Ghana J. Sci. **61** (1), 2020, 96 - 100 : Short Communication https://dx.doi.org/10.4314/gjs.v61i1.8

DETECTION OF HUMAN GENOTYPE "B" *GIARDIA LAMBLIA* IN GHANAIAN CATTLE FROM FRAFRAHA IN ADENTAN MUNICIPALITY OF GHANA

G. T. Mensah*, C. A. Narh, C. A. Brown, P. F. Ayeh-Kumi And I. O. Frempong
(G. T. M. & I. O. F.: CSIR - Water Research Institute ,Accra; P. F. A. & C. A. B.: School of Biomedical and Allied Health Sciences , College of Health Sciences, University of Ghana Korle-Bu; C. N.: Parasitology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon).
**Corresponding author's email: mensgato@yahoo.com*

ABSTRACT

Giardia duodenalis is a common intestinal parasite in humans, a wide range of domesticated and wild animals. There are human and animal specific, as well as zoonotic pathogenic genotypes. It is not clear whether livestock in close proximity to humans could be infected with human specific genotypes, and vice versa. In this study, *Giardia*-positive faecal samples were collected from both humans (n=4) (from Maamobi Polyclinic in the Ayawaso Sub- Metro) and calves (n=8) (from Animal Research Institute Farms, Adentan Municipality), in Ghana. Nested PCR amplification using *Giardia*-specific, *Glutamate dehydrogenase* (GDH) genes and *Triosephosphate isomerase* (TPI) gene primers for human and animal faecal samples respectively was carried out. Results showed that 100% of the calves carried the TPI-B genotype, which is a common pathogenic genotype in humans. This report is based on the unusual results obtained as Giardia *duodenalis* genotype B is known to occur in humans but is being observed in calves for the first time. This suggests that calves in close proximity to humans could be reservoirs and sources of human *Giardia* infections.

Keywords: *Giardia duodenalis*, GDH genes, TPI genes, calves, genotype B

Introduction

Giardia lamblia has been identified as one of the zoonotic intestinal parasite which causes diarrhoea in children less than five years of age and immuno-compromised adults worldwide, this include those who had undergone organ transplant, undergoing chemotherapy and other health challenges which affect the effectiveness of the immune system (Cook, 1996; Cotton *et al.,* (2015)). It is prevalent in both developed (2 - 5%) and under developed (30%) regions of the world (Craun, 1990). Globally, 280 million people are infected every year. Twenty percent of diarrhoea in the developing world is caused by *Giardia* sp (Islam, 1990) and 3% in the developed world (Adam, 1991).

In the recent past, identification of the genus has been based on morphological characteristics which are achieved by microscopy, but there has been an improvement in the methods of identification, involving the use of genetic markers (Durigan *et al.,* 2018). Genetic and phenotypic analyses had gained grounds and that give more accurate results except that these are expensive (Anim- Abaidoo 2013; Durigan *et al.,* 2018). The use of DNA anal-

ysis and iso-enzymes profiles has shown that *G. lamblia* is a complex species which exhibit genetic diversity. Glutamate dehydrogenase (GDH) genes and *Triosephosphate isomerase* (TPI) genes can now be amplified and used to identify the different genotypes and sub-genotypes (Molina *et al.,* 2007). GDH gene is an enzyme, which is present in most micro-organisms as well as the mitochondria of eukaryotes. It is an enzyme required for urea synthesis and it converts glutamate to α-ketoglutarate, and vice versa. In animals, the ammonia produced by the excretory organs/organelles is used as a substrate in the urea cycle. The glutamate dehydrogenase gene (*GDH*) is a useful genetic markers for the genotypic analysis of *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*), the protozoan that widely causes enteric disease in humans (Sprong *et al.,* 2009*).* TPI is an enzyme that in humans, it is encoded by the *TPI1* gene. This gene encodes an enzyme which consist of two identical proteins. The TPII gene catalysis the isomerization of glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) in glycolysis and gluconeogenesis. Mutations in this gene are associated with triosephosphate isomerase deficiency (Feng & Xiao, 2011) Both genes are located on the parasite chromosome and the locations are conserved regions of the genome, this allows the loci to be exploited for the purpose of identifying the parasite (Hopkins *et al.,* 1997) Since the *Giardia* species are zoonotic (there are human species and animal species), GDH and TPI primers were used for the study and the objective of this study was to identify the various genotypes in the human and animal faecal samples (from the cattle). This was to help in tracking zoonotic transmission between the cattle and humans so as to facilitate the break in the transmission cycles.

Experimental

Four human faecal samples (ages five to 17 years) were collected from Mamobi in the Ayawaso West Sub-Metro (Urban community),which has a population of about 20,000 people (Ghana Statistical Service Demographic Report 2014) and eight calves' faecal samples (less than six months) were collected from the CSIR- Animal Research Institute Farms at Fafraha in the Adentan Municipality (which is a Peri-Urban Community with a human population of about 10,000 (Ghana Statistical Service Demographic Report, 2014) for use as positive control in a study) using wide-mouth plastic container with crew lid and this was used as positive control in a study (Mtabo, 1995). All the samples were diarrhoea positive. The collected samples were processed on the same day of collection and the parasite DNA was extracted using the QIAGEN mini stool kit. *Giardia* sp. was detected using the PCR method described elsewhere (Amar *et al.,* 2002; Molina *et al.,* 2007), making use of GDH, TPIA and TPIB primers for amplification of the *Giardia* sp from all the faecal samples collected. The nested PCR primers for the GDH gene amplification were; GDH1a (5'-ATCTTCGAGAAGGATGCTTGAG-3') GH1(5'-ATCTTCGAGAGGATGCTTGAG - 3'), GDH5s(5'-GGATACTTSTCCTTGAACTC-3')to produce a 2324 bp fragment.

In the secondary PCR, a 465 bp fragment was amplified using internal forward primer GDHeF (5'-TACACGTYAAYAAY-CGYGGYTTCCGT-3') and internal reverse primer GDiR (5'-GTTRTCCTTGCA-CATCTCC-3') (Boontanom *et al.,* 2010).
The TPIB primers were;
TPIB-F (5'-GTTGCTCCCTTTGTGC-3') and TPIB-RI (5'-CTCTGCTCATTGGTCTC-GC -3'), (208 bp) (Amar *et al.,* 2002; Molina *et al.,* 2007). All the primers were purchased from New England Biolabs Inc., Ipswich, MA, USA through Iqaba, South Africa.

All PCR amplifications were carried out in a PeQ STAR thermo cycler (USA) After the PCR, 10 µl of PCR product was add

ed to 2 µl of 6 bromophenols blue loading dye and electrophoresed in 2% agarose gels stained with 0.5 µg/ml Ethidium bromide. The gels were prepared and run in 1x TAE buffer at 110V for 30 – 45 minutes. They were then observed and photographed over UV trans illuminator at short wavelength using a Kodak EDAS 290 gel documentation system. The sizes of the PCR products were estimated by comparing with the mobility of a standard 100bp DNA ladder (New England Biolabs Inc., Ipswich, MA, USA).

Statistical analysis: No statistical analysis was made as the study was to identify the species present.

Results

From the work done, it was observed that the 'GDH' primers identified *Giardia* genes in all the four human faecal samples collected and analyzed (Fig. 2 below) whereas the 'TPI' primer identifies *Giardia* genes in all the eight animal faecal samples collected and analyzed (Fig.1 below).

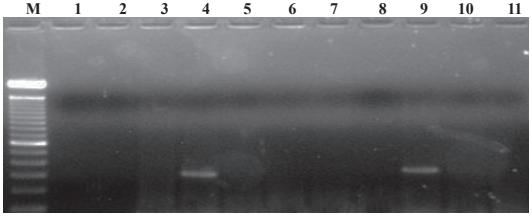

Fig. 1: *Giardia duodenalis* TPI-B gel image (208 bp).

Lane M = 100 bp molecular ladder; Lanes 1-3, 5-8, 10 = negative field faecal samples; Lanes 4 and 9 = positive field faecal samples; Lane 11 = negative control

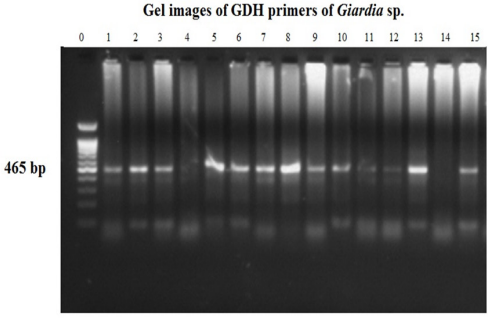

Fig. 2: *Giardia duodenalis* GDH gel image (465 bp)

Lane M = 100 bp molecular ladder; Lanes 1-3 =human positive controls; Lane 4 = negative control

These observations are poorly understood as no study have been able to explain why this happened, (Anim-Baidoo,2013) could not also explain these observations he made in an earlier study.

Discussions and conclusion

Giardia duodenalis genotype B a rare species in animals was identified in faecal samples from Fafraha when diarrhoea positive control samples were collected from the Animal Research Institute Farm. It was identified in calves of about three months of age. Identification was done by the use of TPI primers. Also, GDH genes identified species from human positive control samples identified by microscopy collected from Mamobi. These observations are important in the transmission of *Giardia duodenalis* infections in both humans and domesticated animals, because differentiation between the genotypes A and B has been

found to play effective role in the level of virulence of infection. Whilst genotype A causes intermittent diarrhoea, genotype B is related to persistent diarrhoea (Homan & Mank, 2001). This paper sought to report the first case of genotype B in domesticated animals in Ghana. It is an important observation showing a reverse of zoonosis because a species found only in humans has found its way into animals (Mensah, 2016). This will help in managing the spread of the genotype from animal species to humans.

Acknowledgements

Ethical clearance was sought from the Ethical Review Committee of the Council for Scientific and Industrial Research, Accra Ghana. The authors wish to thank the Laboratory staff of Maamobi Polyclinic of the Ghana Health Service and the Director and Staff of CSIR Animal Research Institute, Accra for supplying the samples for the studies.

References

Adam, R.D. (1991) Biology of *Giardia* spp. Microbiol. Rev. **55**, 706 – 732.

Anim-Baidoo, I. (2013). Epidemiology and molecular characterization of *Giardialamblia* and *Cryptosporidium* sp. infections among children in Accra, Ghana A thesis submitted to the University of Ghana, Legon.

Boontanom, P., Siripattanapipong, S., Munnthin, M., Tan-Ariya, P. & Leelayoova, S. (2010) Improved sensitivity of PCR amplification of Glutamate Dehydrogenase gene for detection and genotyping of Giardia duodenalis in stool specimen. South-East Asian J. Trop. Med Public Health 4.

Cook, G. C. (1996) Manson's Tropical Diseases, 20th ed. W. B. Saunders Company Ltd., London.**1** (2), 280 – 284.

Cotton, J.a., Amat, C.b. & Buret., A. G. (2015) Disruptions of Host Immunity and Inflammation by Giardia Duodenalis: Potential Consequences for Co-Infections in the Gastro-Intestinal Tract. Pathogens.**4,** 764 – 792.

Craun, G.f. (1990) Waterborne giardiasis. In Meyer EA,ed.Human parasitic diseases. Vol.3, Giardiasis, Elsevier: 267 – 293.

Durigan, M., Cardoso-Silva, C.b., Ciampiguillardi M, Toledo-Silva, G., Mori G.m., Franco R.m.b., *et al.,* (2018) Molecular genotyping, diversity studies and high-resolution molecular markers unveiled by microsatellites in Giardia duodenalis. PLoS Negl Trop Dis 12 (11): e0006928.

Feng, Y. & Xiao L. (2011) Zoonotic Potential and Molecular Epidemiology of Giardia Species and Giardiasis. *Clin Microbiol Rev*.**24**(1), 110 – 140. pmid:21233509 Ghana Statistical Service Demographic Report 2014

Homan, W. & Mank, T. (2001) Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parasitol* **31**, 822 – 826.

Hopkins, R. M., Meloni, B. P., Groth, D. M., Wetherall, J. D., Reynoldson, J. A. & Thompson, R. C. A. (1997) Ribosomal RNA sequencing reveals differences between genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. *J. Parasitol.* **83,** 44 – 51.

Islam, A. (1990) Giardiasis in developing world. In Meyer EA, ed. Human parasitic diseases. Vol.3, Giardiasis, Elsevier: 235 – 266.

Mensah, G. T. (2016) Molecular Epidemiology of Cryptosporidium and Giardia spp in Kpong and its environs in Ghana A thesis submitted to the University of Ghana, Legon.

Molina, N., Polverino, D., Minvielle, M. & Basualdo, J. (2007) Pcr amplification of triose phosphate isomerase gene of Giardia lamblia in fomalin fixed feces. Rev. Latinoam *Microbiol* **49** (1 – 2), 6 – 1.

Troll, H., M. Hanspeter & N. Weiss. (1997) Simple Differential Detection of *Entamoeba histolytica* and *Entamoeba dispar* in Fresh Stool Specimens by Sodium Acetate-Acetic Acid-Formalin Concentration and PCR. *J. Clin. Microbiol*. **35**, 1701 – 1705.

MTAMBO, M. M. A. (1995) The prevalence of *Cryptosporidium sp.* in cattle and wildlife in Tanzania. *Int. J. Parasitol.* **28**, 111 – 120.

SPRONG, H., CACCIO, S.M. & GIESSEN, J.W.B.V.D. (2009) Identification of Zoonotic Genotypes of *Giardia duodenalis*. *PloS Negl Trop Dis*. **3** (12), e558. pmid:19956662.

Received 24 Aug 17; revised 13 Mar 20.