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Giardiasis and intestinal pathology: Molecular detection and taxon assemblage typing of *Giardia duodenalis* in school-aged children along the shoreline of Lake Malawi, Malawi

Giardiasis is a waterborne and potentially debilitating intestinal parasitic disease caused by infection with the eukaryotic protozoan *Giardia duodenalis*. Whilst cosmopolitan in distribution, prevalence of human giardiasis can be particularly high in rural areas of low- and middle-income countries (LMICs) lacking adequate water and sanitation hygiene (WASH) infrastructure, including those in sub-Saharan Africa.

Of the eight known morphologically identical but genetically distinct *G. duodenalis* taxon assemblages A through H, the majority of human infections are caused by zoonotic assemblages A and B. Differentiation between human infections with *G. duodenalis* assemblages A and B, as well as between single (A or B)- and mixed (A and B)-assemblage infections, is therefore essential to better understand the pathological impact of infection with either, or both, assemblages and thus also for improved disease surveillance and control.

Using end-point PCR with subsequent genotyping and phylogenetic analyses, as well as real-time PCR, we assessed the prevalence of human infection with either, or both, *G. duodenalis* assemblages A and B using faecal samples provided by 305 school-aged children situated along the southern shoreline of Lake Malawi, Mangochi District, Malawi; an area where only limited data on the prevalence of human giardiasis is currently available. In addition, pathology data was also collected from all study participants in the form of lateral flow rapid diagnostic tests (RDTs) that detect overt blood and calprotectin in faeces, and questionnaire responses to the questions ‘do you currently have abdominal (stomach) pain?’ and ‘do you currently have loose stool (diarrhoea)?’.

Prevalence of *G. duodenalis* infection was 39.3% when using a species-specific 18S diagnostic real-time PCR. When targeting two additional and distinct genetic assemblage-specific loci, 35% of all infections were identified as single *G. duodenalis* assemblage A; 32% were identified as single *G. duodenalis* assemblage B; and 33% were identified as mixed *G. duodenalis* A and B. No infections were identified as *G. duodenalis* assemblages C-H.

Whilst there was no association between single infection with *G. duodenalis* assemblage A and any form of pathology, there was a statistically significant and strong positive correlation between single infection with *G. duodenalis* assemblage B and both self-reporting of abdominal pain and self-reporting of diarrhoea. Additionally, there was a statistically significant and positive correlation

between mixed infection with both *G. duodenalis* assemblages A and B and self-reporting of abdominal pain, but no association between mixed infections and any other form of pathology. Our study therefore further highlights the importance of molecular methods that can be used to identify *G. duodenalis* assemblage types and investigate their impact on human intestinal pathology, whilst also reaffirming the need for improved access to WASH infrastructure in rural areas of low- and middle-income countries.