

**DETECTION OF *Entamoeba histolytica* IN ORGANIC FERTILIZER AND  
LIQUID SUPPLEMENTS FROM SELECTED ORGANIC  
FARMS IN LUZON**

**ISIDRA DE GUZMAN PADUA**

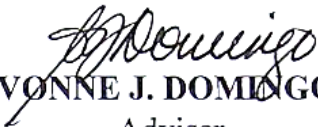
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**ACCEPTANCE SHEET**

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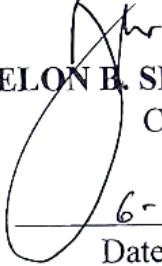
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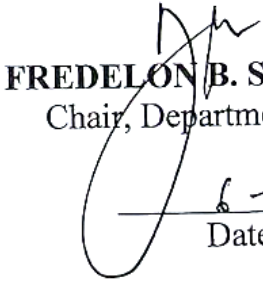
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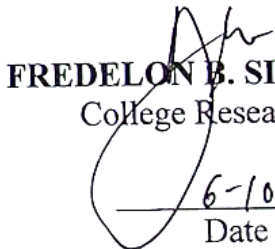
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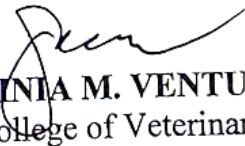
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## **BIOGRAPHICAL SKETCH**

The author, Isidra De Guzman Padua, was born on April 30, 1996 at San Carlos City, Pangasinan. The daughter of Mr. Isidro C. Padua, a farmer and Mrs. Iuminada G. Padua, a vendor. She is the youngest in the family and her siblings are Mc nell Padua, Catherine Padua, Cherrylyn Padua and Estephany Padua.

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## ABSTRACT

**PADUA, ISIDRA DG.**, College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **June 2019**, **DETECTION OF *Entamoeba histolytica* IN ORGANIC FERTILIZER AND LIQUID SUPPLEMENTS FROM SELECTED ORGANIC FARMS IN LUZON.**

Adviser: CLARISSA YVONNE J. DOMINGO, DVM, DrPH.

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The study aimed to detect the presence of *Entamoeba histolytica* in organic fertilizer and liquid supplements from selected farms in Luzon. A total of thirty (30) samples, composed of fifteen (15) organic fertilizer and fifteen (15) liquid supplements were collected and subjected to conventional (Microscopy) and molecular (Polymerase chain reaction). In addition, assessment of the laboratory technique used was also done. However, both laboratory test methods did not yield any positive results for *Entamoeba histolytica* in all of the samples.

Keywords: *Entamoeba sp.*; polymerase chain reaction; Formalin-Ether concentration technique

## LITERATURE CITED

- Andes, A. L. and V. G. V. Paller. 2018. Effect of various composting methods on the concentration and viability of *Ascaris suum* eggs in organic fertilizers. *Tropocal Agricultural Science*. 41(2): 687- 698.
- Andreoli, C.V., M.V. Sperling, F. Fernandes and M. Ronteltap. 2007. Sludge treatment and disposal. IWA Publishing.
- Alvarez, A. J., and E. A. Hernandez-Delgado and G. A. Toranzos. 1993. Advantage and disadvantages of traditional and molecular techniques applied to the detection of pathogens in waters. *Wat. Sci. Tech.* Vol. 27, No. 3-4, pp 253- 256.
- Arredondo, J.L., M.P. Gonzales, A.L. Coria, J.E. Ortega, J. Villarreal, J.L. Pinero and M.R. Vallarta. 2014. *Entamoeba histolytica*: trophozoite, precyst and cyst studied by atomic force microscopy. *Microscopy: Advance in Scientific Research and Education (A.Mendez-Vilas, Ed)*.
- Ayed, L. B. and S. Sabbahi. 2017. *Entamoeba histolytica*. Global water pathogen project part three. Specific excreted pathogens: environmental and epidemiology aspects. <http://www.waterpathogens.org>.
- Bhattacharya, S., A. Bhattacharya, and L.S. Diamond. 1988. Comparison of repeated DNA from strains of *Entamoeba histolytica* and other *Entamoeba*. *Mol. Biochem Parasitol.* 27, pp. 257-262.
- Bhattacharya, S., A. Bhattacharya, L.S. Diamond, and A.T. Soldo. 1989. Circular DNA of *Entamoeba histolytica* encodes ribosomes RNA. *J. Protozoal.* 36, pp. 455-458.
- Blessman, J., H. Buss, P.A. Tonnu, B.T. Dinh, Q.T. Vietngo, A.L. Van, M.D. Abdalla, T.F.H.G. Jackson, J.I. Ravdin and E. Tannich. Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in fecal samples. *Journal of the Clinical Microbiology*. Vol. 40, No. 12, pp. 4413-4417.
- CENTER FOR DISEASE CONTROL AND PREVENTION. 2013. Amoebiasis. DPDx-Laboratory Identification of Parasitic Diseases of Public Health. USA. <https://www.cdc.gov/dpdx/amebiasis/dx.html#molecular>.
- Canellas, L. P., F. L. Olivares, N. O. Aguilar, D. I. Jones, A. Nebbioso, P. Mazzei, and A. Piccolo. 2015. Humic and fulvic acids as biostimulants in horticulture. *Sci Horti.* 196, 15-27.

- Cupic, M., C.L. Lazarev, A. Knezevic, and M. Stanojevic. 2007. Conventional and molecular methods in diagnosing and monitoring of viral infection. 135(9-10): 589-593.
- Day, M., and K. Shaw. 2001. Biological, chemical and physical processes of composting. Compost Utilization in Horticulture Cropping Systems. Boca Raton, FL, Lewis Publisher. pp. 17-50.
- Erlich, H.A., D. Gelfand and J.J. Sninsky. 1991. Recent advance in the polymerase chain reaction. Science, New Series, Vol. 252, No. 5013, pp. 1643-1651.
- Fotedar, R., D. Stark, N. Beebe, D. Marriott, J. Ellis, and J. Harkness. 2007. Laboratory diagnostic techniques for *Entamoeba* species. *Clinical. Microbiology Reviews*. Vol. 20, No. 3, pp. 511-532.
- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2003. On-farm composting method. p. 3.
- Gonzales-Ruiz, A., R. Haque, A. Aguire, G. Castanon, A. Hall, F. Guhi, G. Ruis-Palacios, M. A. Miles and D. C. Warhurst. 1994. Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*. *Journal of Clinical. Pathology*. 47:236-239. In Fotedar *et al.*, 2007.
- Gutierrez-Miceli, F.A., R.C. Garcia-Gomez, M.A. Llaven, G.C. Marcos-Joaquin and D. Luc. 2008. Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* (L.) Moench using vermicompost leachate. *Bioresource Technology* 99:6174-6180.
- Haque, R., L. M. Neville, P. Hahn, and W. A. Petri. 1995. Rapid detection of *Entamoeba histolytica* stool antigen detection kits. *Journal of Clinical Microbiology*. Vol. 33, No10, pp. 2558-2561.
- Hoitink, H. A. and P. C. Fahy. 1986. Basis for the control of soilborne plant pathogens with composts. *Annual Reviews Phytopathol*. 24: 93-114.
- Chen, J. H. 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International Workshop. Taiwan.
- Ji, R., G. Dong, W. Shi and J. Min. 2017. Effects of liquid organic fertilizers on plant growth and Rhizosphere soil characteristics. *Sustainability* (9) 841.
- Kubista, M., J.M. Andrade., M. Bengtsson, A. Forootaan, J. Jonak, K. Lind, R. Sindelka., R. Sjoback, B. Sjogreen, L. Strombom, A. Stahlberg and N.

- Zoric. 2006. The real-time polymerase chain reaction. *Molecular Aspects of Medicine* 27: 95-125.
- Kehl, K. S., H. Cicirello., and P. L. Havens. 1995. Comparison of four detection of *Cryptosporidium* species. *Journal of Clinical Microbiology*. p 416-418.
- Letourneau V., C. Duchaine, C. Core, A. Letellier, E. Topp and D. Masse. 2010. Presence of zoonotic pathogens in physico-chemically characterized manures from hog finishing houses using different production systems. *Bioresource Technology* 101, pp. 4048- 4055.
- Novati, S., M. Sironi, S. Granata, A. Bruno, S. Gatti, and M. Scaglia et al. 1996. Direct sequencing of the PCR amplified USS rRNA gene of *Entamoeba dispar* and the design of primers for rapid differentiation from *Entamoeba histolytica*. *Parasitology*. 112, pp. 363-369.
- Nowak, P., K. Mastalska and J. Loster. 2015. *Entamoeba histolytica*- pathogenic protozoan of the large intestine in humans. *Journal of Clinical Microbiology and Biochemical Technology*. 1(1): 010-017.
- Misra, R.V., R. N. Roy, H. Hiraoka. 2003. On-Farming composting methods. Land and Water Discussion Paper. pp 29-33.
- Mirelman, D., Y. Nuchamonwtz, and T. Stolarsky. 1997. Comparison of use of Enzyme-Link Immunosorbent Assay-Based kits and PCR amplification of rRNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar*. *Journal of Clinical Microbiology*. p. 2405-2407.
- Ogunniyi, T. AB., I. K. Adewumi, A. C. Achudume and A. A. Folayanka. 2012. Parasite count and survival during fecal waste fermentation in a piggery. *J Top Med Parasitol* 35: 6-13.
- PHILIPPINE NATIONAL STANDARD. 2016. (PNS-Organic Agriculture. No. 07/2016) p. 4.
- Parkash, V. and A. J. Saikai. Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of *Capsicum chinensis* Jacq. "Bhut Jolokia". *Biotechnology Research International*. Vol. 2015. pp. 1-7.
- Robertson, L. J., A. T. Campbell, H., and V. Smith. 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental technologies. *Appl Environ Microbial* 58:3494-3500.

- Wittgen, T.D. and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative Ct method. *Nature Protocol*. Vol. 3, No. 6, pp. 1101.
- Wach, D. 1991. Survival of pathogenic micro-organisms and parasites in environmental manure and sewage sludge. *Rev. sci. tech. Off. int. Epiz.* 10(3):813-846.
- Wuyts, M., and W. A. Petri. 2003. Laboratory diagnosis of amoebiasis. *Clinical Microbiology Reviews*. 16: 713-729. In FOTEDAR *et al.*, 2003.
- Wurina, V. M. 2005. Compiled Lecture Notes in Veterinary Protozoology. 2nd Edition. CLSU: CLSU University Press. Pp. 61- 63.
- Wu, J.J., F. Oostvogel, E.A.T. A. Nang-Beifubah,, J. Ziem and A.M. Polderman. 2010. Short communication prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in Northern Ghana. *Tropical Medicine and International Health*. pp. 1153-1156.
- Wu, C., M. Koutero, M.A. Dillies, H. V, C. Lopez-Camarillo, J.Y. Coppehon and N. Guillen. 2016. Extensive transcriptome analysis correlates genetic plasticity of *Entamoeba histolytica* pathogenesis to rapid phenotype change depending on the environment. *Scientific Reports*, 6:35052.