

To his critic, Dr. Zeth Allen B. Santiago for giving time to help him improve this work through her insights and recommendations;

To the college research coordinator, Dr. Fredelon B. Sison for patiently checking the outline and final thesis revisions and for giving suggestions to improve this work;

To the whole PCC Animal Health Lab team, Dr. Claro N. Mingala and Dr. Gabriel Alexis Tubalinal and his thesis mates under the PCC project for their unending help during the writing and conduct of this research;

To his mother, Vivianita O. Sayco, who even though physically not here, inspired the researcher to be persevered enough to face the challenges encountered in the conduct and writing of this work;

To his close friends, Jaydenrich Paul M. Dela Cruz and Jamica Martin for their love, care, support, financially and morally, and their understanding in the conduct and writing of this work;

To all the people whose names have been inadvertently missed but who have helped him achieve his goals, the researcher is deeply grateful; and

Above all, to Almighty God, who endlessly love, blessed and guided him to complete this research and who gave his everlasting strength to overcome the adversities of life to be able to survive until this time.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF APPENDIX TABLE

LIST OF APPENDIX PLATES

ABSTRACT

SAYCO, DENNIS, JR O., College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019**, **DETECTION OF *Burkholderia pseudomallei* USING POLYMERASE CHAIN REACTION (PCR) IN SHEEP (*Ovis aries*) IN NUEVA ECIJA**

Adviser: JAYLORD M. PIOQUINTO, DVM
Co-Adviser: CLARO N. MINGALA, DVM, PhD

Melioidosis is a bacterial disease that affects humans and many species of animals including sheep. It is systemic disease that may affect almost any organ in an affected animal (Benoit et al., 2015) and a disease of public health importance in southeast Asia that is associated with high case-fatality rates in animals and humans (Cheng et al., 2005). The study was performed to detect *Burkholderia pseudomallei* from nasal swab of sheep around Nueva Ecija using Polymerase Chain Reaction/

A total of 40 sheep that are randomly picked at Nueva Ecija were collected with nasal swab samples. Extracted samples were subjected to bacterial culture and DNA extraction. Identification of *Burkholderia pseudomallei* was molecularly confirmed through Polymerase Chain Reaction with a primer of 16S rRNA gene and Tat domain gene primer and were subjected to gel analysis for identification.

Results showed that all 40 samples were negative from 16S rRNA gene and Tat domain gene primer that was used for the detection of *Burkholderia pseudomallei.*

Keywords: *Burkholderia pseudomallei.*; Melioidosis; Polymerase Chain Reaction; 16sr DNA gene; Tat domain gene primer

LITERATURE CITED

Baker Al, Ezzahir J, Gardiner C, Shipton W, and Warner JM. (2015). Environmental attributes influencing the distribution of *Burkholderia pseudomallei* in Northern Australia. *PLoS One* 10(9):e0138953. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26398904

Bauernfeind A, Roller C, Meyer D, Jungwirth R, and Schneider I. (1998). Molecular procedure for rapid detection of *Burkholderia mallei* and *Burkholderia pseudomallei*. *Journal of Clinical Microbiology* 36: 2737-41. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9705426

Benoit TJ, Blaney DD, Doker TJ, Gee JE, Elrod MG, Rolim DB, Inglis TJ, Hoffmaster AR, Bower WA, and Walke HT. (2015). A review of melioidosis cases in the Americas. *American Journal of Tropical Medline and Hygiene*. 93(6):1134-9. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4674224/

Bioterrorism Agents/Diseases. (2016). Retrieved from https://emergency.cdc.gov/agent/agentlist-category.asp

Brett, P. J., Deshazer, D., and Woods, D. E. (1997). Characterization of *Burkholderia pseudomallei* and *Burkholderia pseudomallei*-like strains. *Epidemiol. Infect* 118: 137–148. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9129590

Bureau of Animal Industry. (2018) Meat and Meat Products of 2017. Retrieved from http://www.bai.da.gov.ph/index.php/stakeholders-corner/importation-data

Casas, N. (2018). Raising Sheep in the Philippines. 2017. Retrieved from http://businessdiary.com.ph/430/raising-sheep-in-the-philippines/

Center for Disease Control and Prevention (CDC). (2006). Melioidosis. Retrieved from http://www.cdc.gov.tw/uploads/files/03b6323f-a38c-4e43-9e12a7701e88db52.pdf

Chen, Y., Lin, H., Mu, J., Chiang, C., Chen, C., Buu, L., Lin, Y. E. and Chen, Y., (2010). Distribution of Melioidosis Cases and Viable *Burkholderia pseudomallei* in Soil: Evidence for Emerging Melioidosis in Taiwan. *Journal of Clinical Microbiology* 48 (4): 1432-1434. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2849618

Cheng, A. C. and Currie, B. J. (2005). Melioidosis: Epidemiology, Pathophysiology, and Management. *Clinical microbiology reviews* 18 (2): 383–416. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15831829

Choy, J. L (2017). Overview of Melioidosis. Retrieved from https://www.msdvetmanual.com/generalized-conditions/melioidosis/overview-of-melioidosis#v3273746

Dance, D. A. (2000). Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia* spp. and human-animal hosts. *Acta Trop* 74:159-168. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10674645

Doker TJ, Quinn CL, Salehi ED, Sherwood JJ, Benoit TJ, Glass EM, GEE JE, Shadomy SV, Bower WA, Hoffmaster AR, Walke HT, Blaney DD, and Diorio MS. (2014). Melioidosis Investigation Team. Fatal *Burkholderia pseudomallei* infection initially reported as a Bacillus species. *American Journal of Tropical Medicine and Hygiene* 91(4):743-6. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25092821

Egwuatu. TO., F. T. Ogunsola, I. M. Okodugha, B. Jide, D.G. Arewa, and O. A. Osinupebi. (2014). Effect of Blood Agar from Different Animal Blood on Growth Rates and Morphology of Common Pathogenic Bacteria. *Advancesin Microbiology* 1238-1241. Retrieved from https://file.scirp.org/pdf/AiM_2014123014065959.pdf

Engelthaler DM, Bowers J, Schupp JA, Pearson T, and Ginther J. (2011). Molecular investigations of a locally acquired case of melioidosis in southern AZ, USA. *PLoS Neglected Tropical Diseases* 5(10):e1347. Retrieved from https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001347

Food and Agricultural Organization of United Nations (FAO). (2019). AGA NEWS: Economic analysis of animal diseases. Retrieved from http://www.fao.org/ag/againfo/home/en/news_archive/2016_Economic_analysis_animal_diseases.html

Foong, Y. C., Tan, M., and Bradbury, R.S. (2014). Melioidosis: A Review. *Rural and Remote Health* 14: 2763. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25359677

Garibyan, L. and Avashia, N. (2013). Research Techniques Made Simple: Polymerase Chain Reaction (PCR). *J Invest Dermatol.* 133(3). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4102308/

Garner, G., Saville, P., and Fediavesky, A. (2003). Manual for the recognition of exotic diseases of livestock: A reference guide for animal health staff. Food and Agriculture Organization of the United Nation (FAO). Retrieved from http://www.spc.int/rahs

Gee JE, Sacchi CT, Glass MB, De BK, Weyant RS, Levett PN, Whitney AM, Hoffmaster AR, and Popovic T. (2003) Use of 16S rRNA gene sequencing for rapid identification and differentiation of Burkholderia pseudomallei and B. mallei. *J Clin Microbiol. 2003 Oct;41(10):4647-54.* Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14532197

Haase, A. Brennan, M., Barrett, S., Wood, Y., Huffam, S. O'brien, D., and Currie, B. (1998). Evaluation of PCR for Diagnosis of Melioidosis. *Journal of clinical microbiology* 36 (4): 1039–1041. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC104685/

Hagen, R. M., Gauthier, Y. P., Sprague, L. D., Vidal, D. R., Zysk, G., Finke, E-J., and Neubauer, H. (2002). Technical Report: Strategies for PCR based detection of Burkholderia *pseudomallei* DNA in paraffin wax embedded tissues. *Journal of Clinical Pathology: Molecular Pathology* 55:398–400. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1187279/

Hall CM, Busch JD, Shippy K, Allender CJ, Kaestli M, Mayo M, Sahl JW, Schupp JM, Colman RE, Keim P, Currie BJ, and Wagner DM. (2015). Diverse *Burkholderia* species isolated from soils in the southern United States with no evidence of *B. pseudomallei. PLoS One* 10(11):e0143254. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26600238

Hampton V, Kaestli M, Mayo M, Choy JL, Harrington G, Richardson L, Benedict S, Noske R, Garnett ST, Godoy D, Spratt BG, and Currie BJ. (2011). Melioidosis in birds and Burkholderia pseudomallei dispersal, Australia. *Emerging Infectious Diseases* 17(7):1310-2. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3381411/

Haque, Q. M. (2010). Evaluation of Available Diagnostic Tests for Melioidosis. *Journal of Taibah University Medical Sciences* 5(2): 89-97. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29720430

Heymann DL., (2006). Melioidosis. *Control of Communicable Diseases Manual Edition.* 18: 386-8 Retrieved from https://www.navybmr.com/study%20material/CCDM.pdf

Hillsborough Community College (2018). BLOOD AGAR NOTES. Retrieved from https://www.hccfl.edu/media/580776/blood%20agar%20notes.pdf

Ho C., Lau C., Martelli P., Chan S., Tse C., Wu A., Yuen K., Lau SK., and Woo P. (2011). Novel Pan-Genomic Analysis in Target Selection for Multiplex PCR Identification and Detection of *Bukholderia pseudomallei, Burkholderia thailandensis, and*

Bukrholderia cepacia Complex Species: A Proof of Concept Study. *Journal of Clinical Microbiology*. 49(3):814-821. Doi: 10.1128/JCM.01702-10. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3067743/

Howard, K. and Inglis, T. J. J. (2003). Novel Selective Medium for Isolation of *Burkholderia pseudomallei*. *Journal of Clinical Microbiology* 41 (7): 3312–3316. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC165331

Inglis, T. J. J. (2010). The Treatment of Melioidosis. *Pharmaceuticals* 3: 1296-1303. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4033981

James GL, Delaney B, Ward L, Freeman K, Mayo M, and Currie BJ. (2013). Surprisingly low seroprevalence of *Burkholderia pseudomallei* in exposed healthy adults in the Darwin region of tropical Australia where melioidosis is highly endemic. *Clinical Vaccine Immunology* 20(5):759-60. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3647750

Kaestli M, Harrington G, Mayo M, Chatfield MD, Harrington I, Hill A, Munksgaard N, Gibb K, Currie BJ. (2015). What drives the occurrence of the melioidosis bacterium Burkholderia pseudomallei in domestic gardens? *PLoS Neglected Tropical Diseases* 9(3):e0003635. Retrieved from https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003635

Lau SK, Sridhar S, Ho CC, Chow WN, Lee KC, Lam CW, Yuen KY and Woo PC. (2015). Laboratory diagnosis of melioidosis: past, present and future. *Experimental Biology and Med (Maywood)*. 2015;240(6):742-51. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25908634

Latoza, R. (2015). WV records highest goat, sheep production in PH. Retrieved: http://www.iloilometropolitantimes.com/wv-records-highest-goat-sheep-production-in-ph/

Lazar A, N. R., Govan, B., Cullinane, M., Harper, M., Adler, B., and Boyce, J. D. (2009). The molecular and cellular basis of pathogenesis in melioidosis : how does *Burkholderia pseudomallei* cause disease?. FEMS Microbiology Review 33:1079–1099. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19732156

Lee, N., J. L. Wu, C. H. Lee, and W. C. Tsai. (1985). Pseudomonas pseudomallei infection from drowning: the first reported case in Taiwan. *Journal of Clinical Microbiology* 22:352-354. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC268408/

Limmathurotsakul, D., and Peacock, S. J. (2011). Melioidosis: A clinical Overview. *British Medical Bulletin* 99: 125–139. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21558159

Millan JM, Mayo M, Gal D, Janmaat A, and Currie BJ. (2007) Clinical variation in melioidosis in pigs with clonal infection following possible environmental contamination from bore water. *Veterinary Journal* 174:200-2. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16807011

Mohanty, S., Pradhan, G., Panigrahi, M. K., Mohapatra, P. R., and Mishra, B. (2016). A case of systemic melioidosis: unravelling the etiology of chronic unexplained fever with multiple presentations. *Pneumonol Alergol Pol* 84: 121–125. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27238172

Montúfar Fe, Ochoa Je, Ortega H, Franco L, Montúfar Mc, Monsalve A, Jaramillo C, and Zapata M. (2015). Melioidosis in Antioquia, Colombia: an emerging or endemic disease? A case series. *International Journal of Infectious Diseases* 37:50-7. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26051974

Peacock, S. J., Chieng, G., Cheng, A. C., Dance, D. A. B., Amornchai, P., Wongsuvan, G. Teerawattanasook, N., Chierkul, W., Day, N. P. J. Raja NS, Ahmed MZ, and Singh NN. (2005). Melioidosis: an emerging infectious disease. *Journal of Postgraduate Medicine* 51:140-5. Retrieved from http://www.jpgmonline.com/article.asp?issn=0022-3859;year=2005;volume=51;issue=2;spage=140;epage=145;aulast=Raja

Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD) (2011). Goat/Sheep. Retrieved from http://www.pcaarrd.dost.gov.ph/home/momentum/ruminants/index.php?option=com_content&task=view&id=131&Itemid=175

Philippines Statistics Authority (PSA). (2018). Goat Situation Report, January - June 2018. Retrieved from https://psa.gov.ph/livestock-poultry-iprs/goat/inventory

Rega, P. P. (2015). CBRNE – Glanders and Melioidosis. Retrieved from https://emedicine.medscape.com/article/830235-overview

Samy, R. P., Stiles, B. G., Sethi, G., and Lim, L. H. K. (2017). Melioidosis: Clinical impact and public health threat in the tropics. *Plos: neglected tropical diseases.* 1-28. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28493905

Solanki, G. (2012). Polymerase Chain Reaction. *International Journal of Pharmacologica Research* 2(3): 98 – 101. Retrieved from

https://www.researchgate.net/publication/271729269_Polymerase_Chain_Reactio
n

acio, H. D. (2017). Raising Small Ruminants for food and profit. Retrieved from
http://edgedavao.net/agri-trends/2017/01/18/raising-small-ruminants-food-profit/

ne Swedish University of Agricultural Sciences (SLU). (2016). *Burkholderia*
pseudomallei. Retrieved from http://www.vetbact.org/?artid=49

rakulsomboon, S., V. Vuddhakul, P. Tharavichitkul, N. Na-gnam, Y. Suputtamongkol
and V. Thamlikitkul. (1999). Epidemiology of arabinose assimilation in
Burkholderia pseudomallei isolated from patients and soil in Thailand. *Southeas*
Asian Journal of Tropical Medical Public Health 30:756-759. Retrieved from
https://www.ncbi.nlm.nih.gov/pubmed/10928371

Vuthiekanun, V. (2005). Comparison of Ashdown's Medium, *Burkholderia cepacia*
Medium, and *Burkholderia pseudomallei* Selective Agar for Clinical Isolation o
Burkholderia pseudomallei. *Journal of Clinical Microbiology* 43 (10): 5359–5361
Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1248505/

Vuthiekanun, V. and Dance, D. (2012). Standard Operating Procedure (SOP):
Simplified Method for the Isolation of *Burkholderia pseudomallei* from soil 3-4
Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3264119/

Yabuchi E., Kosako, Y., Oyaizu H., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezak
T., and Arakawa, M. (1992). Proposal of Burkholderia gen. nov. and transfer o
seven species of the genus Pseudomonas homology group II to the new genus, wit
the group species Burkholderia cepacia (Palleroni and Holmes 1981) comb. nov
John Wiley and Sons, 36(12), 1251-1275. Retrieved fro
https://www.ncbi.nlm.nih.gov/pubmed/1283774