MICROBIOLOGICAL ASSESSMENT AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH RAW BUFFALO MILK USING CONVENTIONAL PLATING METHOD AND 3M PETRIFILM

CARLO S. BUENAVENTURA

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree

BACHELOR OF SCIENCE IN BIOLOGY
(Major in Microbiology)

JUNE 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDIX FIGURES

ABSTRACT

BUENAVENTURA, CARLO S., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019, MICROBIOLOGICAL ASSESSMENT AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH RAW BUFFALO MILK USING CONVENTIONAL PLATING METHOD AND 3M PETRIFILM**

Adviser: MARY JHANE G. VALENTINO, M.Sc.
Co-Adviser: MINA P. ABELLA, M.Sc.

Raw buffalo milk from a dairy cooperative in Nueva Ecija was evaluated through its physico- chemical properties and microbial load using conventional plating method and 3M Petrifilm. Organoleptic tests were done alongside with the seven platform tests namely, alcohol precipitation, lactometer, temperature, pH, total titratable acidity, sediment test, and clot on boiling tests.

The microbial load of raw buffalo milk for both methods are in an acceptable range which satisfies the given standard value. Presence of coliform, were also detected and *E. coli* was not observed. Four bacterial isolates were characterized morphologically and culturally

LITERATURE CITED

Asperger, H. (1994). *Staphylococcus aureus*. In: The significance of pathogenic microorganisms in raw milk. *International Dairy Federation,* 24-42.

Beloti, V., Barros, M. A. F., Nero, L. A., Pachemshy, J. A. S., Santana, E. H. W. & Franco, B. D. G. M. (2002). Quality of pasteurized milk influences the performance of ready-to-use systems for enumeration of aerobic microorganisms. *International Dairy Journal,* 12(5), 413-418.

Bramley, A. J. & McKinnon, C. H. (1990). The microbiology of raw milk. In: Robinson RK, editor. *Dairy Microbiology,* 163- 208.

Carvalho, C. M., Dliveira, A. J. G. & Rosa, C. (2002). The Petrifilm system as an alternative for traditional methods for total count of aerobic microorganisms and total coliform in refrigerated raw milk. *Higiene Alimentar Magazine*, 16(100), 116-126.

Centers for Disease Control and Prevention (2003). Multistate outbreak of *Salmonella* serotype *typhimurium* infections associated with drinking unpasteurized milk— Illinois, Indiana, Ohio, and Tennessee. *Morbidity and Mortality Weekly Report,* 52, 613.

Centers for Disease Control and Prevention (2004). Preliminary food net data on the incidence of infection with pathogens transmitted commonly through food—selected sites, United States. *Morbidity and Mortality Weekly Report*, 53,338.

Chambers, J. V. (2002). The microbiology of raw milk. In: Robinson RK editor. *Dairy Microbiology Handbook,* 39-90.

Clark, M. (2005). *Escherichia coli* food poisoning. Retrieved from http://www.about-ecoli.com/.

Desalegn, A. (2014). Assessing the microbiological quality of milk. *World Journal Agronomy; Food Science Technology,* 1-10.

Doyle, M. E., Hartmann, F. A. & Lee Wong, A. C. (2012). Methicillin-resistant staphylococci: Implications for our food supply? *Animal Health Research Reviews,* 13, 157–180.

Ellis, K. A., Innocent, G. T., Mihm, M., Cripps, P., McLean, W. G. & Howard C. V. (2003). Dairy cow cleanliness and milk quality on organic and conventional farms in the UK. *Journal Dairy Research,*74(3), 302-310.

Eberhart, R. J., Wilson, R. A., Oldham, E., & Lmtner, T. (1987). Environmental effects on teat skin microflora, 71. 26th Annual Management National Mastitis Council.

Fadaei, A. (2014). Bacteriological quality of raw cow milk in Shahrekord, Iran. *Veterinary World* 7(4): 240-243.

Freitas, R., Nero, L. A., & Carvalho, A. F. (2009). Technical note: enumeration of mesophilic aerobes in milk: evaluation of standard official protocols and Petrifilm aerobic count plates. *Journal of Dairy Science*, 92(7), 3069-3073.

FAO (1998). Production yearbook Food and Agriculture Organization. Rome, Italy.

Food and Agriculture Organization of the United Nation (2014). Milk and Dairy Products in Human Nutrition, 344, 43-59. Rome, Italy.

Garin-Bastuji, B. & Verger, J. M. (1994). *Brucella abortus* and *Brucella melitensis*. In: The significance of pathogenic microorganisms in raw milk. *International Dairy Federation. IDF, Brussels, Beljium*, 167-1.

Gebra-Eanuael & Teka, G. (1997). Food hygiene-principles and methods of food borne disease control with special reference to Ethiopia, 73-87.1sted, Faculty of Medicine, Department of Community Health, Addis Ababa University.

Gilbert, R. J., & Kramer, J. M. (1986). Bacillus cereus food poisoning, 85-93. In Progress in food safety food research institute (proceedings of symposium). Madison, WI: Food Research Institute, University of Wisconsin. USA.

Godefay, B. & Molla, B. (2000). Bacteriological quality of raw milk from four dairy farms and milk collection center. *International Journal of Food Science and Microbiology,* 113, 13.

Hayes, M. C., Ralyea, R. D., Murphy, S. C., Carey, N. R., Scarlett, J. M. & Boor, K. J. (2001). Identification and characterization of elevated microbial counts in bulk tank raw milk. *Journal Dairy Science*, 84(l), 292-298.

Hendreson, J. L. (1971). The Fluid Milk Industry. *The AVI Publishing Company Incorporated*, 49-79.

Holm, C., Jepsen, L., Larsen, M. & Jespersen, L. (2004). Predominant microflora of downgraded danish bulk tank milk. *Journal Dairy Science,* 87(5), 1151-1157.

Hogan, J. S., Smith, K. L., Hoblet, K. H., Todhunter, D. A., Schoenberger, P. S. & Hueston, W. D. (1989). Bacterial counts in bedding materials used on nine commercial dairies. *Journal .Dairy Science,* 72(l), 250-258.

Hurley, J. (2002). Milk composition. University of Illinois, Department of Animal Sciences. Retrieved from: http://classes.aces.uiuc.edu/AnSci308/milkcomp.html.

Hussain, T. (2015). An introduction to the Serotypes, Pathotypes and Phylotypes of *Escherichia coli.* Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan.

Jang, J. (2017). Environmental *Escherichia coli*: ecology and public health implications—a review. Retrieved from: http://onlinelibrary.wiley.com/doi/10.1111/jam.13468/abstract.

Jeffrey, D. C. & Wilson, J. (1987). Effect of mastitis-related bacteria on the total bacteria counts of bulk milk supplies. *Journal Society Dairy Technology*, 40(2), 23-26.

Kotiranta, A., Lounatmaa, K. & Haapasalo, M. (2000). Epidemiology and pathogenesis of Bacillus cereus infections. *Microbes and Infection*, 2, 189–198.

Mamuad, F. V. & Abella, M. P. (2000). Studies on the manufacture of soft cheese from buffalo milk using abosomal extracts. Unpublished Master's Thesis. CLSU.

McKinnon, C. H., Rowlands, G. J. & Bramley, A. J. (1992). The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds. *Journal Dairy Research*, 57(3), 307-318.

Mehari, T. (1998). Thermoduric and psychrophilic bacteria from raw milk. Master of science thesis, Faculty of Science, Addis Ababa University, Ethiopia. *International Journal of Food Science and Microbiology*, 2(6), 87-91.

Murphy S. W. & Boor, K. J. (2000). Trouble-shooting sources and causes of high bacteria counts in raw milk. *Dairy Food Environment Sanitation*, (2), 606-611.

Nero, L. A., Beloti, V. & Barros, M. A. F. (2000). Métodos rápidos e automatizados para enumeração de microrganismos indicadores em leite - utilização no brasil. Semina: Ciências Agrárias, 21(1), 115-126.

O' Mahony, F. (1988). Rural dairy technology-experiences in Ethiopia. ILCA manual no. 4. Dairy Technology Unit. iLCA, Addis Ababa, Ethiopia. *International Journal of Food Science and Microbiology*, 2 (6), 64.

Ombu J. N. (1995). Quality of raw milk collected and marketed by dairy cooperative societies in Kiambu District, Kenya. Bull. *Animal Health Production Africa*, 43, 277-284.

Pal, M. (2014). Spoilage of dairy products due to fungi. *Beverage and Food World*, 41(3738), 40.

Pal, M. & Awel, H. (2014). Public health significance of *Listeria monocytogenes* in milk and milk products. *Journal of Veterinary Public Health* ,12, 1-5.

Prentice, G. A. (1994). *Listeria monocytogenes*, 101-115. In: the significance of pathogenic microorganisms in raw milk. International Dairy Federation. Brussels, Beljium.

Reinemann, D. J., Wolters, G. M. V. H., Billon, P., Lind, O. & Rasmussen, M. D. (2003). Review of practices for cleaning and sanitation of milking machines. Bulletin 381. International Dairy Federation. Brussels, Beljium.

Reta, M. A. & Addis, A. H. (2015). Microbiological quality assessment of raw and pasteurized milk. *International Journal of Food Science and Microbiology*, 2(6), 87-91.

Rosmini, M. R., Signorini, M. L., Schneider, R. & Bonazza, J. C. (2004). Evaluation of two alternative techniques for counting mesophilic aerobic bacteria in raw milk. *Food Control*, 15(1), 39-44.

Sayo, T. (2004). Selected physico-chemical properties of mastits infected murrah buffalo milk. Undergraduate Thesis. Central Luzon State University. Philippines.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases,* 17, 7–15.

Schreiner, D. A. & Ruegg, P. L. (2003). Relationship between udder and leg hygiene scores and subclinical mastitis. *Journal. Dairy Science*, 86(ll), 3460-3465.

Schimdt, K., Stupar, J., Shirley, J., Adapa, S. & Sukup, D. (1996). Factors Affecting Titratable Acidity in Raw Milk, *Kansas Agricultural Experiment Station Research Reports,* (2), 60-62.

Seo, K. S. & Bohach, G. A. (2013). Staphylococcus aureus. InFood microbiology: Washington, DC: American Society of Microbiology. USA.

Silva, M. P., Cavalli, D. R. & Dliveira, T. C. R. M. (2006). Avaliação do padrão coliformes a 45°C e comparação da eficiência das técnicas dos tubos múltiplos e Petrifilm EC na detecção de coliformes totais e Escherichia coli em alimentos. *Food Science and Technology (Campinas)*, 26(2), 352-359.

Sinha, R. N. (1994a). *Mycobacterium bovis*, 141-161. In: The significance of pathogenic microorganisms in raw milk. *International Dairy Federation.* Brussels, Beljium.

Sinha, R. N. (1994b). *Mycobacterium tuberculosis*, 116-140. In: The significance of pathogenic microorganisms in raw milk. *International Dairy Federation.* Brussels, Beljium.

Solomon, M. (2013). Bacteriological quality of bovine raw milk at selected dairy farms in DebreZeit town, Ethiopia. Comprehensive. *Journal of Food Science Technology Research*, 1(1), 1-8.

Soomro, A. H., Arain, M. A., Khaskheli, M. & Bhuto, B. (2002). Isolation of *E. coli* from raw milk and milk products in relation to public health sold under market conditions at Tandonjam, Pakistan. *Journal of Nutrition,* 1(3), 151-152.

Tanaka, D., Shima, T., Isobe, J., Watahiki, M., Matsumoto, M., Endoh, M. (2006). Epidemiology and molecular analysis of group A streptococci from patients involved in food-borne disease outbreaks in Japan between 1996 and 2003. *Japanese Journal of Infectious Diseases,* 59, 202–203.

Tatini, S. R. & Kauppi, K. L. (2003). , 1, 74-79. Academic press and Elsevier Science. Amsterdam, Boston, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo. *Encyclopedia of Dairy Sciences*, Volume 1, Pages 74-79.

Teka, G. (1997). Food hygiene principles and food borne disease control with special reference to Ethiopia. 1st Ed, Faculty of Medicine, Department of Community Health, Addis Ababa University Ethiopia. *International Journal of Food Science and Microbiology,* 73-86.

Tessema, A. & Tibbo, M. (2009). Milk Quality Control. Retrievedfrom: https://www.researchgate.net/publication/304526125_Milk_quality_control.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. & Fowler, V. G., Jr. (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews,* 28, 603–661.

Van Werven, T., Noordhuizen-Stassen, E. N., Daemen, A. J., Schukken, Y. H., Brand, A. & Burvenich, C. Preinfection in vitro chemotaxis, phagocytosis, oxidative burst, and expression of CDl 1/CD 18 receptors and their predictive capacity on the outcome of mastitis induced in dairy cows with Escherichia coli. *Journal Dairy Science*, 80 (l),67-74.

Vlaemynck, G. (1994). *Salmonella,* 78-90. ln: The significance of pathogenic micro-organisms in raw milk. *International Dairy Federation.* Brussels, Beljium.

Weese, J. S., Reid-Smith, R. J., Avery, B. P. & Rousseau, J. (2010). Detection and characterization of Clostridium difficile in retail chicken. *Letters in Applied Microbiology,* 50, 362–365.

Zadoks, R. N., Gonzalez, R. N., Boor, K. J. & Schukken, Y. H. (2004). Mastitis-causing Streptococci are important contributors to bacterial counts in raw bulk tank milk. *Journal Food Prot*ection, 67(12), 2644-2650.

Zdanowicz, M., Shelford, J. A., Tucker, C. B., Weary, D. M. & von Keyserlingk, M. A. (2004). Bacterial populations on teat ends of dairy cows boused in free stalls and bedded with either sand or sawdust. *Journal Dairy Science,* 87(6), 1694-1701.

Zocche, F., Bersot, L. S., Barcellos, V. C., Paranhos, J. K., Rosa, S. T. M. & Raymundo, N. K. (2002). Microbiological and physicalchemistry quality of pasteurized milks produced in the west region, Parana, Brazil. *Archives of Veterinary Science,* 7(2), 59-67