

Molecular epidemiology of giardiasis: facts and problems

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Giardia is a genus of intestinal flagellates that infects a wide range of vertebrate hosts. The genus currently comprises six species, namely *Giardia agilis* in amphibians, *Giardia ardeae* and *Giardia psittaci* in birds, *Giardia microti* and *Giardia muris* in rodents, and *Giardia duodenalis* in mammals. These species are distinguished on the basis of the morphology and ultrastructure of their trophozoites. *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*) is the only species found in humans, although it is also found in other mammals, including pets and livestock. This parasite has a global distribution, causing an estimated 2.8×10^8 cases per year, and is the most common intestinal parasite of humans in developed countries. Infection is by the typical faecal-oral route, and the epidemiology of giardiasis is complex and involves both direct (human-to-human, animal-to-animal) and indirect (zoonotic, water-borne and food-borne) transmission routes. Furthermore, the lack of reliable morphologic variation among isolates explain why investigation of aspects such as host specificity and transmission patterns requires a direct genetic characterization of cysts/trophozoites from host samples. Compared to other protozoan pathogens, genotyping techniques for *Giardia* spp. are not particularly advanced, and the vast majority of studies have relied on the analysis of few genes, including the small subunit ribosomal RNA, the β -giardin, the glutamate dehydrogenase, the elongation factor 1-alpha, the triose phosphate isomerase, and the GLORF-C4 genes.

A considerable amount of data has shown that *G. duodenalis* should be considered as a species complex, whose members show little variation in their morphology, yet can be assigned to at least seven distinct assemblages (A to G) based on genetic analyses. The analysis of more than a thousand human isolates from different geographical locations, examined by PCR amplification of DNA extracted directly from faeces, demonstrates that in almost all cases, only *G. duodenalis* assemblages A and B are associated with human infections. The prevalence of each assemblage varies considerably from country to country; assemblage B seems more common, overall, but no strong conclusions can be drawn from current data. The remaining assemblages (C to G) are likely to be host-specific, as assemblage C and D have been identified in dogs, cats, coyotes and wolves, assemblage E in cattle, sheep, goats, pigs, water buffaloes and muflons, and assemblages F and G in cats and rats, respectively.

Sequence-based surveys have identified a number of subtypes within assemblages A and B in animal species, and their zoonotic potential seem supported by the fact that identical subtypes are also recovered from humans. More variable loci however are required to resolve the issue of zoonosis and it is possible that the use of these loci will indicate more genetic differences between isolates found in animals and those in humans, as nicely exemplified by recent research on *Cryptosporidium*.

Transmission of *G. duodenalis* through drinking and recreational water is well documented, as are outbreaks of giardiasis following the consumption of contaminated water. Indeed, of the 325 water-associated outbreaks of parasitic protozoan disease reported so far, *G. duodenalis* was the causative agent in about 40%. Despite this, little is known about the *Giardia* spp. or *G. duodenalis* assemblages present in the aquatic environment.

Molecular epidemiologic studies were implicitly based on the assumption that the identified genotypes are stable over time, and that recombination was absent. The real situation may be more complex, as witnessed by the recognition of intra-isolate sequence heterogeneity (i.e., “mixed templates”, that affects identification

of subtypes within each assemblage), and by the fact that different markers support the assignment of isolates to different *G. duodenalis* assemblages (i.e., that a given isolate cannot be unequivocally assigned to a given assemblage). The genetics of parasites of the genus *Giardia* is still poorly understood and the classical view of an asexually replicating organism has been called into question by recent evidence in favour of the occurrence of meiosis and genetic exchange. Therefore, the population genetics of this organism should be re-evaluated to take into account the effect of recombination among members of the *G. duodenalis* species complex. Many questions still remain unanswered and more experimental work is needed to understand how this will impact on studies addressing the epidemiology of the infection based on a molecular genotyping approach.