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Risk factors for sporadic giardiasis: a systematic review and meta-analysis

Anne Thébault [a,*], Loic Favennec [b,c], Pauline Kooh [a], Vasco Cadavez [d], Ursula Gonzales-Barron [d], Isabelle Villena [e]

[a] Risk assessment Department, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort, France
[b] French National Cryptosporidiosis Reference Center, Rouen University Hospital, 1 Rue de Germont, 76031 Rouen Cedex, France
[c] EA 7510, UFR Santé, University of Rouen Normandy, Normandy University, 22 Bd. Gambetta, 76183 Rouen Cedex, France
[d] Centro de Investigação de Montanha – (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
[e] Laboratory of Parasitology-Mycology, EA ESCAPE, University of Reims Champagne-Ardenne, Reims, France

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ABSTRACT

Giardia duodenalis is an important source of gastroenteritis worldwide. Endemic cases have been described in developing and industrialized countries. We analyzed risk factors for sporadic giardiasis by a systematic review and a meta-analysis of the literature. From 72 studies, contact with an infected person, lack of personal hygiene, and attending a child daycare center were identified as risk factors in children and adults.

Feco-oral transmission was significantly associated with exposure to human sewage/waste water, untreated drinking water and recreational waters. Travel abroad was a risk factor in industrialized countries. No hand-washing before eating or preparing food, eating unwashed vegetables, or composite food were significant risk factors. Breastfeeding was a protective factor in developing countries. Interestingly, contact with pets was found as a significant risk factor in children in this meta-analysis. This could be explored in future studies with the comparison of the *Giardia* assemblage isolated from humans and pets. In the future, it would be interesting to investigate more precisely the type of water, vegetable, and whenever possible the method of preparation/treatment. Environmental and epidemiological investigations of specific risk factors by assemblages and types of *Giardia* spp. should be further studied. Finally, host factors in relationship with the severity and sequelae of giardiasis deserve future research.

1. Introduction

The flagellated binucleated protozoan *Giardia duodenalis* (also referred to as *G. intestinalis or G. lamblia*) is an important source of infectious gastroenteritis with a global distribution: the 11th for food (FAO/WHO, 2014) and sharing the first place with *Cryptosporidium* spp. as zoonotic waterborne (Suresh et al., 2012). *Giardia* can infect different hosts, including humans, and animals, such as livestock, wild animals, and pets. *Giardia duodenalis* exhibit genetic diversity with eight genetic assemblages, from A to H (Feng and Xiao, 2011). Humans are infected with assemblages A and B, and assemblage B is predominant worldwide (Feng and Xiao, 2011). Assemblages A and B can also infect mammalian species other than humans. Other assemblages, like F (cats), C and D (dogs), or E (livestock), appear to have a more restricted host range (Solaymani-Mohammadi and Singer, 2010). The reported estimated prevalence is around 20-30% in low- and middle-income countries, and about 1-3% in industrialized countries (Feng and Xiao, 2011; Fletcher et al., 2012). In terms of the number of cases, among enteric protozoa, *Giardia duodenalis* is the most implicated in gastro-enteritis, with approximately 2. $\times$ 10^8 symptomatic cases worldwide annually (Feng and Xiao, 2011), and is responsible for worldwide foodborne and waterborne outbreaks of giardiasis (Adam et al., 2016).

The incubation period of giardiasis is between 3 to 25 days. Around 50% to 70% of infections are asymptomatic. Symptoms include diarrhea, abdominal cramps, bloating, and weight loss (Leung, et al., 2019). Giardia duodenalis is one of the four most prevalent enteropathogens in early life and is implicated in stunting at 2 years of age (Allain and Buret, 2020). There is bimodal peak morbidity at ages 1-9 years and 45-49 years, and a seasonal pattern in summer months and early autumn (Leung et al., 2019). In children, long term sequelae have been described such as cognitive deficits (Berkman et al., 2002).

Giardiasis has been associated with post-infectious irritable bowel syndrome, several years after resolution of the infection, chronic fatigue, and abdominal pain (Morch et al., 2009; Wensaas et al., 2012;

* Corresponding author.
E-mail address: anne.thebault@anses.fr (A. Thébault).

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Progreba-Brown et al., 2020). The clinical outcome could be the result of interactions between the parasite, the host (age and immune factors), gut microbiome, co-infecting enteropathogens, and diet (Halliez et al., 2016; Allain and Buret, 2020). Patients with immunodeficiencies as AIDS are not more prone to symptomatic giardiasis (Klotz and Aebischer, 2015). The understanding of the immunological mechanism and pathophysiology of giardiasis is still challenging (Solaymani-Mohammadi and Singer, 2010; Klotz and Aebicher, 2015; Buret et al., 2020). Giardiasis is a treatable disease; however, treatment may have side effects, and clinical relapse may occur in relationship with the emergence of resistant isolates of *Giardia* (Lemée et al., 2000; Buret et al., 2020).

The detection of *Giardia duodenalis* trophozoites or cysts in stools is generally achieved with microscopic examination of three concentrated stool samples using iodine stain. Immunofluorescent assay microscopy, immunochromatography tests or molecular tools (Polymerase Chain Reaction) are more sensitive than microscopy of concentrated iodine stained samples and require only one stool.These latter methods are more and more frequently used (Fletcher et al., 2012; Plutzer et al., 2018; Leung et al., 2019). Trophozoites of *Giardia* may also be identified on duodenal biopsies by microscopic examination or molecular assay (Maillot et al., 2000).

The parasite is shed in the infected host's feces, and transmitted through feco-oral pathway, with human-to-human transmission. As the parasite is resistant in cool and damp environments, contamination of the environment, as soil, fresh water, salt water, and food can lead to human outbreaks (Feng and Xiao, 2011; Adam et al., 2016). Giardiasis is more prevalent in developing countries than in industrialized countries. However, locally acquired giardiasis, waterborne outbreaks, or daycare outbreaks linked to *Giardia duodenalis* are regularly described in industrialized countries (Feng and Xiao, 2011; Fletcher et al., 2012; Adam et al., 2016). Different case-control studies have been performed to investigate risk factors related to *Giardia duodenalis* associated with other intestinal parasites. The aim of this meta-analysis was to summarize the different risk factors for sporadic giardiasis, considering all scientific studies regardless of the country of origin.

2. Methodology

The protocol of the systematic review and the meta-analysis model are described in depth in our methodological study (Gonzales-Barron et al., 2019).

2.1. Systematic review

The literature search was conducted between March 2017 and December 2017, using a combination of keywords related to (1) *Giardia* OR Giardiasis; (2) "case-control" OR "risk factor" OR "cohort; (3) infection OR disease; joined by the logical connector AND. Relevant studies were identified from five bibliographic search engines, Science Direct, PubMed, Scielo, ISI Web of Science and Scopus. No restrictions were defined for the year of the study or type of article. The search was limited to articles written in English, French, Portuguese and Spanish. A PRISMA diagram showing the detailed selection process is provided in Fig. 1.

Each study was screened for relevance for inclusion in the meta-analysis study, and then, the methodological quality of these studies was assessed using pre-set quality criteria comprising: (1) appropriate selection of controls; (2) adjustment to correct for confounders; (3) comparability between cases and controls; (4) acceptable responses rates for the exposed and control groups; (5) compatibility between data analysis and the study design; (6) provision of Odds ratio (OR) with confidence interval or p-value, or provision of sufficient data to calculate ORs; and (7) overall quality of the study (Gonzales-Barron et al., 2019). Primary studies that passed the screening for relevance were identified as having potential for bias if they failed to meet at least one of the methodological quality assessment criteria.

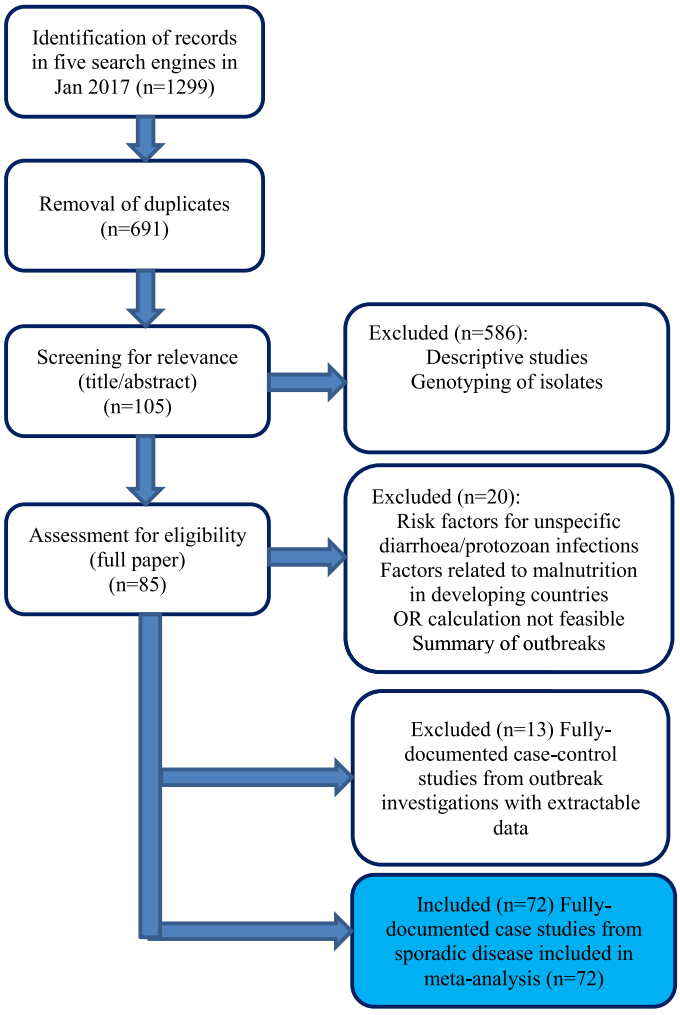

Fig. 1. PRISMA Flow chart of literature search for case-control and cohort studies of human *Giardia* infection.

Data from primary studies were extracted using a standardised spreadsheet. Data from primary studies that passed the previous steps were manually extracted by one trained reviewer using a standardized spreadsheet. Once the data extraction spreadsheet from a foodborne hazard was finalized, a second researcher checked all the collected data against the original studies (Gonzales-Barron et al., 2019). Data extracted from selected primary studies included the relevant study characteristics (location, time period, population, genotype, case definition, design, sample size of the groups, type of model, etc.), risk factors, setting, handling practices and outcome of the study (ORs). The data were entered into a categorization system previously established to hierarchically group risk factors into travel, host-specific factors and pathways of exposure (i.e., person-to-person, animal, environment and food routes) (Gonzales-Barron et al., 2019). For *Giardia duodenalis*, the variable "population" was stratified into 3 populations: mixed (adults or non-age related), "specific" (the elderly and antiretroviral treated HIV/AIDs patients), and children (young children to adolescents).

2.2. Data synthesis

The full meta-analytical data were first described using basic statistics. Next, data were partitioned into meaningful categories of risk factors, such as travel, host-specific factors, and pathways of exposure (i.e., person-to-person, animal, environment and food routes). Meta-analysis meta-regression models were fitted to each of the data partitions, with

group class (e.g. travel abroad/domestic travel) that depends on data partition (e.g. travel) (Gonzales-Barron et al., 2019). The meta-analytical models were fitted separately by population type. For some food classes, the effects of handling (e.g., eating raw, undercooked, unwashed vegetables) and setting (e.g., eating out) on the overall OR were assessed by the calculation of the ratio of the pooled OR of food mishandling to the base OR (e.g., no indication of mishandling).

The statistical analysis was designed to assess the effect of the geographical region and the analysis type (univariate/multivariate) on the final result. The objective of the region-specific meta-analysis was to inform the decision on the geographical regions that should be kept for the sequent pooling of ORs (Gonzales-Barron et al., 2019). Geographical regions (Asia, North America, South America, Africa, Europe, and Oceania) were removed from the meta-analysis when their ORs were different from those associated with the other regions or if fewer than 3 ORs represented the region (Gonzales-Barron et al., 2019).

All meta-analysis models were essentially weighted random-effects linear regression models. The random-effect is taking into account the study effect nested into the risk factor category (Gonzalez-Barron et al., 2019). Once a meta-analysis model was fitted, sensitivity diagnostics statistics (Cook's distance) were assessed to remove any influential observation originating from studies identified as having potential for bias. Publication bias was assessed by funnel plots and a statistical test investigating the effect of the study sample size on the ORs (pbias Tables 1, 2) (Gonzales-Barron et al., 2019). Heterogeneity between studies was assessed by different indicators such as between-study variability (τ^2), the QE test investigating residual heterogeneity between studies, the variance of residuals and the intra-class correlation I^2 (Gonzales-Barron et al., 2019). All analyses were produced in R software (R Development Core Team, 2008) implemented with the *metafor* package (Viechtbauer, 2010). Pooled ORs were considered as significant when the lower bound of the 95% confidence interval (CI) was equal or greater than 1.0, except for breastfeeding where the CI upper bound had to be below 1 for it to be deemed as having a significant protective effect.

3. Results

3.1. Descriptive statistics

In the systematic review of risk factors for human infection with *Giardia duodenalis*, 691 bibliographic sources were identified using the keywords in the five search engines, 85 of which passed the full assessment for eligibility comprising case-control and cohort studies of both sporadic disease and outbreaks (Fig. 1). In this study, the meta-analysis was done in 72 primary studies (case-control, cohort studies, cross-sectional and, 1 ecological study) with a focus on sporadic disease. The references of these 72 studies are detailed in Appendix 1 and their main features in Appendix 2. The eligible studies jointly provided 717 categorized odds-ratios for meta-analysis.

These published studies were conducted between 1977 and 2016 (35% after 2009). The majority of the studies were from Asia (17) and South America (17), then Africa (13), Europe (11), North America (9), and Oceania (5). The majority of the studies were conducted with children (40) and the mixed (adults and non-precise age) population (34). Three studies were conducted in specific populations ("specific" population): two in the elderly (Cohen et al., 2008; Girotto et al., 2013) and one in antiretroviral treated HIV/AIDs patients (Mahmud et al., 2014) (Appendices 1 and 2). Four studies investigated sources of giardiasis in different populations: one study investigated all three populations, while three studies investigated two populations). Cases for all studies were defined as symptomatic with laboratory detection of the pathogen as described earlier.

The potential for selection bias status was assigned to case-control studies where the controls were not healthy individuals but had another enteric disease such as cryptosporidiosis (Firdu et al., 2014, Redlinger et al., 2002), salmonellosis (Marder, 2012), amoebiasis (Ravel et al., 2013), campylobacteriosis (Wilson et al., 2008). For one study, the cases were defined as giardiasis "for which travel outside of the Canada prior to disease onset was recorded and the expected incubation period overlapped with the travel time", and the control group is giardiasis acquired in Canada (Swirski et al., 2016). The last study use enteric controls who were patients whose stool specimens submitted the same day to the same laboratory, were without *Giardia* cysts (Mathias et al., 1992). As it is not clear whether these controls shared routes of exposure with the case-patients, the ORs extracted from the aforementioned studies were identified as having potential for selection bias. The ORs obtained from Cifuentes et al. (2000), Espelage et al. (2010) and Esrey et al. (1989) were regarded as having potential for misclassification bias because it was unclear whether controls were negative or not for *Giardia duodenalis* (potentially asymptomatic cases). The rationale for assigning potential-for-bias status to the association measures extracted from Enserink et al. (2015) and Fraser and Cooke (1991) was related to the statistical approach used: respectively, a Poisson model (adjusted incidence rate ratios used as OR in meta-analysis) and the absence of confounders (Gonzales-Barron et al., 2019).

After methodological quality assessment, twelve case-control studies were identified as potentially biased. Corresponding ORs were removed if sensitivity analysis found an influence on the pool estimate (Gonzales-Barron et al., 2019). The risk factors categorized in this meta-analysis were person-to-person (29 ORs), environmental (367 ORs), contact with animals (105 ORs), food or related to poor hygiene and preparation practices (93 ORs). Risk factors related to the host (73 OR) or travel (50 OR) were also studied in this work.

3.2. Meta-analysis results

All significant results of the meta-analysis are given in Tables 1 and 2. Non-significant pooled ORs are given in Table 3. Travel was a significant risk factor for acquiring giardiasis with high values of ORs in the mixed population for travel abroad (pooled OR=4.381) or to any destination (pooled OR=3.615); these ORs were from studies conducted in developed countries (Western Europe, North America, and New Zealand) (Appendix 2). Travel within a country, with ORs from the same geographical areas, was not a significant risk factor in the mixed population (pooled OR=1.231; 95% CI: [0.686-2.209]) (Table 3), but it was significant in the children population (pooled OR=2.382; 95% CI: [1.788-3.174]).

Few ORs (4) and studies (2) investigated impaired immunity, associated with HIV infection or impaired immunity linked to diseases such as cancer (Espelage et al., 2010). Impaired immunity was associated with giardiasis (pooled OR=7.825; 95% CI: [4.049-15.120]). Regarding host factors in the mixed population, "history of anti-acid therapy", "intestinal surgery", and "medicine for indigestion" (without more precision) were grouped together within one category "other medical digestive conditions" with only 3 ORs, which were associated with *Giardia* cases with a pooled OR of 2.710 (95% CI: [1.625-4.520]); Table 1). Several factors were tested among children, but the only significant factor was protective for children breastfeeding (pooled OR=0.532; 95% CI [0.418-0.677]) measured in African countries. Within host-specific factors in children, the "other medical digestive conditions" category was not found significant in children with heterogeneous conditions, such as "chronic disease", "previous parasitic infection", "previous EnteroAggregative *Escherichia coli* (EAEC) infection", or "previous *Helicobacter pylori* infection"(Table 3). Reported HIV infection in children was not found significant, according to two studies (Mbae et al., 2013, Cohen et al., 2008) (Table 3).

Human-to-human transmission was a significant risk factor with high ORs in the mixed population (pooled OR=3.392; 95% CI: [2.083-5.525]) and in the children (pooled OR=3.404; 95% CI: [1.873-6.187]) (Table 1). This transmission involves contact with an ill person, such as a family member. Lack of hygiene in the mixed population was not a significant risk factor (pooled OR=1.254; 95% CI [0.922-

Table 1
Meta-analysis results of (significant) risk factors for human sporadic giardiasis.

Popula-tion	Geographi-cal area	Risk factor	Pooled OR [95% CI]	N/ n*	p-value risk factor	Publica-tion bias p-value	Points removed **	Heterogeneity analysis***
Travel								
Mixed	**All**	**Abroad**	4.381 [2.351 - 8.164]	10/ 32	<.0001	0.184	1	τ^2= 1.989 QE(df = 38) = 166.3; p-val <.0001 s^2=0.926 I^2=68.25
		Any	3.615 [2.013 - 6.491]	3/4	<.0001			
Children	**All**	**All**	2.382 [1.788 - 3.174]	3/7	<.0001	0.828	0	τ^2=0.002 Q(df = 6) = 7.257; p-val=0.298 s^2=0.537 I^2=0.353
Host-specific								
Mixed	**Asia, removed (1 OR)**	**Impaired immunity**	7.825 [4.049 - 15.12]	2/4	<.0001	0.630	0	τ^2=0 QE(df = 5) = 16.13; p-val=0.006 s^2=1.393 I^2=0
		Other medical conditions	2.710 [1.625 - 4.520]	3/3	<.0001			
Children	**All**	**Breastfeeding**	0.532[0.418-0.677]	4/7	<0.0001	0.381	0	τ^2=1.039 QE(df = 16) = 77.7125, p-val < .0001 s^2=0.614 I^2=62.862
Person-to-person								
All	**Africa removed (3 ORs)**	**Mixed**	3.392 [2.083 - 5.525]	10/ 16	<.0001	0.155	1	τ^2=0.571 QE(df = 23) = 309.3; p-val <.0001 s^2=0.587 I^2=49.28
		Children	3.404 [1.873 - 6.187]	6/9	<.0001			
Personal hygiene								
All	**All**	**Children**	1.462 [1.082 - 1.977]	9/ 14	0.013	0.515	**0**	τ^2= 0.115 QE(df = 16) =40.207, p-val=0.0007 s^2=0.234 I^2=33.03
		Specific population	2.232 [1.318 - 3.778]	2/3	0.003			
Animals								
Mixed	**Oceania removed (2 ORs)**	**Pets**	1.348 [1.060 - 1.715]	16/ 28	0.015	0.805	3	τ^2=0.429 QE(df = 43) = 113.8; p-val <.0001 s^2=0.407 I^2=51.33
		Wild animals	1.705 [1.177 - 2.469]	4/9	0.005			
Children	**All**	**Pets**	1.798 [1.287 - 2.513]	13/ 36	0.001	0.091	2	τ^2=0.501 QE(df = 48) = 171.6; p-val < .0001 s^2=0.568 I^2=46.88
		Farm animals	1.713 [1.217 - 2.411]	5/ 14	0.002			
Environment								
Mixed	**Africa (8 ORs) & South America (3 ORs) removed**	Day care	2.237 [1.809 - 2.765]	6/ 12	<.0001	0.851	4	τ^2=0.808 QE(df = 180) = 1140; p-val <. 0001 s^2=0.535 I^2=60.16
		Untreated drinking water	1.863 [1.499 - 2.316]	21/ 64	<.0001			
		Playground	1.462 [1.214 - 1.762]	7/ 10	<.0001			
		Recreational Water	1.941 [1.552 - 2.427]	15/ 72	<.0001			
		Waste water	2.059 [1.609 - 2.634]	12/ 27	<.0001			
Children	**All**	Day care	1.477 [1.205 - 1.808]	10/ 21	0.002	0.777	0	τ^2=0.952 QE(df=151) =487.878, p-val<0.0001 s^2= 0.467 I^2=67.088
		Untreated drinking water	1.774 [1.411 - 2.230]	24/ 71	<.0001			
		Farm environment	1.728 [1.308 - 2.282]	10/ 13	0.0001			
		Playground	2.221 [1.272 - 3.879]	5/6	0.047			
		Recreational water	1.404 [1.194 - 1.651]	4/ 18	<.0001			
		Waste water	1.680 [1.358 - 2.078]	13/ 28	<.0001			
Food								
Mixed	**All**	Composite	2.606 [2.005 - 3.386]	2/ 14	<.0001	0.362	3	τ^2=0.136 QE(df = 49) = 146.5; p-val < .0001
		Produce	1.499 [1.162 - 1.935]	12/ 33	0.002			

(*continued on next page*)

Table 1 (*continued*)

Popula-tion	Geographi-cal area	Risk factor	Pooled OR [95% CI]	N/n*	p-value risk factor	Publica-tion bias p-value	Points removed **	Heterogeneity analysis***
Children	**All**	Produce	2.192 [1.465 - 3.278]	7/10	0.001	0.001	0	s^2=0.374 I^2=26.60 τ^2=0.425 QE(df = 11) = 27.10; p-val=0.004 s^2=0.483 I^2=46.85
No handwashing before eating or preparing food								
Children	**All**	-	1.631 [1.108 - 2.401]	8/11	0.013	0.004	0	τ^2=0.179 QE(df = 15) = 29.42; p-val=0.014 s^2=0.274 I^2=39.55

* N/n Number of studies/number of OR;
** points removed by sensitivity analysis, all results are given after removing data concerned;
*** Between-study variability (τ^2), test for residual heterogeneity (Q or QE), variance of residuals (s^2), intra-class correlation (I^2).

Table 2
Effect of poor handling on the pooled association between consumption of vegetables and giardiasis.

Risk Factor	Handling	Pooled OR [95% CI]	N/n*	p-value risk factor	Ratio unwashed to base [95% CI]	Points removed **	Publica-tion bias p-value	Heterogeneity analysis***
Vege-tables	**Unwashed**	2.164 [1.121 - 4.177]	5/9	0.086	1.403 [0.953 - 2.065]	0	0.655	τ^2=0.154 QE(df = 31) = 82.02 p-val < .0001 s^2=0.413 I^2=27.19
	Base	1.542 [1.175 - 2.023]	14/24	0.002	-			

* N/n Number of studies/number of OR;
** points removed by sensitivity analysis, all results are given after removing data concerned;
*** Between-study variability (τ^2), test for residual heterogeneity (Q or QE), variance of residuals (s^2), intra-class correlation (I^2).

Table 3
Non significant results for risk factors.

Population	Geographical area	Risk factor	Pooled OR [IC95%]	N/n*
Travel				
Mixed	All	inside	1.231 [0.686 -2.209]	4/5
Hygien				
Mixed	All	poor hygien	1.254 [0.922-1.705]	2/2
Host-specific				
Children	All	Other medical condition	1.204 [0.766-1.892]	5/8
		Impaired immunity	2.319 [0.620-8.681]	2/4
Animals				
Mixed	Oceania removed	Farm animals	1.291 [0.920 - 1.813]	5/9
Food				
Mixed	All	Beverage	1.221 [0.969 - 1.539]	3/4
		Dairy	1.170 [0.308-4.440]	1/2
children	All	Meat	1.080 [0.466 - 2.500]	2/3
No Handwashing before eating or preparing food				
all	all	mixed	1.122 [0.918 - 1.372]	4/6

*N/n Number of studies/number of OR*N/n Number of studies/number of OR;** For isolated studies N=1, if different ORs can be retrieved from the same publication.

1.705]), but it was significant in the children (mostly "not washing hands after toilet") and in the "specific" population (not washing hands with soap) (Table 1). For lack of hygiene in adults, there were only two non-significant ORs derived from two different studies (Table 3).

Working in or attending a child daycare center (school, kindergarten or nursery) rendered a significant association with giardiasis in two populations: mixed (with children daycare and assisting elderly) (pooled OR=2.237; 95% CI: [1.809-2.765]), mixed (with children day care only: pooled OR=2.370; 95% CI: [1.737-3.235]) and children (pooled OR=1.477; 95% CI: [1.205-1.808]); forest plot (with children daycare and assisting elderly) in the mixed population is shown in Fig. 2.

Contact with pets was significantly associated with giardiasis in two populations: mixed (pooled OR=1.348; 95% CI: [1.060-1.715]) and children (pooled OR=1.798; 95% CI: [1.287-2.513]; forest plot in the children population in Fig. 3, (Table 1). For the mixed population, the pets considered were cats or dogs, with varying levels of contact (for instance, not washing hands after playing with animals), and high variability in ORs between studies ranging from 0.56 to 145. Owning dogs or cats (bearing the highest ORs) did not show a strong trend of different ORs (not statistically tested). The so-called category of "wild animals" was identified as a risk factor in the mixed population with a pooled OR of 1.705 (95% CI: [1.177-2.469]), and the sources were birds, reptiles, rabbits, rodents, and working in or visiting a zoo. Contact with farm animals (such as cattle, poultry, goats, and sheep) was associated with giardiasis in the children population (pooled OR=1.713; 95% CI: [1.217-2.411]) but not in the mixed population (pooled OR=1.291; 95% CI: [0.920-1.813]) (Table 3).

In environmental contaminations, elevated ORs were related to water contamination or direct or indirect contact with human or animal excreta. Contact with human fecal matters, mainly defined as "lack of home toilets", "sewage near home", and "occupational exposure to sewage" was a significant risk factor in the children (pooled OR=1.680 (95% CI: [1.358-2.078]) and mixed populations (pooled OR=2.059 (95% CI: [1.609-2.634]); forest plot in the mixed population in Fig. 4. Recreational water was a significant risk factor in the children (pooled OR=1.404; 95% CI: [1.194-1.651]) and mixed populations (pooled OR=1.941; 95% CI: [1.552-2.427]). In this category, children "swimming in the river" or "swallowing water" had the highest OR in comparison with "swimming" without further precision. Adults swimming in

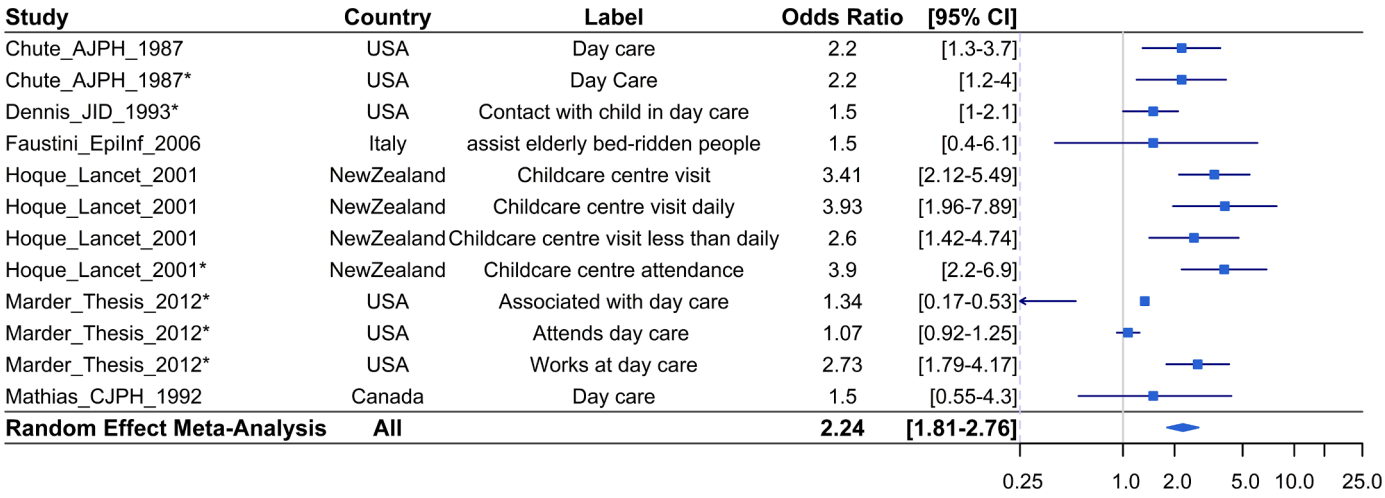

Fig. 2. Forest plot of the association between *Giardia* infection and daycare in the mixed population. Legend: From left to right: first name of study reference with year of study, country of study, label: risk factor as mentioned in publication, OR and its 95% confidence interval and its graphical representation, at the bottom of the graph pooled OR estimate and its 95% confidence interval, * adjusted OR.

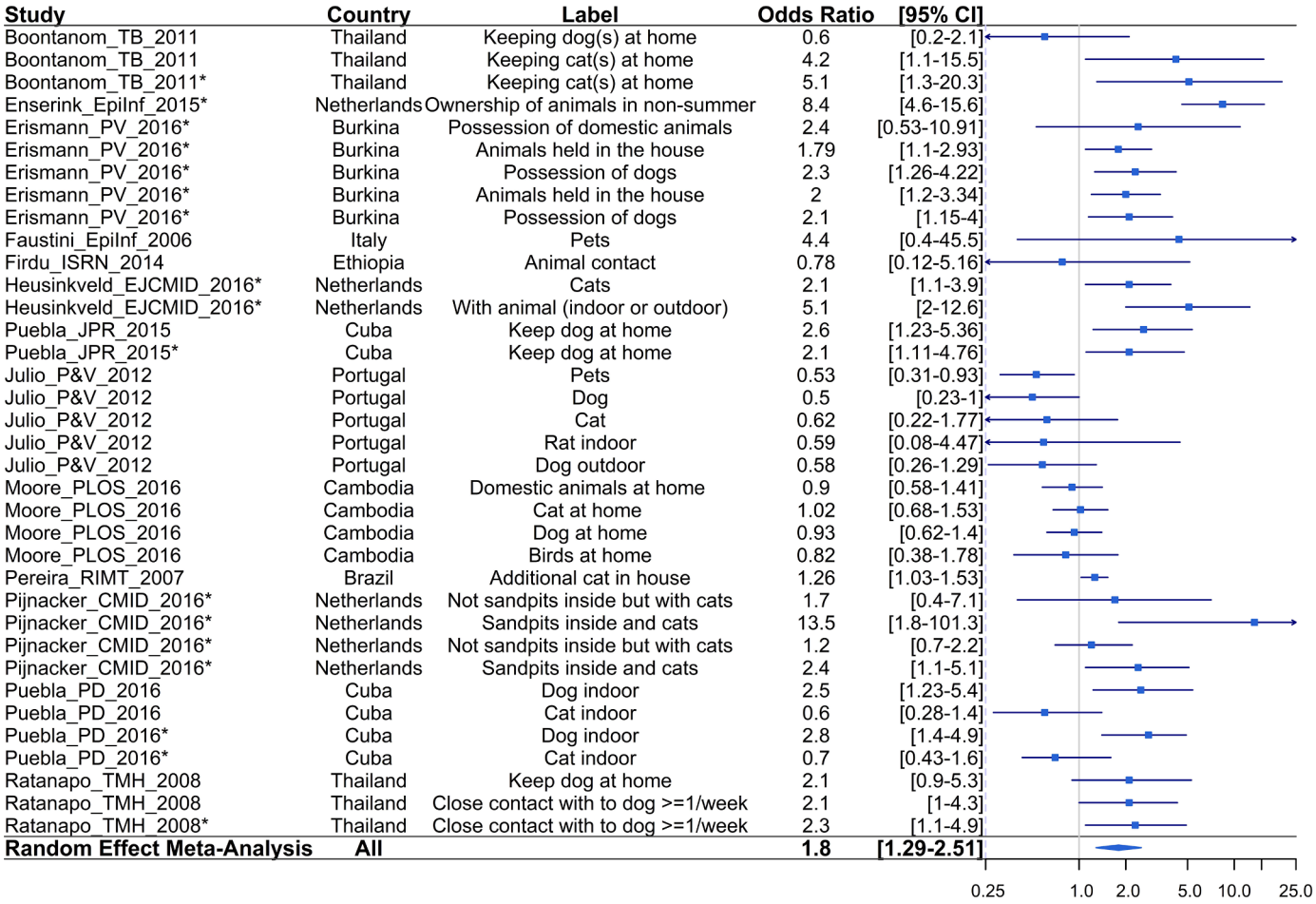

Fig. 3. Forest plot of the association between *Giardia* infection and contact with pets in the children population.
Legend: From left to right: first name of study reference with year of study, country of study, label: risk factor as mentioned in publication, OR and its 95% confidence interval and its graphical representation, at the bottom of the graph pooled OR estimate and its 95% confidence interval, * adjusted OR.

the ocean, lakes or river had higher but variable ORs. Regarding drinking water, conditions were heterogeneous depending on whether the water was from insufficiently-treated sources, or treated at home, or from wells or public networks, with a pooled OR of 1.863 (95% CI: [1.499-2.316]) in the mixed population, and a pooled OR of 1.774 (95% CI: [1.411-2.230]) in the children population. Contact with potentially contaminated soil, defined as the category "playground", including "sandpits at home", "not washing hands after gardening", and

Study	Country	Label	Odds Ratio	[95% CI]
Alyousefi_PLOS_2011	Yemen	Other sewage disposal	1.31	[0.7-2.44]
Anuar_BMC_2014	Malaysia	Indiscriminate defecation	1.1	[0.64-1.91]
Anuar_BMC_2014	Malaysia	Sewage disposal	1.01	[0.6-1.73]
Anuar_BMC_2014	Malaysia	Indiscriminate defecation	1.45	[0.73-2.87]
Anuar_BMC_2014	Malaysia	Sewage disposal	1.7	[0.86-3.34]
Anuar_IJP_2012	Malaysia	Indiscriminate defecation	1.03	[0.87-1.23]
Anuar_IJP_2012	Malaysia	Sewage disposal - common drainage	1.05	[0.83-1.33]
Choy_SR_2014	Malaysia	Not presence of toilet at household	2	[1.4-2.8]
Choy_SR_2014	Malaysia	Indiscriminate defecation	2.3	[1.6-3.2]
Choy_SR_2014	Malaysia	Indiscriminate garbage disposal	2	[1.4-2.8]
Choy_SR_2014*	Malaysia	Not presence of toilet at household	1.5	[1-2.2]
Choy_SR_2014*	Malaysia	Indiscriminate defecation	1.2	[0.7-2]
Choy_SR_2014*	Malaysia	Indiscriminate garbage disposal	1.1	[0.7-1.7]
Ensink_TRSTMH_2006	Pakistan	Absence of toilets/use defecation sites	1.6	[1.3-2.1]
Ensink_TRSTMH_2006*	Pakistan	Contact with wastewater	3.3	[2.5-4.4]
Espelage_BMC_2010	Germany	Contact with waste water	0.62	[0.15-2.53]
Faustini_EpiInf_2006	Italy	Sewage, manure/animal husbandry	13.3	[1.5-116.2]
Hoque_IJID_2002*	NewZealand	Occupational exposure to human waste	5.26	[2.27-12.2]
Hoque_IJID_2002*	NewZealand	Non-occupational exposure to human waste	3.33	[1.14-9.74]
Hoque_IJID_2002*	NewZealand	Occupational exposure to human waste	2.85	[1.07-7.6]
Hoque_IJID_2002*	NewZealand	Occupational exposure to human waste	4.04	[1.85-8.85]
Hoque_IJID_2002*	NewZealand	Non-occupational exposure to human waste	2.11	[0.7-6.36]
Hoque_IJID_2002*	NewZealand	Non-occupational exposure to human waste	3.45	[1.27-9.36]
Mahdy_AT_2009	Malaysia	Does not use toilet	2.1	[0.95-4.66]
Mahdy_TRSTMH_2008	Malaysia	Not using toilet	1.8	[1.1-3.03]
Norhayati_SAJTMPH_1998	Malaysia	Non usage of toilets	1.04	[0.51-1.98]
Wilson_EPI_2008	NewZealand	Contact with human faecal matter	3.6	[2.9-4.4]
Random Effect Meta-Analysis	**All**		**2.06**	**[1.61-2.63]**

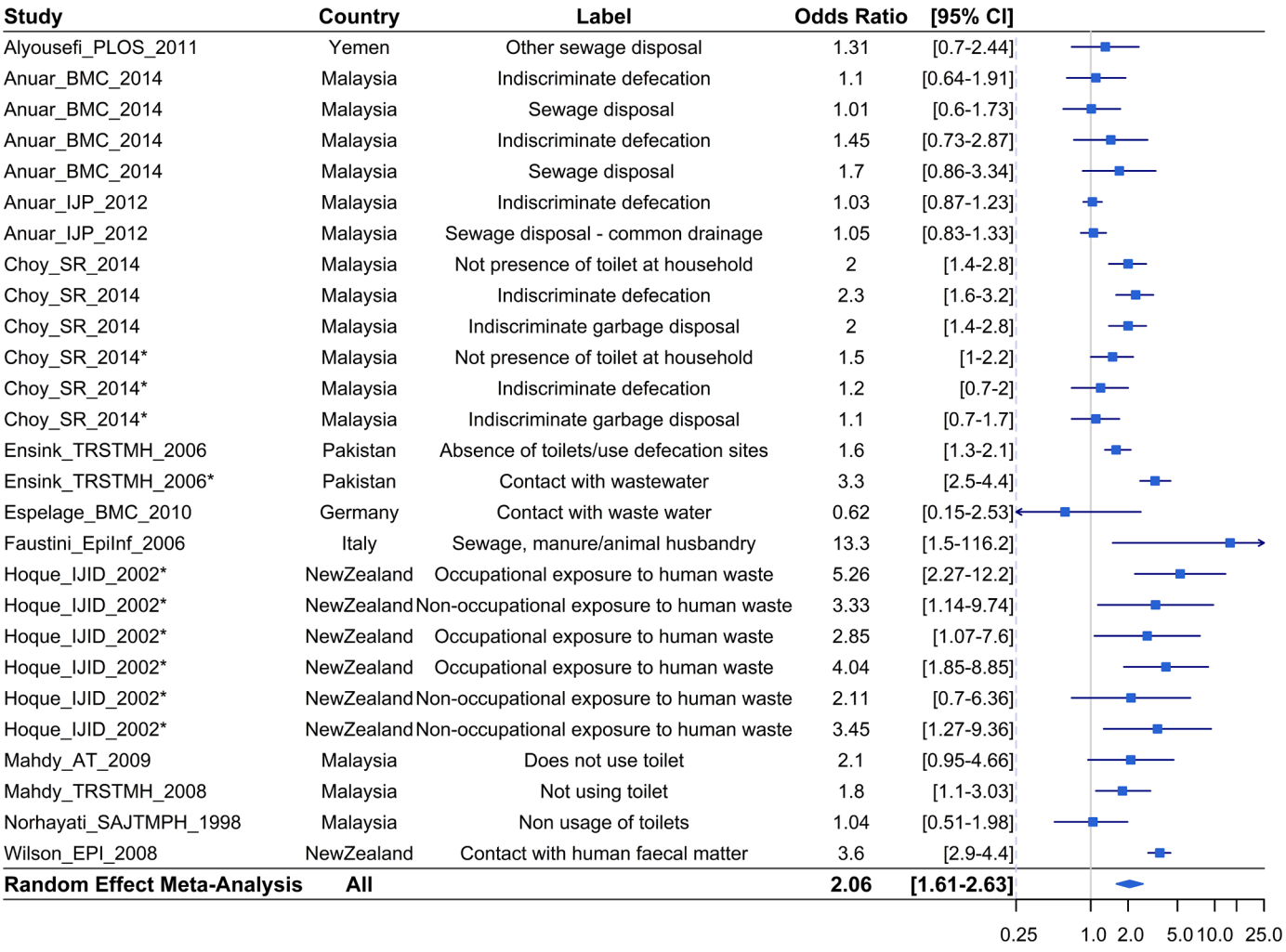

Fig. 4. Forest plot of the association between *Giardia* infection and sewage and waste water exposure in the mixed population.
Legend: From the left to the right: first name of study reference with year of study, country of study, label: risk factor as mentioned in publication, OR and its 95% confidence interval and its graphical representation, at the bottom of the graph pooled OR estimate and its 95% confidence interval, * adjusted OR.

Study	Country	Label	Odds Ratio	[95% CI]
Bello_ATMP_2011	Cuba	Unwashed raw vegetables	4.84	[2.33-10.14]
Bello_ATMP_2011*	Cuba	Unwashed raw vegetables	2.9	[1.4-6.2]
Faustini_EpiInf_2006	Italy	Raw vegetables	2	[0.3-13.8]
Puebla_JPR_2015	Cuba	Not washed vegetables/fruits before consumption	1.2	[0.68-2.04]
Puebla_JPR_2015*	Cuba	Not washed vegetables/fruits before consumption	1.3	[0.8-1.97]
Nunez_CSP_2003	Cuba	Not washing vegetables	12.16	[1.41-104.76]
Osman_PLOS_2016	Lebanon	Raw fruits and vegetables	2.7	[1.2-6.2]
Pereira_RIMT_2007	Brazil	Raw cabbage	2.9	[1.34-6.43]
Puebla_PD_2016	Cuba	Not washed vegetables/fruits before consumption	1.6	[0.87-3]
Puebla_PD_2016*	Cuba	Not washed vegetables/fruits before consumption	1.3	[0.71-2.8]
Random Effect Meta-Analysis	**All**		**2.19**	**[1.47-3.28]**

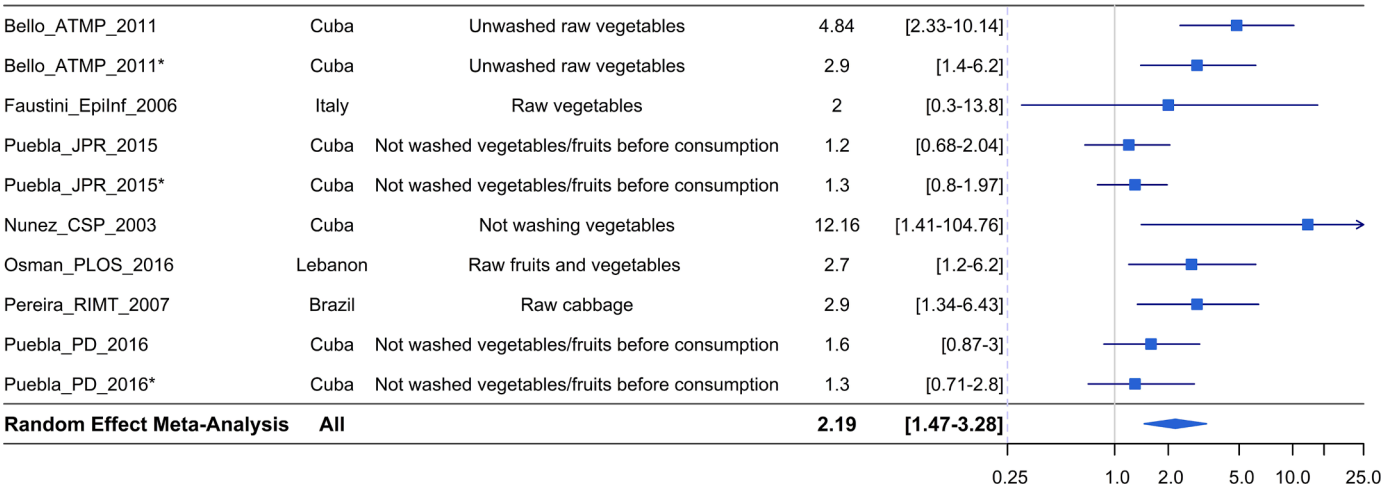

Fig. 5. Forest plot of the association between *Giardia* infection and produce in the children population.
Legend: From left to right: first name of study reference with year of study, country of study, label: risk factor as mentioned in publication, OR and its 95% confidence interval and its graphical representation, at the bottom of the graph pooled OR estimate and its 95% confidence interval, * adjusted OR.

"camping", was identified as a risk factor for giardiasis with a pooled OR of 1.462 (95% CI: [1.214-1.762]) in the mixed population, and a pooled OR of 2.221 (95% CI: [1.272-3.879]) in the children population. Farm or rural environment was a risk factor in the children population (pooled OR 1.728; 95% CI: [1.308-2.282]), but was not studied in adults (outside exposure to farm animals, described in Table 3). For children, farm environment was mainly defined as "living on a farm" or "in a rural residence".

With regard to food-related risk factors, consumption of produce was identified as a risk factor in the children (pooled OR=2.192; 95% CI: [1.465-3.278]) and mixed populations (pooled OR=1.499; 95% CI: [1.162-1.935]). However, the kind of produce was mostly not specified in the primary studies (except for some salads or some fresh fruits) (Fig. 5). Composite foods, ready for consumption and/or out-of-home catering was studied in the mixed population, but only in Canadian studies (Swirski et al., 2016; Ravel et al., 2013), and this factor was highly significant with a pooled OR of 2.606 (95% CI: [2.005-3.386]). For children, only one OR was available (Hoque et al., 2003) and, hence, could not be assessed by meta-analysis. Outcomes of ORs associated with beverages (bottled water or unpasteurized juice), meats (the children population only) and dairy products (the mixed population only, in only one study) were not significant (Table 3).

With regard to the influence of food preparation patterns, the impact of not washing hands before eating or preparing food was only found significant in the children population (pooled OR=1.631; 95% CI: [1.108-2.401]). In the mixed population, this source was not significant with a pooled OR of 1.122 (95% CI: [0.918-1.372]) (Table 3). These hygiene measures were only studied in Asia, Africa, and South America. The consumption of unwashed vegetables increased the pooled ORs associated with plants by a factor of 1.403 (Table 2). However, the consumption of unwashed vegetables was reported only in Cuba and Malaysia.

For most of the meta-analytical models reported in Tables 1 and 2, the statistical tests indicate the absence of potential for publication bias at a significance level of 5%. An exception was observed in partitions related to food in children and no handwashing before eating (Table 1). For better assessing the publication bias, the funnel plots for the two risk factors are given in Fig. 6. In the meta-analysis of "food in children", there was an asymmetry in the distribution of studies' outcomes, indicating a lack of large studies with low ORs. In the meta-analysis of "no handwashing before eating", the asymmetry was not as obvious as in the previous case. The intra-class correlation I^2 (Table 1) was always below high heterogeneity (<75%). Most often, significant residual between-study heterogeneity (p-value below 0.05 for Q or QE) was observed for the data partitions.

4. Discussion

The risk factors identified as significant in this meta-analysis are consistent with the known modes of contamination for *Giardia duodenalis*. Humans excrete this parasite and are infectious for each other. Human-to-human contact, non-compliance with basic hygiene rules, and attending a child daycare centre, were expected risk factors. This first meta-analysis of *Giardia* provides the first overview of risk factors for sporadic cases.

Human-to-human transmission was studied in case-control studies as contact with an infected person at the family level. The significance of the OR should help to define specific recommendations for the prevention of transmission in the family context. Poor personal hygiene, such as not washing hands after going to the toilet, was also detected as a risk factor and could contribute to person-to-person transmission. Child daycare centers (as mentioned above) were the setting of numerous *Giardia* outbreaks (Steketee et al., 1989; Fletcher et al., 2012; Enserink et al., 2015). We confirm the significance of this factor for sporadic cases in adults and children.

Another type of contact, as sexual activity, could also be involved in transmission (Fletcher et al., 2012); however, this risk factor could not be studied in this meta-analysis because it was only informed (OR available) in the most recent case-control studies (Reses et al., 2018).

Concerning host-specific factors, *Giardia* is not considered to be an opportunistic infection in AIDS patients. Exposure to risky sexual practices, or MSM (Men who have Sex with Men) may explain why more HIV infected people were also infected with *Giardia* than controls (Stark et al., 2009; Solaymanni-Mohammadi and Singer 2010). "Other medical conditions" ("history of anti-acid therapy", "intestinal surgery" and "medicine for indigestion") is a heterogeneous category, and should be better explored in the future, in particular their association with potential case severity.

Breastfeeding was a significant protective factor for children, only studied in African and Asian geographical areas. Unsaturated fatty acids present in human milk showed an anti giardial activity (Rohrer et al., 1986; Crouch et al., 1991). Moreover, it is likely that in highly endemic countries, specific secretory IgA antibodies, potentially effective against giardiasis (Samra et al., 1991), are provided by breast milk of seropositive mothers (Ignatius et al., 2012). Breastfeeding also protects from exposure to contaminated milk.

Travel, a well-known risk factor in developed countries, is only studied for those countries, and is, by extension, considered in those countries as a "traveler's disease" (Currie et al., 2017). Most of the time, this travel item only indicates the destination, as "a risk country", "Asia" or "Africa"; it would be more informative if a list of countries, taking into

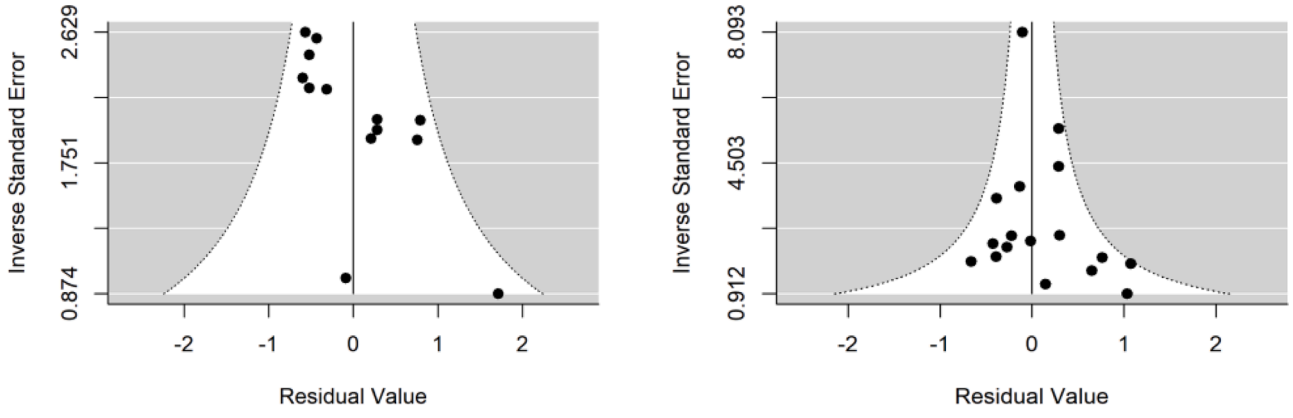

Fig. 6. Funnel plots of meta-analyses investigating food (left) and no handwashing before eating or preparing food (right) in the children population.
Legend: plot shows the residuals of the model ('observed - fitted' values) on the x-axis against their corresponding standard errors. A vertical line indicates the estimate based on the model. A pseudo confidence interval region is drawn around this value with bounds equal to ± *1.96 SE*, where *SE* is the standard error value from the y-axis (assuming level=95). A lack of symmetry around the vertical line is an indicator of publication bias (see text).

account the level of known endemicity (prevalence), was established to facilitate comparison between geographical areas. Among different kinds of travel, it would be useful to provide travelers with the conditions and length of the journey, exploring items such as "camping", "caravanning", "eating street food", or "drinking tap or bottled water".

Giardia duodenalis has been considered for a long time as restricted to humans. The issue of the zoonotic potential of *Giardia duodenalis* has been a topic of interest for many years. Interestingly, in children, contact with farm animals or pets was found as a risk factor in this meta-analysis (pets in Fig. 3) with heterogeneous ORs. The duration of exposure, the type of contact or hygiene practices, the animal species, and the types of *Giardia* spp. involved in the contamination could explain the heterogeneity between ORs (Fig. 3). Assemblage A and B have been commonly described in cattle (Geurden et al., 2012) and in cats and dogs (Bouzid et al, 2015). Moreover, human cases with assemblage F and C in Europe have recently been reported (Štrkolcová G et al., 2015; Pipiková et al., 2020): A survey of contamination in pet zoo animal stools (Conrad et al., 2018) revealed the presence of *Giardia* assemblage A in different mammalian species. Concerning other pets, a petting zoo with rabbits, and pet reptiles in a long term facility were identified as a source of *Giardia duodenalis* outbreaks (Adam et al., 2016).

Investigating the type of assemblage and even the group in animals and humans (Minetti et al., 2014; Plutzer et al., 2018) could lead to a better analysis of the zoonotic role of transmission (Feng and Xiao, 2011; Coelho et al., 2017; Mughini-Gras et al., 2019) Only some case-control studies included in this meta-analysis considered the type of assemblage in cases (Mahdy et al., 2009; Minetti et al., 2015; Choy et al., 2014; Anuar et al., 2014; Moore et al., 2016) or studied the type of risk factor associated with the corresponding assemblage (Mahdy et al., 2009; Anuar et al.,2014; Minetti et al., 2015).

Molecular characterization with more discriminatory tools, as whole-genome sequencing (WGS), may also help to establish patterns of transmission in cases from outbreaks (Tsui et al.,2018); and sporadic cases of giardiasis (Tsui et al.,2018; Thompson and Ash, 2019)

Farm environments and exposure to farm animals are mostly mentioned with no adjusted OR or not adjusted with other known potential risk factors. Therefore, the significance of these factors could result from correlation with other factors such as unsanitary toilets, insufficient/low treatment of drinking water, or unsafe sewage systems. Thus, more data are needed to confirm farm animals as risk factors.

Environmental sources such as water or soil contaminated by human stools, and, consequently, insufficiently treated drinking water, bathing water, and contact with soil (including sandpits) are confirmed as risk factors for sporadic cases. However, exposure to contaminated water and soil is not the same. *Giardia* was shown to be less resistant in soil than in water (Karim et al., 2004) and in particular, not resistant to the winter in the North of Europe (Robertson and Gjerde., 2004).

Contaminated drinking (Adam et al., 2016; Horton et al., 2019) or recreational waters (Adam et al., 2016) are known sources of outbreaks. It is well known that usual chlorine treatment at low water temperature is not effective to treat *Giardia* cysts (Jarroll et al., 1981).

Giardia contamination in drinking water is a regular topic of quantitative risk assessment, requiring water treatment regulations (Regli et al.,1991, Schijven et al., 2011, Murphy et al., 2016; Daniels et al., 2018). Drinking water protocols in epidemiological studies could be more informative and could include categories. A common hierarchical framework could be useful to establish categories with the type of water, as well water, surface water, private storage tank water or treatment type. The baseline of drinking water varies between studies and countries, as the safety of "tap source" used as a reference category, which may explain some OR variability. Environmental surveys, including detection but also estimates of viability and infectivity (Rousseau et al., 2018) could also complement epidemiological studies, to reinforce causal pathway and comparison with quantitative risk assessment (QRA).

Exposure to wastewater, sewage, and faecal matter should be considered as a source of sporadic cases. Preventing contamination includes good hygiene practices and safe wastewater treatment. In developing countries, increased wastewater safety should be accompanied by other global measures, as clean water, sanitation and, good hygiene practices (Ngure et al., 2014). In developed countries, occupational exposure to wastewater could be further explored. Recently, the use of UV in wastewater has been shown to be effective to treat *Giardia* cysts (Adeyemo et al., 2019).

Composite foods represent a myriad of food products. This meta-analysis includes food eaten outside the home (street food, restaurant, cafeteria, fast food) or ready to eat foods, and deli foods. More precise definitions are missing in the case-control studies to identify if the product was initially contaminated or cross-contaminated during food preparation or consumption, (e.g. unwashed hands, and inadequate cleaning or storing in a contaminated environment) (Adam et al., 2016) or to investigate more precisely the seller type (i.e. cafeteria, street food, and fast food). Not washing hands before meals or during food preparation is a risk factor. A recent outbreak underscores the risk associated with asymptomatic food handlers (Figgatt et al., 2017). It is essential to highlight that this meta-analysis shows the importance of the recommendation of washing vegetables and washing hands before eating or before preparing food.

As food products exposed to contaminated soil or contaminated irrigation water, produce – particularly unwashed – were expected to be a risk factor. In the USA, within 15 foodborne outbreaks with food sources identified, produce ("raw vegetables", "salad bar"), ice and fruits were implicated (Adam et al., 2016). Vegetables are regularly found contaminated (Berrouch et al., 2020; (Robertson and Gjerde, 2000; 2001; Amoros et al., 2010). *Giardia* in vegetables was the subject of QRA in a context of wastewater reuse in agriculture (Mota et al., 2009; Kouame et al., 2017). For future epidemiological studies, it would be interesting to evaluate the type of vegetables and washing practices that are used by consumers. For instance, it would be useful to investigate whether leafy greens like lettuce, cabbage, rocket, coriander, or mint, are more susceptible to retain contamination in comparison with other vegetables with a smooth surface (Berrouch et al., 2020).

Sometimes, eating produce is perceived as a protective factor in some publications. For instance, eating salads or raw vegetables was found to exert protection against giardiasis in North West England (Minetti et al., 2015). It was shown that after two waterborne giardiasis outbreaks separated over a period of 5 years, the number of persons infected during the first outbreak decreased during the second outbreak (Isaac-Renton et al., 1994).

Innate and adaptative components could explain the immune response to *Giardia*. Solaymani-Mohammadi and Singer (2010) suggest that immune response might not be protective against infection but rather that symptoms could be less severe during re-infection. Even if the mechanism of immune response after *Giardia* infection is only partially understood (Klotz and Aebisher, 2015; Buret et al., 2020).It may be interesting to search for immunological response in the control group as a marker of past (or at least recent) infection, however this technique is not available in routine for the moment.

Eating raw mollusks or shellfish, as filtering animals, was not included in case-control questionnaires; however, due to the fecal-oral pathway of transmission, these can be suspicious food sources. Among 38 foodborne outbreaks in the USA, one is attributed to oysters (Adam et al., 2016). *Giardia* cysts were detected in mussels (Gómez-Couso et al., 2005) and oysters (Schets et al., 2007).

We investigated "children", mixed (mainly adults), and "specific" populations separately. The children population included very young children as toddlers, and adolescents. In the future, it could be interesting to study separately different age classes in a meta-analysis to investigate different types of exposure. In industrialized countries, analyzing risk factors in travelers and endemic cases separately (Swirski et al., 2016) may help to identify different or emerging sources of contamination between those populations. However, since locally

acquired giardiasis cases may be underreported, Giardiasis diagnosis and risk factors other than travel could be further investigated in gastroenteritis cases "whatever the setting" (Currie et al., 2017; Escobedo et al., 2018; Horton et al., 2019).

Due to the high level of prevalence of giardiasis in developing countries, and the role of inter-human transmission as shown with high OR in this meta-analysis, it would be useful to explore quantitative microbial risk assessment or dynamic modeling of disease burden relative to a specific source as water or food, taking into account the dynamics of the disease in the population (Daniels et al., 2018), but also the dynamics of inter-human transmission as secondary cases, as already done for other pathogens such as *Cryptosporidium* in Eisenberg, et al., (1996), or norovirus (Amoueyan et al., 2020). However, quantifying inter-human transmission parameters for *Giardia* would probably be challenging.

5. Conclusion

In conclusion, this first meta-analysis of risk factors for sporadic giardiasis provides an overview of the risk factors involved and confirms the known pathways of transmission of giardiasis. These risk factors should now be considered when investigating cases-controls to assign a source of infection. The results from multivariate analysis could be compared between studies, thus avoiding some confounding factors. This meta-analysis provides some clues for future research in particular in pets and other mammalian species. However, more detailed items including the frequency of consumption or better specifying the exposure (the type of food, cooked or washed) could help for management purposes.

Some potential risk factors were missing in some studies in the meta-analysis (such as shellfish consumption and venereal pathways). A common hierarchical framework of risk factors, studying transmission pathways by network analysis, and prioritizing them based on biological plausibility, reported association, and potential exposure (Reses et al. (2018), in relationship with management/risk assessment possibilities (Wang et al., 2018) could be helpful for future meta-analyses and their applications. Further investigation is needed to explore host factors in relationship with severe and chronic consequences of infection. In the future, epidemiological studies and environmentalanalysis could investigate risk factors in relation to the assemblage and type of *Giardia* spp. Inter-human transmission should not be neglected in the disease burden of *Giardia*, and microbial risk assessment of specific sources should not overlook cases of secondary transmission.

CRediT authorship contribution statement

Anne Thébault: Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Loic Favennec:** Writing - review & editing, Supervision. **Pauline Kooh:** Methodology, Project administration, Writing - original draft, Writing - review & editing. **Vasco Cadavez:** Methodology, Investigation, Formal analysis. **Ursula Gonzales-Barron:** Methodology, Investigation, Formal analysis, Writing - review & editing. **Isabelle Villena:** Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2020.100158.

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