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Ecological characterization of interspecific relationships between human parasites: conflict, cooperation or independence?

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Keywords

Tropical parasites;
Multiparasitism;
Ecological relationship;
Control strategies.

Abstract

Interspecific associations among parasite species occur at the single host individual, population and community levels. The detection and understanding of multiparasitism are crucial to prevent and manage infectious diseases. In order to find out potential interspecific interactions among parasite species at a host population level, a cross-sectional study was carried out from September 2017 to July 2018 on schoolchildren aged from 4 to 15 years. Primary schools were randomly selected in the Nyong-et-Mfoumou Division. Stool samples and blood smears were analysed to detect parasitic forms of protozoa and helminths. Parasite interspecific associations were explored by ecological indices of association: Dice (D), Forbes (F) and tetrachoric coefficient (ϕ). The parasitological analysis revealed the presence of 13 parasite species belonging to 11 families, 9 orders, 7 classes and 5 phyla. A cooperation or positive association was found between *Entamoeba coli* and *E. histolytica/* dispar, *Ascaris lumbricoides* and *Trichiuris trichiura*, *E. coli* and *Plasmodium falciparum*, *E. histolytica/* dispar and *P. falciparum*, *E. coli* and *A. lumbricoides*, and *E. coli* and *T. trichiura*. The above pairs of parasites co-occurred together more frequently than expected by chance. The conflict or negative association was noticed between *Giardia intestinalis* and both *A. lumbricoides* and *T. trichiura*, and between *A. lumbricoides* and *P. falciparum*. The independence was found between *G. intestinalis* and both *E. histolytica/* dispar and *E. coli*; and between *Mansonella perstans* and *Endolimax nana*, *G. intestinalis*; *E. coli* and *E. histolytica/* dispar. Further studies are needed to identify the real interaction mechanisms between parasite species and to evaluate the consequences of multiparasitism for both parasite species and the host.

Historic

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1. Introduction

Multiparasitism is the simultaneously presence of more than one parasite species in a single host individual. Increasingly, it is recognised that this situation is the rule in most biological models, including humans [1]. Within the host individual, co-infecting parasites can interact with each other, modifying the intra-host and/or inter-host dynamics of each other. Such interspecific interactions increase or decrease the susceptibility of the host to other parasite species, the inter-host transmission rate of the interacting parasites, and/or the severity of the disease symptoms they induce. They may also greatly influence the evolution of the parasites themselves, in particular that of their virulence [2].

Interspecific interactions among parasites that occur at the single host individual level, can spill over to population and community host levels. At the single host level, parasite species can interact with each other either directly through several mechanisms such as mechanical facilitation, interference, cooperation, and competition or indirectly through resource quest or through the host immune system [3, 4]. The result may be on the one hand, co-occurrence or synergy i.e., individuals of one species create conditions that favour the arrival, settlement and development of subsequent parasite species [5]; or on the other hand, the exclusion or antagonism i.e. individuals of one parasite species create an unfavourable terrain for the installation or persistence of other parasite species [6]. At the host population level, the presence of several parasites may play an important role for the successive steps of the transmission of one parasite: exit from the host, contact with susceptible hosts and successful infection. At the host meta-population or community level, the presence of several parasites may affect the probability of successful transmission of a given species to different sub-populations of the same host species [2]. Interactions at the intra-host level have been much more extensively documented than those at the upper levels.

Actually, the challenge for epidemiologist is moving from a "one parasite – one host" approach towards an ecosystem view: "multi-parasites – multi-hosts"; this can help to have a clear vision of the complexity of natural systems [7]. The study of multiparasitism in order to elucidate the interactions between parasite species in

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different infracommunities is crucial to master their transmission dynamics, the pathogenesis of parasitic diseases, and to develop more effective strategies of fighting against parasitic diseases [8]. In some cases, taking multiparasitism into account can be helpful for the identification of the causes of epidemics or declines, and the development of tools and methods for epidemiological surveillance [9].

In sub-Saharan Africa, despite the efforts of governments, schoolchildren are still most vulnerable to several parasitic diseases such as malaria and intestinal parasitosis; they are particularly exposed to multiparasitism and its potential consequences [10]. Furthermore, environmental changes and anthropic activities in different settings lead to modify ecological barriers creating favourable conditions for the transmission of several parasite species [11]. During multiparasitism, the interspecific interactions among parasites impact on host health, parasite circulation and pathogen management [12, 13, 14]. Therefore, their detection and understanding are crucial to prevent and manage infectious diseases. The identification of synergies between parasite species may help to prevent population decline or extinction. On the contrary, in an antagonistic interaction, measures targeting only one parasite species may result in unexpected increase of a second co-circulating parasite species [15, 16]. The present study aims to determine blood and gastrointestinal protozoan and helminth parasite fauna in a population of schoolchildren in order to find out potential interspecific interactions among parasite species.

2. Material and methods

2.1. Study area

The Nyong-et-Mfoumou is one of the ten divisions in the Centre region of Cameroon. It is situated between 3°16' – 4°19' north latitudes and 11°48' – 12°51' east longitudes. It covers an area of about 6,172 Km^2 and has over 130,300 inhabitants [17]. It comprises 5 subdivisions: Ayos, Kobdombo, Mengang, Endom and Akonolinga. The climate is typically equatorial with four discontinuous dry and wet seasons. The annual average rainfall is 2000 mm with an annual average temperature of 24.2°C [18]. The hydrographic network is dense, the Nyong and the Mfoumou rivers being the two main watercourses.

2.2. Study population and sample collection

The protocol used in this study was approved by the National Ethical Committee of Research for Human Health (Ethical Clearance N°2018 /01/968/CE/CNERSH/SP) and the management staff of the Yaoundé University Teaching Hospital (Authorization N°: 894/AR/CHUY/DG/DGA/DMT). A cross-sectional survey was carried out from September 2017 to July 2018; schoolchildren aged from 4 to 15 years were recruited in five primary schools in different settings (2 and 3 primary schools in urban and rural areas respectively) randomly selected in the Nyong-et-Mfoumou division. The sample size was computed according to Cochran [19] to make it representative with the formula n=(Z^2 x p x q)/e^2 (Z = confidence level at 95% i.e., 1.96; e = level of precision i.e., 0.05; p = estimated proportion of an attribute that is present in the population (p = 25.12 %, [20]); and q = 1-p).

The pupils enrolled in this study were asked to provide fresh stool samples in a 60 mL plastic screw-cap vial labelled with the participant's anonymous number. Few drops of blood sample were also collected by finger prick using a single-use vaccinostyle. This blood sample was used to perform blood smears (thick and thin films).

2.3. Parasitological analysis

All the samples collected in the field were analysed in the Parasitology, Mycology and Parasitic Immunology laboratory of the Yaoundé University Teaching Hospital. Stool samples were analysed using the direct examination technique in (1) saline and (2) 2% Lugol solution, and the formalin ether concentration technique [21] for the diagnosis of intestinal protozoa and helminths. Blood smears (thick and thin films) realized in the field were examined after staining using May-Grünwald-Giemsa [22].

A participant was considered positive for intestinal parasites if at least one of the two techniques revealed the presence of protozoan cysts or oocysts and/or helminth eggs or larvae in the stool sample. Blood smears were negative if no parasite (*Plasmodium* trophozoites or microfilariae) was found after investigation of at least 20 fields of the thick smears. Thin smears were used for identification of hemoparasites. All the parasite species were identified using the identification keys available in the laboratory [23, 24].

2.4. Data analysis

The prevalence and the infracommunity were defined according to Bush et al. [25] and Combes [26] respectively. The Chi-square test and Fisher's exact test made it possible to compare the prevalence. The difference was considered significant when P-value < 0.05. Parasite interspecific associations were explored by ecological indices of association: Dice (D), Forbes (F) and tetrachoric coefficient (ϕ) [27].

The Dice (D) index assessed the actual level of a binary interspecific association; D=(2 a)/(2a+b+c) and varies from 0 to 1. When D ≤ 0.09 the association is very weak, 0.10 ≤ D ≤ 0.24: weak association; 0.25 ≤ D ≤ 0.49: moderate association; and D ≥ 0.5 means very strong association [27].

The Forbes (F) index measured the deviation from chance of binary association between parasite species: F=(a n)/(a+b)(a+c) . When F < 1, the association is less frequent than expected by chance; F ≈ 1, the association is in accordance with the laws of chance; F > 1, the association is more frequent than expected [27].

The tetrachoric coefficient (ϕ) assessed the correlation between two parasite species: ϕ=(ad-bc)/√((a+b)(a+c)(c+d)(b+d)) . ϕ varies from -1 to +1. ϕ = -1 for perfect exclusion (one parasite excludes another); ϕ = +1 for perfect association (one parasite favors the presence of another); ϕ = 0 when the presence of one parasite does not influence that of the other.

For each ecological association index, we had: a = number of individual hosts harbouring the two parasite species; b = number of individual hosts harbouring the first parasite; c = number of individual hosts harbouring the second parasite; d = number of individual hosts without any parasite, and n = total number of individual hosts examined [27].

3. Results and Discussion

3.1. Results

Study Population

A total of 416 pupils (average 9.17 ± 0.27 years) were enrolled for this study. Among them, 135 (32.45%), 169 (40.63%), and 112 (26.92%) were [4 - 8[, [8 - 12[and [12 - 16[years old respectively. Girls were slightly more represented (54.33%) than boys (45.67%); the boys/girls ratio was 0.8.

Parasitic fauna: prevalence, species richness and infracommunities

The parasitological analysis revealed the presence of 13 parasite species belonging to 11 families, 9 orders, 7 classes and 5 phyla. The phyla Sarcomastigophora and Nematoda were the most diverse with 5 species each; the other (Apicomplexa, Heterokonta and Platyhelminths) were represented by only 1 species each (Table I).

Tableau I : Diversity of parasite species in the study population

Phyla	Classes	Orders	Families	Species
Sarcomastigophora	Lobosea	Amoebida	Entamoebidae	*Entamoeba histolytica*, Schaudinn 1903/ *E. dispar* Brumpt, 1925
				Entamoeba coli Loesch, 1875
				Endolimax nana Kuenen et Swellengrebel, 1917
	Zoomastigophorea	Diplomonadida	Hexamitidae	*Giardia intestinalis* Kofoid et Christiansen, 1915
			Retortamonadidae	*Embadomonas intestinalis* Grassi, 1879
Apicomplexa	Aconoidasida	Haemosporida	Plasmodiidae	*Plasmodium falciparum* Welch, 1897
Heterokonta	Blastocystea	Blastocystida	Blastocystidae	*Blastocystis* sp. Alexieff, 1911
Nemathelminths	Nematoda	Ascaridida	Ascarididae	*Ascaris lumbricoides* Linnaeus, 1758
		Strongylida	Ancylostomidae	*Necator americanus* Stiles, 1902/ *Ancylostoma duodenale*, Dubini, 1843
		Spirurida	Onchocercidae	*Mansonella perstans* Manson, 1891
			Filariidae	*Loa loa* Cobbold, 1864
		Trichocephalida	Trichuridae	*Trichiuris trichiura* Linnaeus, 1771
Plathelminths	Cestoda	Cyclophyllidea	Hymenolepididae	*Hymenolepis nana* Siebold, 1852

Overall, 309/416 children were infected at least by one parasite species, and the general infection rate was 74.28 %. *Plasmodium falciparum* Welch, 1897 was the most prevalent species in the study population (prevalence: 37.26 %), followed by *Entamoeba coli* Loesch, 1875 (29.33 %), *Entamoeba histolytica*, Schaudinn 1903/ *E. dispar* Brumpt, 1925 (23.80 %), Ascaris lumbricoides Linnaeus, 1758 (21.39 %) and *Trichuris trichiura* Linnaeus, 1771 (18.51 %) see Table 2. In general, the prevalence of most parasite species was significantly higher in rural areas than in urban areas ($P < 0.0001$), except for *E. histolytica*/dispar whose prevalence seems to be higher ($P = 0.389$) in urban areas than in rural areas, but the difference was not significant. Details of the above results have been published elsewhere [28].

A total of 185 (44.47%) participants harboured 2 or more parasite species; the frequency of multiparasitism was significantly higher ($P = 0.017$) than that of monoparasitism. The maximum number of parasite species found in one host was five, the average species richness being 1.43 ± 0.01 species/host. Fifty-four (54) infracommunity types were found among which 19, 18, 14 and 3 comprised 2, 3, 4, and 5 species.

Ecological relationships between parasite species

Associations between protozoa species

Many protozoan species were found colonizing the gastrointestinal tract in the study population; on one hand a moderate association was found between the main amoeba species *E. coli* and *E. histolytica*/dispar ($D = 0.35$). These parasite species appeared twice more frequent than expected by chance ($F = 2.04$), and the tetrachoric coefficient revealed a significant association between them ($\phi = 0.32$). On the other hand, associations between the latter parasite species (*E. coli* and *E. histolytica*/dispar) and the other gastrointestinal protozoa (*En. nana* and *G. intestinalis*) were very week ($D \leq 0.09$). Nevertheless, some of them (*E. coli* and *Endolimax nana*, *E. coli* and *G. intestinalis*) were more frequent than expected by chance ($F > 1$); the others (*E. histolytica*/dispar and *En. nana, E. histolytica*/dispar and *G. intestinalis*) were less frequent than expected by chance ($F < 1$) and no influence appeared between them ($\phi = -0.04$ and $\phi = -0.01$, respectively).

Among gastrointestinal and blood protozoans, moderate associations appeared between *P. falciparum* and both *E. histolytica*/dispar and *E. coli* ($D = 0.35$ and $D = 0.32$, respectively); these associations occurred almost twice more frequently than expected by chance ($F = 1.87$ and $F = 1.81$, respectively), although they seem not to influence each other ($\phi = 0.15$ and $\phi = 0.14$ respectively). *P. falciparum* was associated very weakly ($D = 0.05$) and weakly ($D = 0.10$) with *En. nana* and *G. intestinalis*, respectively. Its co-occurrence with the latter was more frequent ($F = 1.26$), while it was less frequent ($F = 0.82$) with En. nana. In both cases no influence appeared between these parasites (Table 3).

The absence of association was noticed between *G. intestinalis* and both *E. histolytica*/dispar ($\phi = 0.03$) and *E. coli* ($\phi = 0.01$), and *P. falciparum* and *En. nana* ($\phi = -0.02$) (Table 3).

Tableau 2 : Prevalence of parasite species in the study population

Parasite species	Total (N = 416)	Living areas		
	n_p (%)	Rural n_1 = 209 (%)	Urban n_2 = 207 (%)	*P*
P. falciparum	155 (37.26)	90 (43.06)	65 (31.40)	0.0139*
M. perstans	18 (4.32)	13 (6.22)	05 (2.41)	0.0565
L. loa	2 (0.48)	02 (0.96)	00 (0.00)	/
E. coli	122 (29.33)	89 (42.58)	33 (15.94)	0.0001*
E. histolytica/dispar	99 (23.80)	46 (22.00)	53 (25.60)	0.389
G. intestinalis	17 (4.09)	13 (6.22)	04 (1.93)	0.0272*
Blastocystis sp.	06 (1.44)	06 (2.87)	00 (0.00)	/
Em. intestinalis	01 (0.24)	01 (0.48)	00 (0.00)	/
En. nana	13 (3.13)	08 (3.83)	05 (2.42)	0.407
A.lumbricoides	89 (21.39)	70 (33.49)	19 (9.17)	0.0000*
T. trichiura	77 (18.51)	65 (31.10)	12 (5.79)	0.0000*
Hoockworms	04 (0.96)	02 (0.96)	02 (0.96)	/
H. nana	01 (0.24)	00 (0.00)	01 (0.48)	/

np = infected ; n1 and n2 = examined per area, N = examined; * : Significant

Tableau 3 : Ecological characterization of relationships between protozoan species found in the study population

Location	Parasitic association Type	Ecological indexes F	ϕ	D	χ2
Gastro-intestinal tract	*E. coli – E. histolytica/dispar*	2.04[a]	0.32	0.35***	6.35 +
	E. coli – En. nana	1.57[a]	0.08	0.08*	1.83
	E. coli – G. intestinalis	1.20[a]	0.03	0.08*	0.30
	E. histolytica/dispar – En. nana	0.65[c]	-0.04	0.03*	0.52
	E. histolytica/dispar – G. intestinalis	0.49[c]	-0.01	0.04*	0.007
Blood versus Gastro-intestinal tract	*P. falciparum– E. histolytica/dispar*	1.87[a]	0.15	0.35***	0.95
	P. falciparum– E. coli	1.81[a]	0.14	0.32***	0.62
	P. falciparum– G. intestinalis	1.26[a]	0.04	0.10**	0.72
	P. falciparum– En. nana	0.82[c]	-0.02	0.05*	0.24

Legend : D: Dice index ; F: Forbes index; χ^2 : Chi square ; ϕ : Tetrachoric coefficient; * : Very weak association ; ** : weak association ; *** : Moderate association; + : Significant ; a : Association more frequent than expected by chance ; ; c : Association less frequent than expected by chance ; *P. Plasmodium* ; *E*: *Entamoeba* ; *G.*: *Giardia* ; *En.*: *Endolimax*.

Associations between helminths species

The association of A. lumbricoides and *T. trichiura*, the two main gastrointestinal helminths found in this study, was moderate (D = 0.42). These parasite species were diagnosed together twice more frequently than expected by chance (F = 2.12) and significantly associated (ϕ = 0.48, P < 0.05).

Mansonella perstans was weakly (D = 0.18) and very weakly (D = 0.06) associated with *A. lumbricoides* and *T. trichiura*, respectively (Table 4). The co-occurrence of *M. perstans* with *A. lumbricoides* was however significantly positive (ϕ = 0.378) and more frequent than expected by chance (F = 2.59). While that of *M. perstans* with *T. trichiura* tended to be in accordance with the chance (F = 0.90) and null (ϕ = - 0.01). *M. perstans* and *T. trichiura* were independent (ϕ = -0.01) (Table 4).

Associations between helminth and protozoan species

Among gastrointestinal parasites, associations of *E. coli* with *T. trichiura* on one hand and *A. lumbricoides* on the other hand were moderate (D = 0.41 and D = 0.39 respectively), more frequent than expected by chance (F = 2.82 and F = 2.61), and significantly positive (ϕ = 0.45 and ϕ = 0.40). Also, the co-occurrence of *E. histolytica/*dispar firstly with *T. trichiura* and secondly with *A. lumbricoides* was weak (D = 0.15 and D = 0.19) though more frequent than expected (F = 1.79 and F = 1.63) and positive (ϕ = 0.11 and ϕ = 0.10). In the other cases, associations between protozoans and helminths were very weak (D <0.09), less frequent than expected (F < 1), and negative (ϕ < 0) especially between *G. intestinalis* and *T. trichiura*, *G. intestinalis* and *A. lumbricoides*, except that between *En. nana* and *A. lumbricoides* which was more frequent (Table 5).

Tableau 4 : Ecological characterization of relationships between the helminth species found in the study population

Location	Parasitic association Type	Ecological indexes F	ϕ	D	χ2
Gastrointestinal tract	*A. lumbricoides – T. trichiura*	2.12[a]	0.48	0.42***	32.52+
Blood versus Gastrointestinal	*M. perstans – A. lumbricoides*	2.59[a]	0.38	0.18**	13.05+
	M. perstans – T. trichiura	0.90[b]	-0.01	0.06*	0.36

Legend : D: Dice index ; F: Forbes index; χ^2 : Chi square ; ϕ : Tetrachoric coefficient; * : Very weak association ; ** : weak association ; *** : Moderate association; + : Significant ; a : Association more frequent than expected by chance ; b : Association in accordance with the chance ; *M.*: *Mansonella* ; *A.*: *Ascaris* ; *T.*: *trichiura*.

Tableau 5 : Ecological characterization of relationship between the protozoan and helminth species found in the study population

Location	Parasitic association Type	Ecological indexes F	ϕ	D	χ2
Gastro intestinal tract	*E. coli – T. trichiura*	2.82[a]	0.45	0.41***	26.08+
	E. coli – A. lumbricoides	2.61[a]	0.40	0.39***	17.43+
	E. histolytica/dispar – T. trichiura	1.79[a]	0.11	0.15**	2.49
	E. histolytica/dispar – A. lumbricoide.	1.63[a]	0.10	0.19**	1.79
	A. lumbricoides – En. nana	1.43[a]	0.04	0.08*	0.70
	G. intestinalis – T. trichiura	0.59[c]	-0.50	0.08*	1.39
	G. intestinalis – A. lumbricoides	0.55[c]	-0.32	0.04*	0.97
	En. nana – T. trichiura	0.42[c]	-0.05	0.02*	1.04
Gastro Intestinal tract versus Blood	*En. nana – M. perstans*	1.78[a]	0.03	0.06*	0.46
	G. intestinalis – M. perstans	1.35[a]	0.02	0.06*	0.10
	E. coli – M. perstans	1.17[a]	0.010	0.13**	1.87
	T. trichiura – P. falciparum	1.08[b]	0.03	0.17**	0.36
	A. lumbricoides – P. falciparum	0.99[b]	-0.10	0.07*	0.001
	E. histolytica/dispar – M. perstans	0.70[c]	-0.03	0.05*	0.52
Blood	*M. perstans – P. falciparum*	1.10[b]	0.00	0.01*	0.41

Legend : D: Dice index ; F: Forbes index; χ^2 : Chi square ; ϕ : Tetrachoric coefficient; * : Very weak association ; ** : weak association ; *** : Moderate association; + : Significant ; a : Association more frequent than expected by chance ; b : Association in accordance with the chance ; c : Association less frequent than expected by chance ; *P. Plasmodium* ; *M.*: *Mansonella* ; *A.*: *Ascaris* ; *T.*: *trichiura* ; *E*: *Entamoeba* ; *G.*: *Giardia* ; *En.*: *Endolimax*.

Protozoans in the gastrointestinal tract and helminths in the blood or vice versa on one hand, and blood pathogens of both groups on the other hand developed very weak or weak associations (0 < 0.24). These interactions appeared slightly negative only between *A. lumbricoides* and *P. falciparum, M. perstans* and *E. histolytica/* dispar (ϕ ≈ 0). The co-occurrence was less frequent than expected (*E. histolytica/* dispar and *M. perstans*), in accordance with the chance (*A. lumbricoides* and *P. falciparum*; *T. trichiura* and *P. falciparum*), and more frequent than expected for the others (Table 5).

A lack of association was found between *En. nana* and both *A. lumbricoides* (ϕ = 0.04) and *T. trichiura* (ϕ = -0.05), *M. perstans* and *En. nana* (ϕ = 0.03), *G. intestinalis* (ϕ = 0.02), *E. coli* (ϕ = 0.01) and *E. histolytica/* dispar (ϕ = -0.03), between *P. falciparum* and both *T. trichiura* and *M. perstans* (Table 5).

3.2. Discussion

The main objective of this study was to determine blood and gastrointestinal protozoan and helminth parasite fauna of a population of schoolchildren in order to find out multiparasitism and potential interspecific interactions (conflict or cooperation) among parasite species. During this study, 309 pupils of the 416 enrolled (74.28%) were infected with at least one parasite species. This percentage is lower than 92% found by Kimbi et al. [29] but close to 63.2 % obtained by Ngo Ngué et al. [30] among schoolchildren in the Southwest and South Regions of Cameroon, respectively. Our result is higher than those found in the general population in several Regions of Cameroon i.e., 26.6% in the Littoral Region [31], 57.2% in the Centre Region [32], and 8.5% in West Region [33]. All these results show that parasitic diseases remain endemic in the large part of the country and that prevalences of these diseases are still high among schoolchildren despite the efforts made by the government since several decades to control parasitic diseases [34].

Herein, the infection rate was found significantly higher (P < 0.0001) in the rural area (85.65%) compared to the urban area (62.80%). This would be due to the fact that populations in rural areas face several difficulties such as limited access to potable drinking water, non-respect of elementary hygiene rules, and lack of toilets, which expose them more to parasitic diseases. When toilets exist, they are often poorly constructed, poorly maintained, and therefore not used by children; these ones prefer to defecate in the environment, enriching it with resistant infective forms of parasites when they are carriers. It is well known that living in urban areas (with a sanitized environment, high level of education and relatively better conditions) reduces the level of transmission of some parasite species [35, 36]. In addition, houses in rural areas are built with semi-hard or clay, with crevices and joints facilitating the entry of anthropophilic mosquitoes. Much more the agricultural activities favor encounter with arthropod vectors of pathogens, in particular *Chrysops* spp. and *Culicoides* spp. which transmit the human blood filariae [37].

The analysis of the binary interspecific associations between parasite taxa found in this work reveals that the majority of the species were associated either weakly or very weakly with each other. However, we found moderate associations between *E. coli* and *E. histolytica/* dispar, *A. lumbricoides* and *T. trichiura, E. coli* and *P. falciparum, E. histolytica/* dispar and *P. falciparum, E. coli* and *A. lumbricoides*, and *E. coli* and *T. trichiura*. These latter associations, especially those involving intestinal parasites, could be favoured by their common mode of transmission i.e., ingestion of infective forms via drinking water, soil, fruits and vegetables [38]. Indeed, the co-endemicity of these parasite species, the resistance and persistence of the infective forms of intestinal parasites in the external environment could exacerbate the likelihood of being ingested as in procession by the same individual host. Moreover, all these moderate associations appeared more frequently than expected by chance. The interspecific association between *A. lumbricoides* and *M. perstans*, two species with different modes of transmission, also appeared more frequently than expected by chance. This could be due to the fact that these parasites species were prevalent in this rural area; thus, they can be harboured by the same hosts.

In the course of this work, no clear negative correlations were observed between different species except *G. intestinalis* and both *A. lumbricoides* and *T. trichiura*, and a bit between *A. lumbricoides* and *P. falciparum*. Blackwell et al. [39] also reported an antagonism between these helminths species (*T. trichiura* and *A. lumbricoides*) and *G. intestinalis*. This apparent antagonism between these parasites is the expression either of competitive exclusion or of cross-immunity between them. Indeed, in a murine model, it has been shown that *G. intestinalis*, which attacks microvilli of the small intestine, is inhibited by *T. trichiura* when the latter is found in the small intestine but not when it is localized in muscle tissue. This response suggests the existence of active competition between these parasite species [40]. Furthermore, Abdul-Wahid and Faubert [41], Matowicka-Karna et al. [42] and Jimenez et al. [43] suggested that the clearance of *G. intestinalis* and protective immunity against this parasite require the production of mixed cytokines Th1 and Th2 (characterized by both the production of *INF*-gamma and IL-4) as well as the production of antibodies of the IgA, IgG and IgE types. This argument could also be valid to explain the negative association between *G. intestinalis* and *A. lumbricoides*. Intestinal helminthiasis are generally chronic infections that can create an immune environment in their hosts, characterized by the hyperactivity of cytokines and Th2-type antibodies. Under these conditions, *G. intestinalis* could be negatively affected and its installation made difficult. Our result revealed a bit of negative correlation between *A. lumbricoides* and *P. falciparum*. This result is in discordance with Nacher et al. [44] who indicated that the induction by a parasite of a Th2 response (*A. lumbricoides*) in its host reduces host ability to fight against a parasite inducing a Th1 response (*P. falciparum*).

In the majority of cases where there seems to be a certain independence between parasite species (ϕ ≈ 0), it is found that they colonize different sites in the host. Indeed, infrapopulations of different parasite species that prefer different regions (or sites) in the host are not in contact and therefore don't compete for any resource [45]; the only possibility of interaction for parasite

species occupying different regions in the host is the indirect competition via the host immune system [2].

Conclusion

The ecological characterization of the relationships between parasite species found during this study revealed mainly 3 types of association: cooperation or positive, conflict or negative and independence or hazardous associations. Positive associations found between *E. coli* and *E. histolytica/* dispar, *P. falciparum* and *E. histolytica/* dispar, *A. lumbricoides* and *E. coli, T. trichiura* and *E. coli, T. trichiura* and *E. histolytica/* dispar, *T. trichiura* and *A. lumbricoides*, and between *A. lumbricoides* and *M. perstans* and these were due either to the common mode of transmission or favored by the same rural environment. The negative associations between *G. intestinalis* and both *A. lumbricoides* and *T. trichiura* were due either to interspecific competition or to the host immune system. Independence found between *G. intestinalis* and both *E. histolytica/* dispar and *E. coli,* and between *M. perstans* and *En. nana, G. intestinalis*, *E. coli* and *E. histolytica/* dispar were due to the fact that they colonize different sites in the host. Further studies are needed to identify the real interaction mechanisms between parasite species and to evaluate the consequences of multiparasitism for the host.

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