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Review

Molecular epidemiology of giardiasis

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ABSTRACT

Giardia duodenalis is a widespread parasite of mammalian species, including humans. Due to its invariant morphology, investigation on aspects such as host specificity and transmission patterns requires a direct genetic characterization of cysts/trophozoites from host samples. A number of molecular assays have been developed to help unravel the complex epidemiology of this infection. A coherent picture has emerged from those studies, indicating the existence of seven genetic groups (or assemblages), two of which (A and B) are found in both humans and animals, whereas the remaining five (C–G) are host-specific. Sequence-based surveys have identified a number of genotypes within assemblages A and B in animal species, some of which may have zoonotic potential. Recently, however, molecular approaches have been complicated by the recognition of intra-isolate sequence heterogeneity (i.e., "mixed templates", that affects identification of subtypes within each assemblage), and by the unreliable assignment of isolates to *G. duodenalis* assemblages generated by different genetic markers. This raises concerns about previous interpretation of genotyping data, especially when single genetic markers have been used. The mechanisms that may be responsible for these findings, including allelic sequence heterozygosity and meiotic recombination, are discussed.

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1. Introduction

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 [1].

G. 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 [2]. 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–G) based on genetic analyses [3]. 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 (Table 1) [4–17]. 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

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Table 1
Prevalence of *Giardia duodenalis* assemblages A and B in humans

Origin	Nature of the samples (no. of isolates)	Loci tested	Assemblage A (%)	Assemblage B (%)	A + B (%)	Reference
Italy	Sporadic (120)	*ssu-rRNA*, *bg*	65 (54%)	39 (32.5%)	16 (13.5%)	[4]
UK	Nursery outbreak (21)	*tpi*		21 (100%)		[5]
The Netherlands	Population survey (18)	*gdh*	9 (50%)	9 (50%)		[5]
The Netherlands	Sporadic (98)	*ssu-rRNA*, *gdh*	34 (35%)	64 (%)		[6]
France	Sporadic (25)	*tpi*	9 (36%)	16 (64%)		[7]
Spain	Case control study (108)	*tpi*	43 (39.8%)	61 (56.5%)	4 (3.7%)	[8]
Norway	Waterborne outbreak (21)	*bg*, *gdh*		21 (100%)		[9]
Norway	Sporadic (63)	*bg*, *gdh*, *tpi*	3 (5%)	60 (95%)		[10]
Albania	Sporadic (22)	*ssu-rRNA*	10 (45%)	12 (55%)		[11]
Uganda	Sporadic (3)	*ssu-rRNA*	3 (100%)			[5]
Ivory Coast	Soldiers (14)	*tpi*		14 (100%)		[7]
Ethiopia	Sporadic (59)	*bg*, *gdh*	31 (52%)	13 (22%)	15 (25%)	[12]
Peru	Sporadic (25)	*tpi*	6 (24%)	19 (76%)		[5]
Brazil	Sporadic (37)	*gdh*	29 (78%)	8 (22%)		[13]
Brazil	Sporadic (62)	*bg*	62 (100%)			[14]
USA	Sporadic (14)	*ssu-rRNA*	14 (100%)			[5]
USA	Sporadic (2)	*tpi*		2 (100%)		[5]
Mexico	Sporadic, children (9)	*bg*	9 (100%)			[15]
Canada	Waterborne outbreak (6)	*ssu-rRNA*	6 (100%)			[5]
Australia	Sporadic (8)	*ssu-rRNA*, *gdh*	2 (25%)	6 (75%)		[5]
Australia	Population survey (23)	*ssu-rRNA*	7 (30%)	16 (70%)		[5]
Australia	Sporadic (12)	*ssu-rRNA*		11 (92%)	1 (8%)	[5]
Turkey	Sporadic (44)	*tpi*	19 (43%)	25 (57%)		[5]
Bangladesh	Case–control study (267)	*tpi*	20 (7.5%)	231 (86.5%)	16 (6%)	[16]
India	Sporadic (10)	*tpi*		10 (100%)		[5]
India	Sporadic (19)	*tpi*, *EF1-α*	6 (32%)	9 (47%)	4 (21%)	[5]
India	Sporadic (12)	*gdh*	5 (42%)	7 (58%)		[17]
Laos	Sporadic (5)	*ORF-C4*		5 (100%)		[5]
China	Sporadic (8)	*ssu-rRNA*	4 (50%)	4 (50%)		[5]
Korea	Sporadic (5)	*ssu-rRNA*	5 (100%)			[5]
			391 (**35**%)	**671** (**60**%)	**56** (**5**%)	

assemblages (C–G) are likely to be host-specific, as assemblages 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 [5].

Giardia duodenalis has a global distribution causing an estimated 2.8×10^8 cases per year [18], and is the most common intestinal parasite of humans in developed countries. In Asia, Africa and Latin America, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year [19].

Clinical manifestations of giardiasis are quite variable, and range from the absence of symptoms to acute or chronic diarrhoea, dehydration, abdominal pain, nausea, vomiting, and weight loss [20]. The severity of disease is determined by the interplay between the virulence of the parasite, and the developmental, nutritional and immunological status of the host. However, studies on the possible association between *G. duodenalis* assemblages and the severity of the disease have proved thus far inconsistent. Indeed, assemblage B has been associated with non-symptomatic infections in children less than 5 years of age [8] but with persistent diarrhoeal complaints in the general Dutch population [21]. Another study in Bangladesh reported that patients with assemblage A were twice as likely to have diarrhoea than patients with assemblage B and that assemblage B infections were statistically associated with asymptomatic *Giardia* infection [16].

In addition to the factors discussed above, several other characteristics of *G. duodenalis* influence the epidemiology of infection: (i) in humans, the infective dose is about 10–100 cysts [22]; (ii) cysts are immediately infectious when excreted in faeces, and can be transmitted by person-to person or animal-to-animal contact [2]; (iii) cysts are remarkably stable and can survive for weeks to months in the environment [23]; and (iv) environmental contamination can lead to the contamination of drinking water and food [24].

In this review, we will first introduce the existing methodology for genotyping *G. duodenalis* isolates and then critically explore, in the light of recent investigations, some controversial aspects, such as zoonotic transmission and the occurrence of allelic sequence heterozygosity and meiotic recombination.

1.1. Molecular typing of G. duodenalis: Genetic loci, their variability and the issue of nomenclature

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 the small subunit ribosomal RNA (*ssu-rRNA*), the β-giardin (*bg*), the glutamate dehydrogenase (*gdh*), the elongation factor 1-alpha (*ef-1*), the triose phosphate isomerase (*tpi*), the GLORF-C4 (*C4*) genes [5] and recently, the inter-genomic rRNA spacer region (IGS) [25]. As the genome of a *G. duodenalis* isolate (WB, assemblage A, subgroup A1) has been completely sequenced [26], it is possible to locate those genes on chromosomes or on large contigs. This shows that the genes mentioned above are unlinked in the *G. duodenalis* genome, at least in the assemblage A genome, which is a desirable property for genetic studies. Indeed, the *tpi* gene is at position 95921–96694 on the 200 kb-long contig ctg02_19, the *bg* gene is at position 55484–56302 on the 90 kb-long contig ctg02_35, the *gdh* gene is at position 60579–61928 on the 231 kb-long contig ctg02_15, the *ef-1* gene is at position 40230–41558 on the 61 kb-long contig ctg02_53, and the *C4* gene is at position 68643–69239 on the 80 kb-long contig ctg02_44 (data taken from http://www.giardiadb.org). In previous studies based on hybridization on chromosomes separated by pulsed-field gel electrophoresis, the *tpi* gene was mapped to chromosome 5, the *gdh* and *bg* were mapped to chromosome 4, and the majority of *ssu-rRNA* gene copies were mapped to chromosome 1 (reviewed in [1]).