# Subject 1: Systematic Review and Meta-Analysis on Risk Factors for Sporadic Food-Borne Illnesses

# Part VI: Giardiasis

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## EXECUTIVE SUMMARY

The quality assessment stage was passed by 72 primary studies investigating risk factors for sporadic infection with *Giardia duodenalis*, which were conducted between 1977 and 2016. From these, forty studies investigated exposures in children/infants, thirty-four in the adult or mixed population while only three studies focused on the susceptible population (elderly and immunocompromised). These primary studies provided 736 ORs categorised for meta-analysis, being 63% of the meta-analytical data produced by case-control and cohort studies from Brazil (8), Malaysia (6), New Zealand (5), UK (5), USA (5), Canada (4), and Cuba (4). Sixty-five case-control studies (~90%) employed an unmatched experimental design and produced a total of 651 ORs. From these, 295 reported ORs were not adjusted by any confounder (i.e., crude ORs by Chi-square test), while the others were adjusted by using either Mantel-Haenzel method (35 ORs) or unconditional logistic regressions (321 ORs). On the other hand, only seven studies (~10%) employed a matched experimental design, providing for meta-analysis a total of 85 ORs. From these, most of them were adjusted ORs estimated by Mantel-Haenzel method (22 ORs) or logistic regressions (54 ORs). After methodological quality assessment, twelve case-control studies were marked as being below standards, which yielded a total of 146 potentially-biased ORs. With regards to the risk factor classes, sporadic illness investigations focused more on the multiple pathways of exposure (594 ORs) than on host specific factors (92) and travel (50).

According to this meta-analysis study, the most important transmission routes of giardiasisis in the mixed population are international travel (overall OR=4.85; 95% CI: 3.30 – 7.12) and the host-specific factors of suffering from a gastrointestinal condition (overall OR=4.28; 95% CI: 2.18 – 8.41) and chronic immunocompromising disease (overall OR=3.93; 95% CI: 1.76 – 8.78). In the mixed population, person-to-person transmission bears a significantly higher association with giardiasis (overall OR=2.54; 95% CI: 1.52 – 4.25) than both dishes/fast-food eaten out (overall OR=2.21; 95% CI: 1.51 – 3.24) and environmental pathways of exposure to

human waste/waste water (overall OR=2.03; 95% CI: 1.57 – 2.63), recreational water (overall OR=1.87; 95% CI: 1.50 – 2.33), drink water (overall OR=1.86; 95% CI: 1.50 – 2.30), day care (overall OR=1.71; 95% CI: 1.21 – 2.44) and camping/gardening (overall OR=1.59; 95% CI: 1.14 – 2.21). In the mixed population, the animal-related pathways, such as contact with wild animals (overall OR=1.66; 95% CI: 1.01 – 2.73) and contact with pets (overall OR=1.30; 95% CI: 1.06 – 1.60) are not as strong determinants of giardiasis as the environmental routes of waste water, recreational water and drink water.

In the children population, the highest associations with giardiasis are posed by person-to-person transmission (overall OR=2.79; 95% CI: 1.48 – 5.25), travel (overall OR=2.38; 95% CI: 1.80 – 3.15) and consumption of fresh/unwashed vegetables (overall OR=2.19; 95% CI: 1.47 – 3.28). Unlike the mixed population, in children, the animal-related routes of transmission of farm animals (overall OR=1.92; 95% CI: 1.35 – 2.73) and pets (overall OR=1.87; 95% CI: 1.38 – 2.53) are, on meta-analysis, at least as important vehicles of giardiasis as the environmental routes of playground (overall OR=1.85; 95% CI: 1.12 – 3.05), drink water (overall OR=1.78; 95% CI: 1.47 – 2.16) and exposure to human waste (overall OR=1.74; 95% CI: 1.35 – 2.25). Other significant pathways of transmission of giardiasis in children were, in decreasing order: living on a farm (overall OR=1.70; 95% CI: 1.24 – 2.34), suffering from chronic disease (overall OR=1.62; 95% CI: 0.97 – 2.70), no handwashing before eating (overall OR=1.57; 95% CI: 1.18 – 2.10), attending day care/kindergarten (overall OR=1.53; 95% CI: 1.12 – 2.08), other medical conditions (overall OR=1.41; 95% CI: 1.00 – 2.00), recreational water (overall OR=1.39; 95% CI: 0.93 – 2.09) and not washing hands after using toilet (overall OR=1.18; 95% CI: 1.03 – 1.35).

Routes of exposure that on meta-analysis were not proven to be significantly associated wtih giardiasis in the mixed population were: poor hygiene habits (overall OR=1.53; 95% CI: 0.71 – 3.29), consuming fresh or unwashed vegetables (overall OR=1.28; 95% CI: 0.88 – 1.88), contact with farm animals (overall OR=1.25; 95% CI: 0.86 – 1.83) and not washing hands before eating (overall OR=1.11; 95% CI: 0.84 – 1.40); while in children the least important route of exposure to giardiasis was poor hygiene habits (overall OR=1.33; 95% CI: 0.94 – 1.88). Breastfeeding protected children against acquiring giardiasis since, on meta-analysis, children who were breastfed presented approximately half the odds (95% CI: 0.29 – 0.91) of becoming infected than those who were not. Not washing fresh vegetables before consumption was found to pose a significant risk of acquiring giardiasis (p=0.086), since people who admitted having eaten unwashed vegetables had 1.404 (95% CI: 0.954 – 2.065; Table 11) greater probability of acquiring giardiasis than those who stated having consumed *simply* fresh vegetables (without having being specific as to whether it was unwashed).

# 3. Results

## 3.6 *Giardia duodenalis*

### *3.6.1 Descriptive statistics*

In the systematic review of risk factors pertaining to human infection with *Giardia duodenalis*, a total of 691 bibliographic sources were identified using the keywords in the five search engines, from which 85 passed the full assessment for eligibility comprising case-control and cohort studies from both sporadic illnesses and outbreaks (Figure 1). A total of 13 fully-documented case-control studies investigated the source(s) of outbreaks and were kept in the JabRef file as their data can be readily extracted. In this study, meta-analysis was undertaken using 72 primary studies – case-control and cohort studies – with focus on sporadic disease (Figure 1). These published studies were conducted in years spanning from 1977 and 2016. Table 1 compiles a list of the case-control studies along with their main features. The eligible studies jointly provided 736 categorised odds-ratios for meta-analysis. A total of 201 ORs were retrieved from 21 case-control studies performed before the year 2000, while 535 ORs were excerpted from 51 case-control studies performed after 2000. Meta-analytical data were obtained from case-control and cohort studies conducted in 34 countries, although studies from only 10 countries provided ~66% of the ORs retrieved. These were: Brazil (8 studies), Malaysia (6), USA (5), UK (5), New Zealand (5), Canada (4) and Cuba (4) (Figure 2, top left).

Forty studies investigated risk factors for giardiasis in children whereas thirty-two primary studies were conducted in the mixed population. By contrary, only two studies focused on the adult population (Cohen et al., 2008 and Girotto et al., 2013) while three studies attempted to find associations of exposures with disease in the susceptible population: the elderly (Cohen et al., 2008 and Girotto et al., 2013) and the immunocompromised (Mahmud et al., 2014). Because of the very few OR measures available for these two population classes – adult and susceptible (Figure 2, top right), it was not possible to undertake separate meta-analyses on these data. Instead, the adult population data (6 ORs) was merged with the mixed population data (389 ORs) while the susceptible data (15 ORs) was incorporated to the children data (326 ORs). By doing this, two population types were created: the mixed population (395 ORs), made up of 53.7% of the data, and the children/susceptible population (341 ORs), comprising 46.3% of the meta-analytical data. As a rule, because of their distinct routes of exposure, separate meta-analyses were adjusted on the mixed population and children. Data from both age groups were merged only when the number of observations belonging to the children population was too few to run a separate meta-analysis model.

In all of the primary studies, the giardiasis cases were laboratory-confirmed. Only 7 case-control studies employed a matched experimental design and produced a total of 85 categorised ORs, which represented 11.5% of the data. Of these, 22 ORs were computed in multivariate analysis whereas 63 were obtained in univariate analysis. ORs from matched designs were mostly estimated using logistic regressions (54) and MH (22), and only a few by the simple chi-square test (9). From the studies that applied an unmatched design (65 studies), mostly cohort studies, a total of 651 ORs were extracted, which constituted 88.5% of the meta-analytical data. A total of 499 ORs were computed in univariate analysis while 152 in multivariate analysis. Most of the ORs from unmatched designs were estimated using chi-square (295) and UL (321) whilst only 35 ORs were MH-estimates adjusted by a confounder's strata within a frequency-matched experimental design. Bringing together the matched and unmatched designs, 430 extracted ORs (58.4% of the data) were not adjusted by any confounder (crude ORs), while only 306 ORs (41.6%) were adjusted using either MH or logistic regressions (Figure 3).

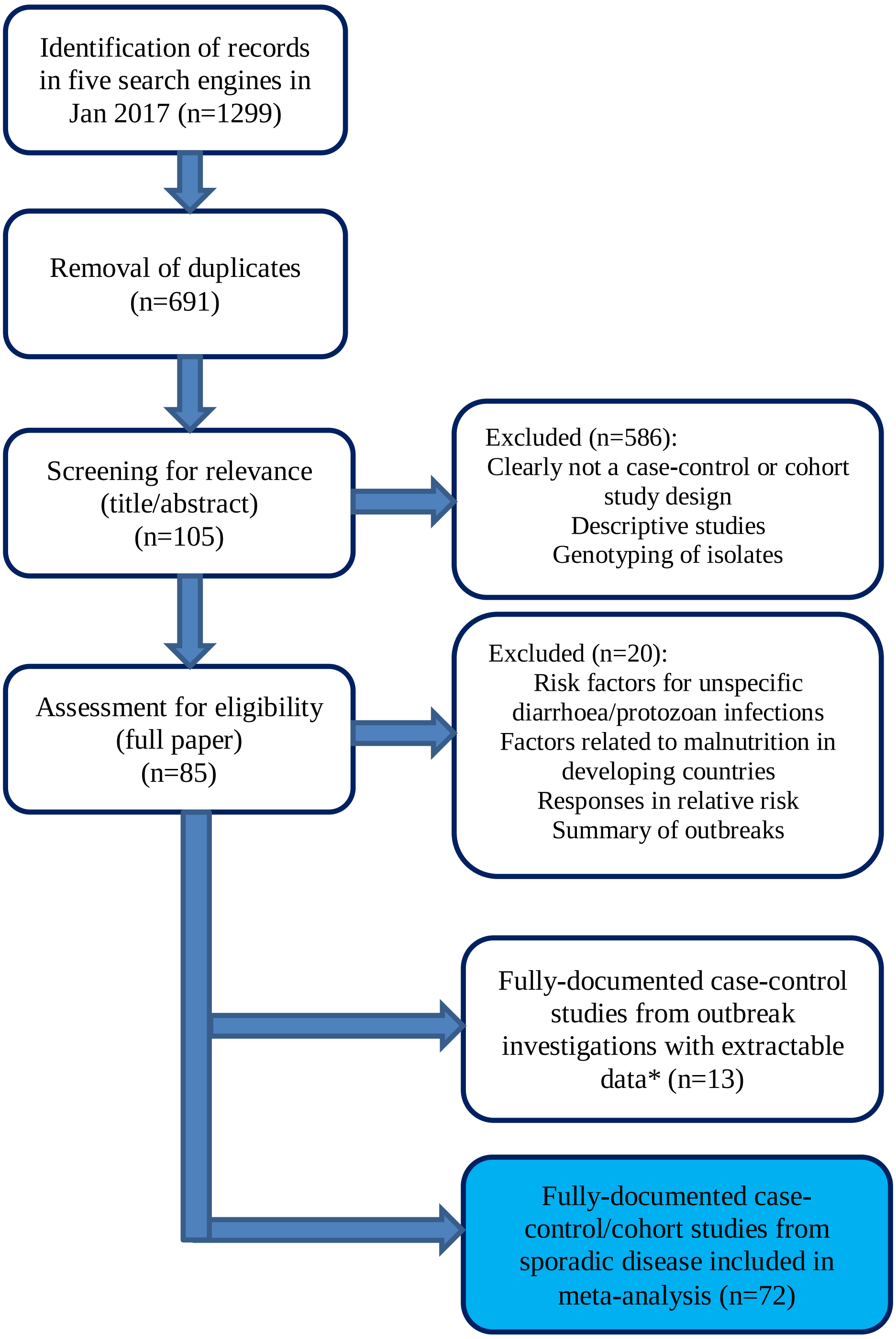


Figure 1. Flow chart of literature search for case-control studies of human giardiasis
*Records kept in JabRef with PDFs files annexed

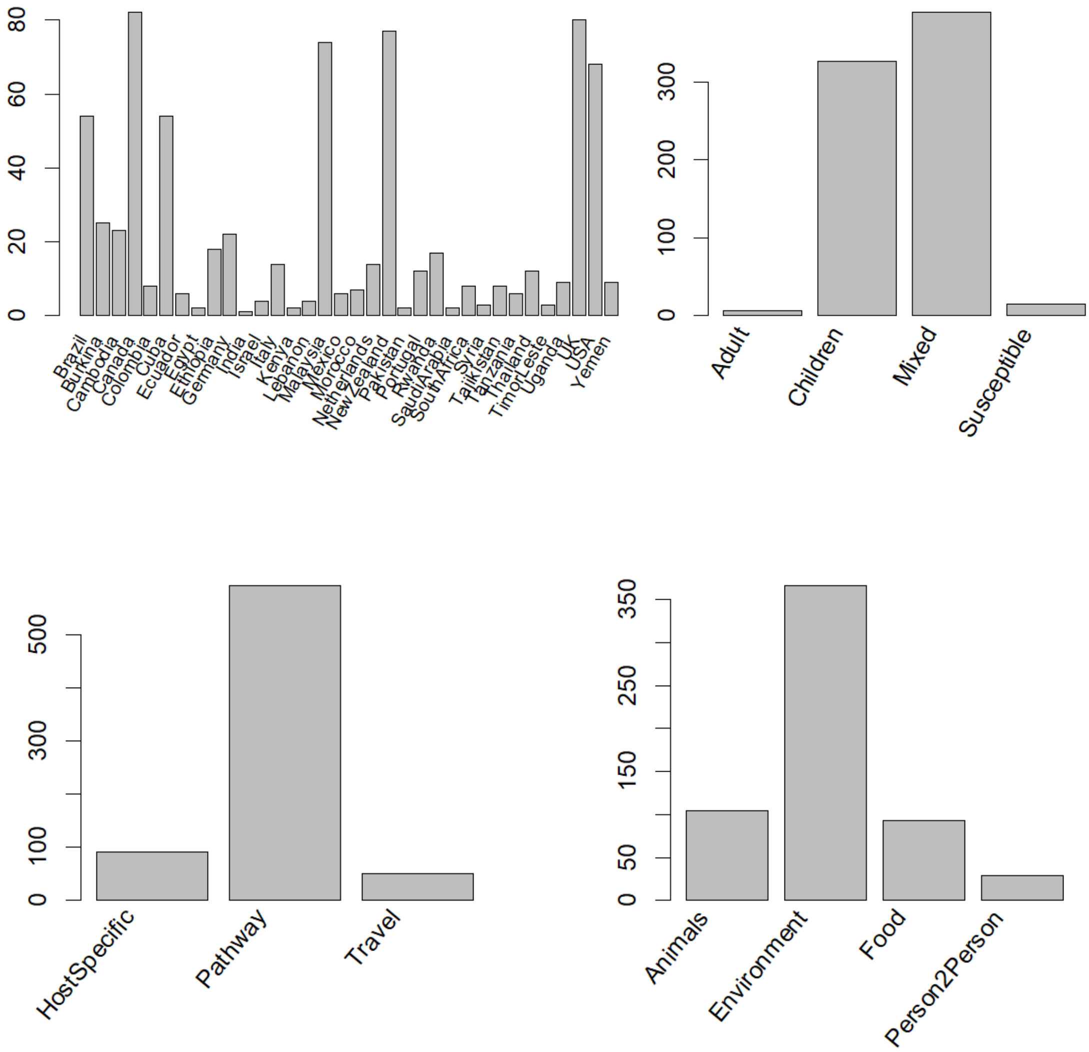


Figure 2. Distribution of the number of odds-ratios (y-axis) extracted from case-control studies of sporadic giardiasis by country (top left), population type (top right), risk factor (bottom left) and pathway of exposure (bottom right)

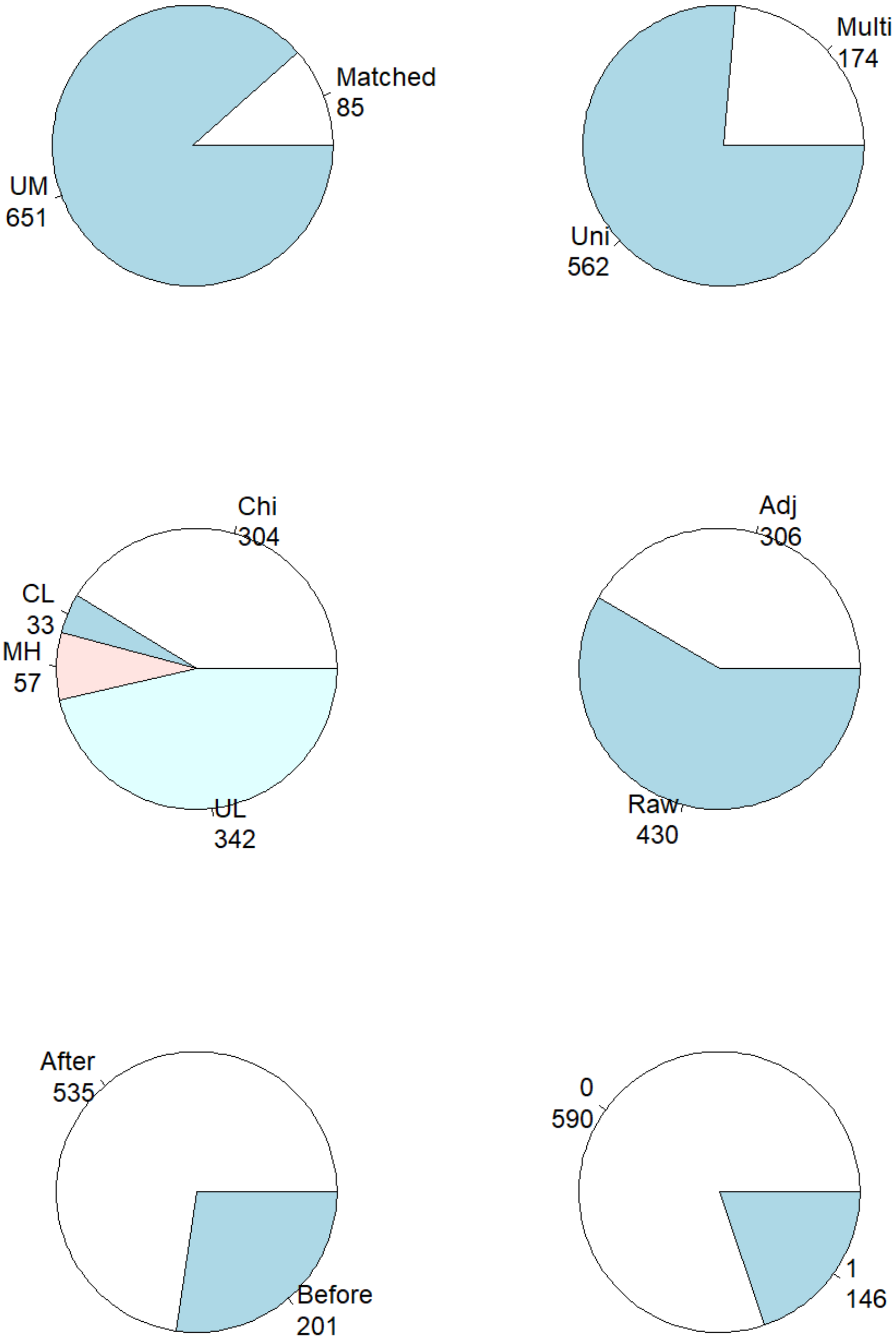


Figure 3. Pie-diagrams of the number of odds-ratios extracted from case-control and cohort studies of sporadic giardiasis by design (top left), analysis type (top right), model type (middle left), odds-ratio type (middle right), study period cut off year 2000 (bottom left) and assigned potential for bias (bottom right)

Table 1. Characteristics of primary studies investigating risk factors for acquiring sporadic giardiasis included in the meta-analysis

| **Study ID** | **Country** | **Study period** | **Population** | **Design** | **Analysis & model** | **# cases/ controls** | **Quality Final ORs /removed** |
|---|---|---|---|---|---|---|---|
| Almerie et al. 2008 | Syria | Mar-Jun 2006 | Children | Unmatched | Uni-UL | 206 cases 1263 controls | Good 3 |
| Alyousefi et al. 2011 | Yemen | 2011 | Mixed | Unmatched | Uni-Chi Multi-UL | 89 cases 414 controls | Good 9 |
| Anuar et al. 2014 | Malaysia | Jun-Dec 2011 | Mixed | Unmatched<br>Unmatched | Uni-Chi Multi-UL<br>Uni-Chi Multi-UL | 62 Assemb A 549 controls<br>36 Assemb B 575 controls | Good 23 |
| Anuar et al. 2012 | Malaysia | Jun-Dec 2011 | Mixed | Unmatched | Uni-UL | 100 cases 400 controls | Good 10 |
| Bello et al. 2011 | Cuba | 2003 | Children | Unmatched | Uni-Chi Multi-UL | 94 cases 257 controls | Good 17 |
| Boontanom et al. 2011 | Thailand | Feb 2007 | Children | Unmatched | Uni-Chi Multi-UL | 11 cases 178 controls | Good 6/1 |
| Campbell et al. 2016 | Timor Leste | May 2012-Oct 2013 | Mixed | Unmatched | Multi-UL | 111 cases 557 controls | Good 3 |
| Chaves et al. 2007 | Colombia | 1995, 2001, 2005 | Children | Unmatched | Uni-Chi | 19 cases 121 controls | Good 5 |
| Choy et al. 2014 | Malaysia | Apr 2011-Feb 2013 | Mixed | Unmatched | Uni-Chi Multi-UL | 154 cases 1176 controls | Good 22 |
| Chute et al. 1987 | USA | Jan 1977- Jun 1984 | Mixed | Matched | Uni-Chi Uni-UL | 171 cases 684 controls | Good 16 |
| Cifuentes et al. | Mexico | Jul-Sep 1990 | Mixed | Unmatched | Uni-UL Uni-Chi | 601 cases 6147 controls | Poor 4 |
| Cohen et al. 2008 | USA | 1993-2002 | Children Adult Susceptible (elderly) | Unmatched | Uni-UL Multi-UL | ? cases ? controls | Good 12 |
| Coles et.al 2009 | Israel | Dec 1994-Mar 1997 | Children | Unmatched | Uni-Chi Multi-UL | 29 c/163 c 55 c/278 c 92 c/307 c | Good 4 |
| Delfino et al. 2016 | Brazil | 2003 2010 2011 | Children | Unmatched | Uni-UL Multi-UL | 92 cases 307 controls | Good 6 |
| Dennis et al. 1993 | USA | 1984 | Mixed | Unmatched | Multi-UL | 273 cases 375 controls | Good 6 |
| Enserink et al. 2015 | Netherlands | 2010-2013 | Children | Unmatched | Multi-UL | 504 cases 4693 controls | Poor 3 |
| Ensink et al. 2006 | Pakistan | Aug 2002-Jul 2003 | Mixed | Unmatched | Uni-UL Multi-UL | 1145 cases 561 controls | Good 2 |
| Erismann et al. 2015 | Burkina | Feb 2015 | Children | Unmatched | Multi-UL Uni-UL | 108 cases 277 controls | Good 25/2 |
| Espelage et al. 2010 | Germany | Feb 2007- Jan 2008 | Mixed | Matched | Uni-MH Multi-CL | 120 cases 240 controls | Poor 22 |

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|---|---|---|---|---|---|---|---|
| Esrey et al. 1989 | South Africa | Jul 1984- Feb 1985 | Children | Unmatched | Uni-Chi Multi-UL | 63 cases 204 controls | Poor 8 |
| Faustine et al. 2006 | Italy | Jan 2000- May 2001 | Mixed<br>Children | Unmatched | Uni-UL Multi-UL<br>Uni-UL | 28 cases 54 controls<br>13 cases 11 controls | Good 14 |
| Firdu et al. 2014 | Ethiopia | Feb-Aug 2011 | Children | Unmatched | Uni-Chi | 18 cases 11 cryptosp | Poor 10 |
| Fonseca et al. 2014 | Brazil | Apr-May, Oct 2010 | Children | Unmatched | Uni-UL Multi-UL | 45 cases 531controls | Good 9 |
| Fraser et al. 1991 | New Zealand | 1986-1988 | Mixed | Matched | Uni-Chi | 21 cases 63 controls | Poor 1 |
| Gagnon et al. 2006 | Canada | 2001-2002 | Children<br>Mixed | Matched | Uni-UL Multi-UL<br>Uni-UL Multi-UL | 23 cases 77 controls<br>113 cases 301 controls | Good 13 |
| Girotto et al. 2013 | Brazil | 2009- 2010 | Susceptible (elderly)<br>Adult | Unmatched | Uni-Chi | 12 cases 281controls<br>3 cases 60 controls | Good 5 |
| Habbari et al. 1999 | Morocco | Jan-Mar 1999 | Children | Unmatched | Uni-Chi | 38 cases 702 controls | Good 5 |
| Heimer et al. 2015 | Rwanda | May 2012 | Children | Unmatched | Uni-Chi Multi-UL | 132 cases 231 controls | Good 9 |
| Heusinkveld et al. 2016 | Netherlands | Apr 2013- Oct 2014 | Children | Unmatched | Multi-UL | 44 cases 913 controls | Good 4 |
| Hoque et al. 2003 | New Zealand | Nov 1999- Jun 2000 | Children | Unmatched | Uni-UL Multi-UL Uni-MH | 69 cases 98 controls | Good 24/1 |
| Hoque et al. 2002 | New Zealand | Jul 1998- Jun 1999 | Mixed | Unmatched | Uni-MH Uni-UL Multi-UL | 183 cases 336 controls | Good 40 |
| Hoque et al. 2001 | New Zealand | Jul 1998-Jun 1999 | Mixed | Unmatched | Uni-Chi Uni-UL | 183 cases 336 controls | Good 4 |
| Ignatius et al. 2012 | Rwanda | Jan - Mar 2010 | Children | Unmatched | Uni-UL Multi-UL | 353 cases 229 controls | Good 8 |
| Jerez-Puebla et al. 2015 | Cuba | Jan-Dec 2013 | Children | Unmatched | Uni-Chi Multi-UL | 76 cases 639 controls | Good 12 |
| Johnston et al. 2010 | Uganda | May-Jun 2007 | Mixed | Unmatched | Uni-Chi | 44 cases 64 controls | Good 9/1 |
| Julio et al. 2012 | Portugal | Feb 2002- Oct 2008 | Children | Unmatched | Uni-Chi | 57 cases 787 controls | Good 12/3 |
| Kettani et al. 2006 | Marocco | 2005 | Mixed | Unmatched | Uni-Chi | 28 cases 305 controls | Good 2 |
| Lander et al. 2012 | Brazil | Aug-Nov 2010 | Children | Unmatched | Uni-UL | 42 cases 283 controls | Good 3 |
| Lora-Suarez et al. 2002 | Colombia | Jan 2000- Jul 2001 | Children | Unmatched | Uni-Chi Uni-MH | 131 cases 86 controls | Good 3 |
| Mahdy et al. 2009 | Malasysia | Feb-Mar 2006 | Mixed | Unmatched | Uni-Chi Multi-UL | 32 cases 208 controls | Good 8 |

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|---|---|---|---|---|---|---|---|
| Mahdy et al. 2008 | Malasysia | Feb-Mar 2006 | Mixed | Unmatched | Uni-Chi | 67 cases<br>218 controls | Good<br>7 |
| Mahmud et al. 2001 | Egypt | 2000 | Children | Unmatched | Uni-Chi<br>Multi-UL | 114 cases<br>312 controls | Good<br>2 |
| Mahmud et al. 2014 | Ethiopia | 2013 | Susceptible *(immuno-compromised)* | Unmatched | Uni-Chi<br>Multi-UL | 47 cases<br>327 controls | Good<br>8 |
| Marder 2012 | USA | 2003-2010 | Mixed | Unmatched | Uni-UL | 12194 cases<br>36386 salmon | Poor<br>19 |
| Mathias et al. 1992 | Canada | 1990 | Mixed | Unmatched | Uni-Chi | 180 cases<br>180 enteric | Poor<br>4 |
| Matthys et al. 2011 | Tajikistan | 2009 | Children | Unmatched | Uni-Chi<br>Multi-UL | 157 cases<br>437 controls | Good<br>8 |
| Mbae et al. 2013 | Kenya | 2010-2011 | Children | Unmatched | Uni-Chi<br>Multi-UL | 90 cases<br>1810 controls | Good<br>2 |
| Minetti et al. 2011 | UK | Feb 2012-Aug 2013 | Mixed | Unmatched | Uni-UL<br>Multi-UL | 118 cases<br>226 controls | Good<br>33/4 |
| Mitra et al. 2016 | India | 2016 | Mixed | Unmatched | Uni-Chi | 30 cases<br>262 controls | Good<br>1 |
| Moore et al. 2016 | Cambodia | Apr-Jun 2012 | Children | Unmatched | Uni-Chi | 132 cases<br>336 controls | Good<br>23 |
| Norhayati et al. 1998 | Malaysia | 1998 | Mixed | Unmatched | Uni-Chi | 176 cases<br>741 controls | Good<br>4 |
| Novotny at al. 1990 | USA | 1990 | Children | Unmatched | Multi-UL<br>Uni-Chi | 40 cases<br>270 controls | Good<br>15 |
| Nunez et al. 2003 | Cuba | 2003 | Children | Unmatched | Uni-Chi | 43 cases<br>76 controls | Good<br>5 |
| Omar et al.1995 | Saudi Arabia | 1992 | Mixed | Unmatched | Multi-UL | 268 cases<br>1149 controls | Good<br>2 |
| Osman et al. 2016 | Lebanon | Jan 2013 | Children | Unmatched | Uni-UL | 71 cases<br>178 controls | Good<br>4/1 |
| Pereira et al. 2007 | Brazil | Aug 1998-May 1999 | Children | Unmatched | Uni-UL | 44 cases<br>401 controls | Good<br>5 |
| Pijnacker et al. 2016 | Netherlands | Mar 2010-Mar 2013 | Children | Unmatched | Multi-UL | 62 Assemb A<br>76 Assemb B<br>4789 controls | Good<br>7 |
| Prado et al. 2003 | Brazil | 1998 | Children | Unmatched | Uni-Chi<br>Multi-UL | 95 cases<br>618 controls | Good<br>12 |
| Puebla et al. 2016 | Cuba | Jan-Jun 2013 | Children | Unmatched | Uni-Chi<br>Multi-UL | 45 cases<br>372 controls | Good<br>20 |
| Ratanapo at al. 2008 | Thailand | Feb 2005 | Children | Unmatched | Uni-Chi<br>Multi-UL | 33 cases<br>498 controls | Good<br>6 |
| Ravel et al. 2013 | Canada | Jun 2005-May 2009 | Mixed | Unmatched | Uni-Chi | 143 cases<br>54 amoebiasis | Poor<br>36/3 |
| Redlinger et al. 2002 | Mexico | Aug 1999-Mar 2000 | Children | Unmatched | Uni-Chi | 345 cases<br>298 cryptosp | Poor<br>2 |
| Sackey et al. 2003 | Ecuador | Jun-Aug. 2000 | Children | Unmatched | Uni-Chi<br>Multi-UL | 72 cases<br>172 controls | Good<br>6 |
| Santos ate al. 2012 | Brazil | May 2007-Mar 2008 | Children | Unmatched | Uni-Chi | 127 cases<br>118 controls | Good<br>11 |
| Gray et al. 1994 | UK | Jul 1992-May 1993 | Mixed | Matched | Uni- CL<br>Multi-CL | 74 cases<br>108 controls | Good<br>11/2 |
| Stuart et al. 2003 | UK | Apr 1998-Mar 1999 | Mixed | Matched | Uni- CL<br>Multi-CL | 192 cases<br>492 controls | Good<br>20/2 |

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|---|---|---|---|---|---|---|---|
| Swirski et al. 2015 | Canada | 2006-2012 | Mixed | Unmatched | Uni-UL | 220 cases<br>281 cases | Poor<br>29 |
| Takaoka et al. 2016 | UK | Sep 2008-May 2010 | Mixed | Unmatched | Uni-Chi<br>Multi-UL | 92 cases<br>3214 controls | Good<br>14 |
| Teixeira et al..2007 | Brazil | 2007 | Children | Unmatched | Multi-UL | 106 cases<br>484 controls | Good<br>3 |
| Tellevik et al. 2015 | Tanzania | Aug 2010-Jul 2011 | Children | Unmatched | Uni-Chi<br>Multi-UL | 701 cases<br>558 controls | Good<br>6 |
| Warburton et al. 1994 | UK | 1994 | Mixed | Matched | Uni-MH | 33 cases<br>112 controls | Good<br>2 |
| Wilson et al. 2008 | New Zealand | 2006 | Mixed | Unmatched | Uni-Chi | 591 cases<br>5395 campyl | Poor<br>8/1 |

During methodological quality assessment, twelve case-control studies were marked as being possibly affected by bias. The potential for selection bias status was assigned to six case-control studies where the controls were not healthy individuals but affected by 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), giardiasis domestically acquired (Swirski et al., 2015) or a group of them (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 marked 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 potentially having misclassification bias because of the possibility that controls may have been classified incorrectly as negative for *Giardia duodenalis*. The rationale for assigning potential-for-bias status to the association measures extracted from Enserink et al. (2015) and Fraser and Cooke (1991) related to the statistical approach employed, which, in the former was a Poisson model and, in the latter, the absence of counfounders. These aforementioned case-control studies provided 146 potentially-biased ORs whose influence on the meta-analysed OR estimates was appraised by means of the Cook's distance.

With regards to the risk factor classes, sporadic illness investigations focused much more on the multiple pathways of transmission (594 ORs) than on host specific factors (92 ORs) or travel (50 ORs) (Figure 2, bottom left). Within the pathways of transmission, the environment-related routes were more frequently investigated (367 ORs) than the animal-related (105 ORs), food consumption (93 ORs) and person-to-person routes (29 ORs) (Figure 2, bottom right). General information on the distribution of the number of ORs available for meta-analysis is presented in Figure 3 by study design, type of analysis, model, type of odds-ratio, study period and potential for bias status.

### *3.6.2 Meta-analysis for host-specific risk factors and travel-, environment-, animal-, food-related and person-to-person pathways of transmission*

Assessing the effect of study period on the pooled ORs in the meta-analysis models, it was found that the risks of giardiasis due to travel (p=0.826; Table 2B), host-specific factors (p=0.273; Table 3B), animal-related routes (p=0.556; Table 4B), environmental pathways (p=0.639; Table 5B) and food consumption (p=0.180; Table 7B), as measured in the earlier studies (before the year 2000), did not change significantly in the most recent years (after 2000). Therefore, in all of the models pertaining to the above data partitions, the variable “study period” was dropped from the full meta-analysis models, and the ORs were pooled to represent data from both study periods. By contrast, study period was found to be significant only in the person-to-person contagion as a risk factor for giardiasis (p=0.058; Table 6B). This meta-analysis revealed that, in recent years, the risk of giardiasis due to person-to-person transmission came down to about half (1/exp(0.740)=0.48; Table 6B) the risk that was estimated two to three decades ago. In the past, person-to-person contagion must have been one of the most important pathways for acquiring giardiasis.

The meta-analysis on travel by geographical region positioned travel as an important risk factor for acquiring giardiasis (p<.01) in the mixed population of North America (pooled OR=3.019), Oceania (pooled OR=3.927) and Europe (pooled OR=2.280; Table 2A). In children, travel was equally an important risk factor of giardiasis (p<.0001) in both regions for which data was available: North America (pooled OR=1.993) and Oceania (pooled OR=3.476; Table 2A).

Table 2A. Meta-analysis of travel as a risk factor for acquiring giardiasis by geographical region for the mixed (n=42 ORs) and children (n=7 ORs) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| North America | 1.105 | 0.291 | 9 | [0.536 – 1.675] | <.0001 |
| Europe | 0.824 | 0.276 | 27 | [0.282 – 1.366] | 0.003 |
| Oceania | 1.368 | 0.404 | 6 | [0.575 – 2.160] | 0.001 |
| Children | | | | | |
| North America | 0.690 | 0.185 | 3 | [0.326 – 1.053] | <.0001 |
| Oceania | 1.246 | 0.197 | 4 | [0.860 – 1.632] | <.0001 |

On average, people who travelled recently, either out of or within the country, had 3.158 times (95% CI: 2.075 – 4.802) greater probability of becoming infected with giardiasis than people who did not travel at all (Table 2B). For nationals of USA, Canada, Germany, New Zealand, UK and Italy, travelling abroad increased their odds of infection with *Giardia duodenalis* by an average of 4.845 (95% CI: 3.297 – 7.120). Although still significant, domestic travelling conveyed a lower risk at an overall OR of 1.824 (95% CI: 0.951 – 3.501), as meta-analysed from nationals of Italy, New Zealand, Canada and UK (Table 2B). However, children from USA, Italy and New Zealand who travelled inside the country presented a higher odds of infection (overall OR=2.377; 95% CI: 1.795 – 3.149) than the mixed population (Table 2B). No data was found for children travelling abroad. The meta-analyses conducted on the travel data partition did not suggest any publication bias either in the mixed (p=0.744) or in the children population (p=0.828), which was also supported by the acceptable spread of observations within the funnel plots (Figure 4). Only one potentially-biased OR was removed from the mixed population meta-analysis after assessing influential observations by Cook's distance.

Table 2B. Meta-analysis of travel as a risk factor for acquiring giardiasis in the mixed (n=42 ORs) and children (n=7 ORs) population

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | All Travel | 1.150 | 0.214 | | [0.730 – 1.569] | <.0001 |
| (12 stud) | Fixed effects | | | | | |
| | Abroad | 1.578[c] | 0.197 | 32 | [1.193 – 1.963] | <.0001 |
| | Any | 0.903[b] | 0.302 | 5 | [0.309 – 1.496] | 0.029 |
| | Inside | 0.601[a] | 0.332 | 5 | [-0.050 – 1.253] | 0.070 |
| | Before 2000 | | | | | 0.826 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.247 | QE(df=38) | | QM(df=3) | |
| | $s^2$ (residual) | 0.8073 | p=0.001 | | p<.001 | |
| | Publication bias | -0.0000 | 0.0002 | | | 0.744 |
| | Points removed | 1 | | | | |
| Children | All Travel* | 0.866 | 0.143 | | [0.585 – 1.147] | <.0001 |
| (3 stud) | Fixed effects | | | | | |
| | Inside | 0.866 | 0.143 | 7 | [0.585 – 1.147] | <.0001 |
| | Before 2000 | | | | | 0.787 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0000 | QE(df=6) | | | |
| | $s^2$ (residual) | 0.5369 | p=0.298 | | | |
| | Publication bias | -0.0005 | 0.0024 | | | 0.828 |
| | Points removed | 0 | | | | |

(*) Pooled OR for All Travel is equal to pooled OR for travel inside since it was the only subcategory for which data was available in the children population

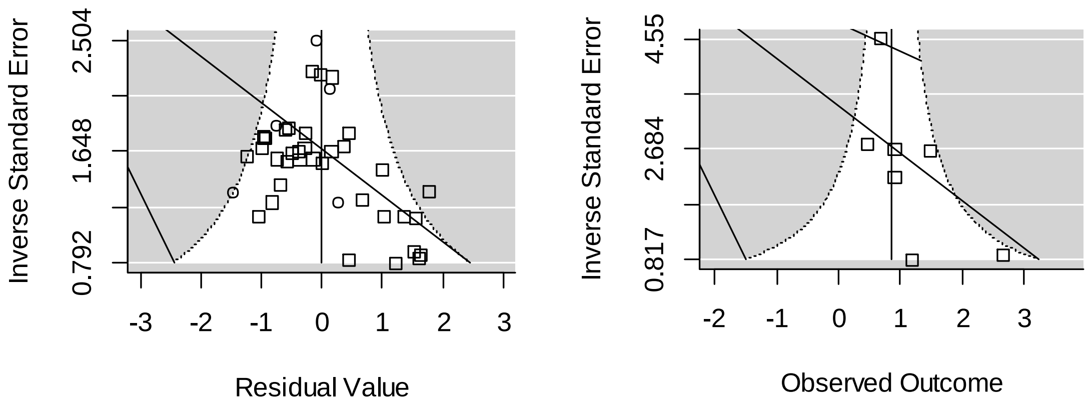



Figure 4. Funnel plots of the meta-analyses of travel as a risk factor for giardiasis in the mixed (left) and children (right) population. Circle markers belong to OR measures with potential for bias

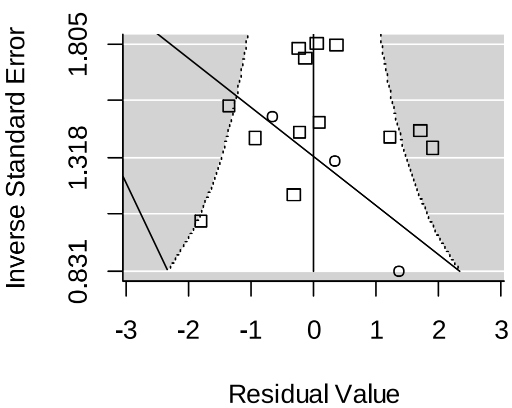
The meta-analysis on host-specific factors revealed that in the North American (pooled OR=6.271) and European countries (pooled ORs from 3.267 to 6.258), suffering from chronic diseases and gastrointestinal conditions exacerbated the risk of acquiring giardiasis to a greater extent than in African and Asian countries (pooled ORs of 1.133 and 1.533, respectively; Table 3A). In the children population, except for North America, all of the other regions (namely, Africa: pooled OR=1.409; Asia: pooled OR=1.349; South America: pooled OR=1.331; and South Europe: pooled OR=1.485) presented comparable overall association between host-specific factors and giardiasis. In North America, the pooled OR was significantly higher (pooled OR=5.073; p<.0001 in Table 3A) because the only host-specific factor meta-analysed (4 data points) was the predisposition of children with HIV to giardiasis. For the full meta-analysis on host-specific factors by subcategory, the data from Africa were removed from the mixed population since their pooled OR was the lowest and the only non-significant among the five geographical regions (Table 3A).

The meta-analysis by subcategory suggested that, in the mixed population, having HIV (chronic) or any gastrointestinal medical condition (Other med) equally predisposed people to acquiring giardiasis, as per the comparable overall ORs of 3.931 (95% CI: 1.761 – 8.776) and 4.284 (95% CI: 2.181 – 8.415), respectively (Table 3B). Poor hygiene habits seemingly barely predisposed the mixed population to acquiring giardiasis (overall OR=1.525; 95% CI: 0.706 – 3.293), although this is not conclusive by any means since the extracted ORs in this subcategory were only four (Table 3B). On meta-analysis, children who suffered from any chronic disease or malnutrition, or with poor hygiene habits presented on average 1.412 times (95% CI: 1.114 – 1.790) greater probability of becoming infected with *Giardia duodenalis* than those who were not exposed by these means (Table 3B). Similar levels of risk of giardiasis were exhibited for children having HIV (Chronic: overall OR=1.621; p=0.064), previous gastrointestinal infections or malnutrition (Other med: overall OR=1.413; p=0.051) and those with poor hand hygiene habits (Hygiene: overall OR=1.332; p=0.102). Infants who were not breastfed presented nearly twice (overall OR=1.945; 95% CI: 1.094 – 3.462) the probability of acquiring giardiasis than those who were breastfed. Thus, breastfeeding had a significant protective effect (p=0.024; Table 3B) against giardiasis. Publication bias was not evident in the children partition meta-analysis given both the lack of effect of study size on the measured ORs (p=0.424), and the scattered distribution of observations within the funnel plot (Figure 5, right). In the mixed population, however, there was evidence of publication bias (p=0.002 in Table 3B), likely to arise from the fewer data points found in the literature (Figure 5, left).

Table 3A. Meta-analyses of host-specific factors for giardiasis by region for the mixed (n=19) and children population (n=65, ORs for breastfeeding removed)

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| Africa | 0.125 | 0.308 | 3 | [-0.478 – 0.729] | 0.684 |
| Asia | 0.427 | 0.100 | 5 | [0.231 – 0.622] | <.0001 |
| North America | 1.836 | 0.370 | 3 | [1.112 – 2.561] | <.0001 |
| North Europe | 1.834 | 0.192 | 5 | [1.459 – 2.210] | <.0001 |
| West Europe | 1.184 | 0.304 | 3 | [0.588 – 1.779] | <.0001 |
| Children* | | | | | |
| Africa | 0.343 | 0.189 | 25 | [-0.028 – 0.713] | 0.070 |
| Asia | 0.300 | 0.227 | 12 | [-0.150 – 0.741] | 0.194 |
| North America** | 1.624 | 0.471 | 4 | [0.701 – 2.546] | 0.001 |
| South America | 0.286 | 0.175 | 22 | [-0.057 – 0.629] | 0.102 |
| South Europe | 0.396 | 0.501 | 2 | [-0.585 – 1.377] | 0.429 |

*The breastfeeding category was removed for the meta-analysis to make pooled ORS by region comparable to those of the mixed population; all breastfeeding OR data (protective effect) were evaluated in African studies
** The four data points assessed the predisposition of children with HIV to giardiasis



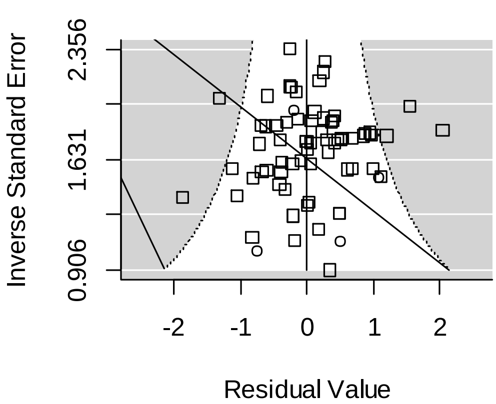




Figure 5. Funnel plot of the meta-analyses of host-specific risk factors for giardiasis in the mixed (left) and children (right) population

Table 3B. Meta-analysis of host specific factors for giardiasis in the mixed (n=16) and children population (n=72) – Africa removed from the mixed population

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | All host-specific | 1.132 | 0.267 | | [0.608 – 1.656] | <.0001 |
| (8 stud) | Fixed effects | | | | | |
| | Chronic | 1.369[b] | 0.409 | 5 | [0.566 – 2.172] | 0.001 |
| | Hygiene | 0.422[a] | 0.393 | 4 | [-0.347 – 1.192] | 0.282 |
| | Other med | 1.455[b] | 0.344 | 7 | [0.780 – 2.130] | <.0001 |
| | Before 2000 | | | | | 0.273 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.424 | QE(df=13) | | QM(df=3) | |
| | $s^2$ (residual) | 1.1189 | p<.001 | | p<.001 | |
| | Publication bias | 0.0003 | 0.0001 | | | 0.002 |
| | Points removed | 0 | | | | |
| Children | All host-specific* | 0.345 | 0.121 | 65 | [0.108 – 0.582] | 0.004 |
| (25 stud) | Fixed effects | | | | | |
| | Breastfeeding | -0.666[a] | 0.294 | 7 | [-1.241 – -0.090] | 0.024 |
| | Chronic | 0.483[b] | 0.261 | 11 | [-0.028 – 1.000] | 0.064 |
| | Hygiene | 0.287[b] | 0.176 | 19 | [-0.057 – 0.631] | 0.102 |
| | Other med | 0.346[b] | 0.177 | 35 | [-0.001 – 0.693] | 0.051 |
| | Before 2000 | | | | | 0.424 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.2635 | QE(df=68) | | QM(df=4) | |
| | $s^2$ (residual) | 0.4545 | p<.0001 | | p=0.005 | |
| | Publication bias | -0.0004 | 0.0003 | | | 0.156 |
| | Points removed | 0 | | | | |

(*) Computed from 23 primary studies since breastfeeding data was excluded for this calculation

Regarding the animal-related pathways of transmission in the mixed population, animal contact appeared to be an important source of giardiasis infection in Europe (pooled OR=1.408; p=0.102) and Asia (pooled OR=1.391; p=0.030 in Table 4A). In the North America mixed population, however, contact with animals did not represent a significant source of transmission of giardiasis (pooled OR=1.096; p=0.566). By contrast, the children from Africa (pooled OR=1.980; p=0.026), Asia (pooled OR=1.592; p=0.038) and South America (pooled OR=1.844; p=0.010) displayed a similar (significant) level of risk when exposed to any contact with animals. Interestingly, in Western European children, the association between animal contact and disease was the highest (pooled OR=3.758; p<.0001) whereas in Southern European children no association between giardiasis and animal contact could be established (pooled OR=0.931; p=0.865 in Table 4A). For the subsequent meta-analysis by animal contact subcategory, the data from Oceania were removed from the mixed population since they produced the lowest pooled OR (1.003; p=0.994) significantly different from that of Europe (Table 4A).

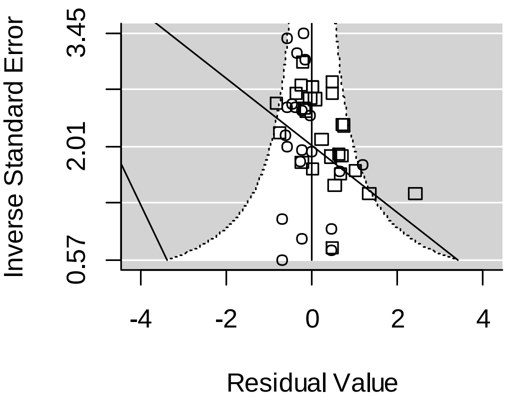
Table 4A. Meta-analyses of pathways related to animal contact for giardiasis by region in the mixed (n=51) and children (n=54) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| Asia | 0.330 | 0.152 | 12 | [0.033 – 0.627] | 0.030 |
| North America | 0.092 | 0.160 | 21 | [-0.222 – 0.406] | 0.566 |
| Oceania | 0.003 | 0.330 | 2 | [-0.644 – 0.650] | 0.994 |
| Europe | 0.342 | 0.209 | 16 | [-0.068 – 0.753] | 0.102 |
| Children | | | | | |
| Africa | 0.683 | 0.307 | 9 | [0.082 – 1.284] | 0.026 |
| Asia | 0.465 | 0.223 | 20 | [0.027 – 0.904] | 0.038 |
| South America | 0.612 | 0.236 | 10 | [0.148 – 1.075] | 0.010 |
| South Europe | -0.071 | 0.416 | 7 | [-0.887 – 0.745] | 0.865 |
| West Europe | 1.324 | 0.289 | 8 | [0.757 – 1.890] | <.0001 |

The risk of transmission of giardiasis posed by any kind of contact with animals is, on average, higher in the children population (overall OR=1.885; 95% CI: 1.409 – 2.512) than in the mixed population (overall OR=1.342; 95% CI: 1.117 – 1.614; Table 4B). Within the mixed population, contact with wild animals, encompassing visiting/working in a zoo and contact with birds, rabbits, reptiles or rodents (pooled OR=1.655; 95% CI: 1.005 – 2.726), bears overall a significantly greater association with giardiasis than having direct contact with livestock/poultry (overall OR=1.252; 95% CI: 0.856 – 1.835) or contact with a pet (overall OR=1.305; 95% CI: 1.064 – 1.600). Yet, in comparison to the mixed population, children are significantly more susceptible to acquiring giardiasis through both animal-related exposures, contact with farm animals (overall OR=1.921; 95% CI: 1.351 – 2.729) and contact with pets (overall OR=1.868; 95% CI: 1.381 – 2.529; Table 4B). Applying the Cook's distance diagnostics on the animal-contact meta-analyses, one potentially-biased influential OR was deleted from the mixed population data and one from the children data (Table 4B). Furthermore, in both meta-analyses, there was a significant effect of study size on the published OR measures (p=0.009 and 0.042). Thus, despite the funnel plots did not signal any strong evidence of publication bias (Figure 6), the formal test for publication bias evidenced the plausible existence of small case-control studies (or OR values) that remained unpublished given their insufficient power to quantify a significant association between animal exposure and giardiasis.

Table 4B. Meta-analyses of pathways related to animal contact for acquiring giardiasis in the mixed (n=49) and children (n=54) population – Oceania removed from the mixed population

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | All animal | 0.294 | 0.094 | | [0.111 – 0.479] | 0.002 |
| (17 stud) | Fixed effects | | | | | |
| | Farm | 0.225[a] | 0.195 | 10 | [-0.156 – 0.607] | 0.245 |
| | Pets | 0.266[a] | 0.104 | 30 | [0.062 – 0.470] | 0.011 |
| | Wild | 0.504[b] | 0.255 | 9 | [0.005 – 1.003] | 0.048 |
| | Before 2000 | | | | | 0.556 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0990 | QE(df=45) | | QM(df=3) | |
| | $s^2$ (residual) | 0.4007 | p<.0001 | | p=0.008 | |
| | Publication bias | -0.0000 | 0.0000 | | | 0.009 |
| | Points removed | 1 | | | | |
| Children | All animal | 0.634 | 0.148 | | [0.343 – 0.921] | <.0001 |
| (18 stud) | Fixed effects | | | | | |
| | Farm | 0.653[a] | 0.179 | 15 | [0.301 – 1.004] | <.0001 |
| | Pets | 0.625[a] | 0.154 | 39 | [0.323 – 0.928] | <.0001 |
| | Before 2000 | | | | | 0.967 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.2477 | QE(df=49) | | QM(df=2) | |
| | $s^2$ (residual) | 0.6725 | p=0.049 | | p=0.995 | |
| | Publication bias | 0.0002 | 0.0001 | | | 0.042 |
| | Points removed | 1 | | | | |



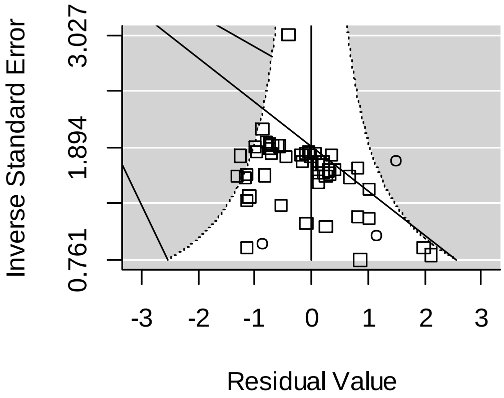




Figure 6. Funnel plots of the meta-analyses of animal contact pathways as risk factors for giardiasis in the mixed (left) and children population (right). Circle markers belong to OR measures with potential for bias

Most of the data recovered from primary studies belonged to the diverse environmental sources of transmission, amounting to 202 ORs for the mixed population and 165 ORs for the children population. In children, the environmental pathways of exposure were strongly associated with giardiasis in nearly all geographical regions: Africa (pooled OR=1.499; p=0.007), Asia (pooled OR=1.336; p=0.094), North America (pooled OR=1.543; p=0.034), Oceania (pooled OR=3.016; p=0.001), South America (pooled OR=1.848; p<.0001) and Western Europe (pooled OR=3.077; p<.0001) (Table 5A). However, in the mixed population, the combined environmental pathways were not proven to be determinant of giardiasis in all of the above geographical regions: while no associations were found in case-control studies from Africa, South America and Western Europe, in the other regions the significant pooled ORs varied from 1.452 (in Asian countries) to 2.707 (Oceanian countries) (Table 5A). Thus, for the general meta-analysis on environmental sources by subcategory, the data from Africa and South America were not included.

The general meta-analyses demonstrated the relevance of the environmental pathways in the transmission of giardiasis in both the mixed (overall OR=1.826; 95% CI: 1.534 – 2.171) and the children population (overall OR=1.718; 95% CI: 1.465 – 2.013; Table 5B). On average, the mixed population exposed to waste water (i.e., exposure to human waste or sewage, common drainage system, and no toilet facilities at home) bore the greatest risk of acquiring giardiasis (overall OR=2.034; 95% CI: 1.571 – 2.633) among all the other environmental pathways. A significantly lower association with giardiasis was found for the exposures related to drinking water (overall OR=1.857; 95% CI: 1.502 – 2.293) and recreational water (overall OR=1.874; 95% CI: 1.504 – 2.335 in Table 5B). While the drinking water exposure encompassed drinking untreated water, private/shallow well/river and bore water and unboiled water in deprived regions; the recreational water exposure included bathing or swimming in river, lake, ocean, swimming pool, any stream and practising water sports. On meta-analysis, all of these water-related exposures were equally likely to cause giardiasis in the mixed population. At a numerally lower risk laid the day care pathway, which grouped attending to day care centre and adult’s contact with child, with an overall OR of 1.714 (95%

CI: 1.207 – 2.435). Camping, gardening and contact with manure (viz. playground) bore the lowest risk of acquiring giardiasis in the mixed population at an overall OR of 1.587 (95% CI: 1.141 – 2.208 in Table 5B).

Table 5A. Meta-analyses of environmental pathways for acquiring giardiasis by region in the mixed (n=202) and children (n=165) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| Africa | 0.280 | 0.248 | 8 | [-0.205 – 0.765] | 0.257 |
| Asia | 0.373 | 0.117 | 43 | [0.144 – 0.601] | 0.001 |
| North America | 0.668 | 0.126 | 63 | [0.421 – 0.914] | <.0001 |
| North Europe | 0.703 | 0.202 | 34 | [0.306 – 1.098] | <.0001 |
| Oceania | 0.996 | 0.186 | 43 | [0.629 – 1.362] | <.0001 |
| South America | 0.214 | 0.335 | 3 | [-0.442 – 0.869] | 0.523 |
| West Europe | 0.238 | 0.307 | 8 | [-0.365 – 0.840] | 0.439 |
| Children | | | | | |
| Africa | 0.405 | 0.150 | 38 | [0.110 – 0.700] | 0.007 |
| Asia | 0.290 | 0.173 | 16 | [-0.049 – 0.630] | 0.094 |
| North America | 0.434 | 0.206 | 18 | [0.031 – 0.837] | 0.034 |
| Oceania | 1.104 | 0.335 | 18 | [0.447 – 1.761] | 0.001 |
| South America | 0.614 | 0.098 | 64 | [0.422 – 0.805] | <.0001 |
| South Europe | -0.169 | 0.316 | 6 | [-0.787– 0.450] | 0.593 |
| West Europe | 1.124 | 0.273 | 5 | [0.589 – 1.660] | <.0001 |

In children, the playground exposure – represented by playing on a soiled floor and having a sandpit or garden at home (overall OR=1.846; 95% CI: 1.117 – 3.049) – bore the greatest risk of acquiring giardiasis. On meta-analysis, children appeared equally likely to acquiring giardiasis when exposed to human waste (including open sewage near home, no toilet or latrine at home, toilet in yard and absence of network sewage system; pooled OR=1.740; 95% CI: 1.346 – 2.250), living on a farm (pooled OR=1.700; 95% CI: 1.236 – 2.342) or drinking untreated water (i.e., unboiled or unfiltered tap water, river/well/borehole/surface/spring/pond water; overall OR=1.781; 95% CI: 1.468 – 2.160 in Table 5B). Significantly lower levels of risk of giardiasis in children were born for the pathways of attending day care/kindergarten/school (overall OR=1.526; 95% CI: 1.122 – 2.088) and recreational water (comprising playing with water, fishing, swimming and sailing; pooled OR=1.392; 95% CI: 0.927 – 2.092). After Cook's distance assessment, only one potentially-biased and influential OR measure was removed from the meta-analysis on the mixed population (Table 5B). In addition, the OR measures appeared to be well distributed within the funnel plots from both, the mixed and children population meta-analyses (Figure 7). Publication bias was very unlikely since the measures of OR were not found to depend upon the case-control study size (p=0.180 and p=0.539; Table 5B).

Table 5B. Meta-analyses of environmental pathways for acquiring giardiasis in the mixed (n=189) and children (n=165) population – African and South American countries removed from the mixed population

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | All environment | 0.602 | 0.088 | | [0.428 – 0.775] | <.0001 |
| (26 stud) | Fixed effects | | | | | |
| | Day care | 0.539[b] | 0.179 | 13 | [0.188 – 0.890] | 0.003 |
| | Drink water | 0.619[c] | 0.108 | 66 | [0.407 – 0.830] | <.0001 |
| | Playground* | 0.462[a] | 0.168 | 10 | [0.132 – 0.792] | 0.006 |
| | Rec water | 0.628[c] | 0.112 | 73 | [0.408 – 0.848] | <.0001 |
| | Waste water | 0.710[d] | 0.131 | 27 | [0.452 – 0.968] | <.0001 |
| | Before 2000 | | | | | 0.639 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.2000 | QE(df=183) | | QM(df=5) | |
| | $s^2$ (residual) | 0.5515 | p<.0001 | | p<.0001 | |
| | Publication bias | 0.0000 | 0.0000 | | | 0.180 |
| | Points removed | 1 | | | | |
| Children | All environment | 0.541 | 0.081 | | [0.382 – 0.700] | <.0001 |
| (34 stud) | Fixed effects | | | | | |
| | Day care | 0.423[a] | 0.159 | 21 | [0.115 – 0.736] | 0.008 |
| | Drink water | 0.577[b] | 0.098 | 73 | [0.384 – 0.770] | <.0001 |
| | Farm | 0.531[b] | 0.163 | 13 | [0.212 – 0.851] | 0.001 |
| | Playground | 0.613[c] | 0.256 | 6 | [0.111 – 1.115] | 0.016 |
| | Rec water | 0.331[a] | 0.208 | 18 | [-0.076 – 0.738] | 0.110 |
| | Waste water | 0.554[b] | 0.131 | 34 | [0.297 – 0.811] | <.0001 |
| | Before 2000 | | | | | 0.891 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.1869 | QE(df=159) | | QM(df=6) | |
| | $s^2$ (residual) | 0.4529 | p<.0001 | | p<.0001 | |
| | Publication bias | 0.0001 | 0.0001 | | | 0.539 |
| | Points removed | 0 | | | | |

(*) Camping, gardening and contact with manure

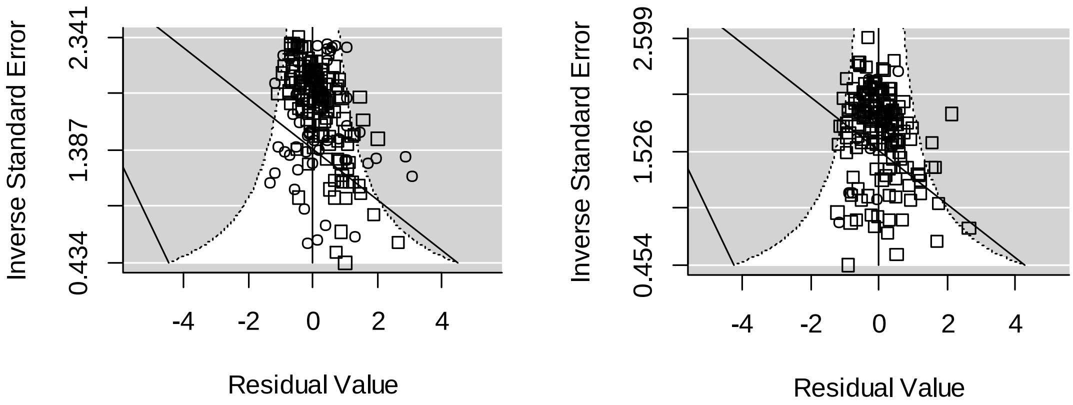



Figure 7. Funnel plots of the meta-analyses of environmental pathways as risk factors for giardiasis in the mixed (left) and children population (right). Circle markers belong to OR measures with potential for bias

The contact with a person having giardiasis is a very important pathway of transmission of disease, confirmed – on meta-analysis – to have significant pooled OR measures of association in the Asian (pooled OR=3.293; p<.0001), North American (pooled OR=2.889; p=<.0001), Oceania (pooled OR=6.228; p<.0001), South American (pooled OR=2.149; p=0.100) and Western European case-control studies (pooled OR=2.071; p=0.061 in Table 6A). Pooling the results from Africa, contact with an ill person was not strongly associated with giardiasis (pooled OR=1.722; p=0.271 in Table 6A). For this reason, the outcomes from the African case-control studies were removed from the data set for the subsequent general meta-analysis by population.

Table 6A. Meta-analysis of person-to-person transmission of giardiasis by region for the combined mixed (n=18) and children (n=11) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed and children | | | | | |
| Africa | 0.544 | 0.494 | 3 | [-0.425 – 1.512] | 0.271 |
| Asia | 1.192 | 0.304 | 7 | [0.600 – 1.788] | <.0001 |
| North America | 1.061 | 0.279 | 9 | [0.513 – 1.609] | <.0001 |
| Oceania | 1.829 | 0.494 | 3 | [0.862 – 2.797] | <.0001 |
| South America | 0.765 | 0.465 | 4 | [-0.146 – 1.676] | 0.100 |
| West Europe | 0.728 | 0.389 | 3 | [-0.034 – 1.490] | 0.061 |

On meta-analysis, children who had contact with any giardiasis case presented 2.787 times (95% CI: 1.480 – 5.254) greater risk of contagion than those who did not. The risk of person-to-person contagion in the mixed population (overall OR=2.542; 95% CI: 1.521 – 4.246 in Table 6B) was not significant different than that of children. In this data partition, only one potentially-bias OR turned out to an influential data point in the meta-analytical regression. The corresponding funnel plot (Figure 8) did not hint any strong evidence of publication bias, which was also supported by the non-significant effect of study size on the measured OR (p=0.355 in Table 6B).

Table 6B. Meta-analysis of person-to-person transmission of giardiasis in the combined mixed (n=16) and children (n=9) population – Africa was removed

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Both | Fixed effects | | | | | |
| (17 stud) | Children | 1.025[a] | 0.323 | 9 | [0.392 – 1.659] | 0.002 |
| | Mixed | 0.933[a] | 0.262 | 17 | [0.420– 1.446] | <.0001 |
| | Before 2000 | 0.740 | 0.390 | | [-0.024 – 1.504] | 0.058 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.2365 | QE(df=22) | | QM(df=2) | |
| | $s^2$ (residual) | 0.5705 | p<.0001 | | p=0.790 | |
| | Publication bias | -0.0000 | 0.0000 | | | 0.355 |
| | Points removed | 1 | | | | |

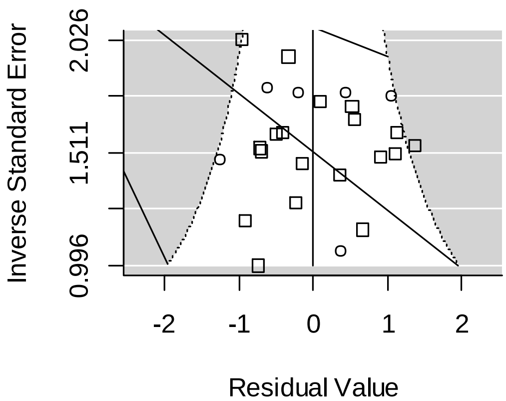



Figure 8. Funnel plot of the meta-analysis of person-to-person transmission of giardiasis in the combined mixed and children population. Circle markers belong to OR measures with potential for bias

In the mixed population, the ingestion of contaminated foods was associated with acquiring giardiasis in the case-control studies from Asia (pooled OR=1.584; p=0.001) and North America (pooled OR=2.186; p<.0001), whereas in Northern (pooled OR=1.040; p=0.841) and Western Europe (pooled OR=1.235; p=0.438), food did not appear to be an important vehicle for the infection with *Giardia duodenalis* (Table 7A). Nonetheless, in children from Latin America, the ingestion of infested food was a significant determinant of disease (pooled OR=2.179; p=0.002 in Table 7A). No geographical region was removed for the subsequent meta-analysis by food subcategory.

Table 7A. Meta-analyses of food pathways for acquiring giardiasis by geographical region in the mixed (n=56) and children (n=11) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| Asia | 0.460 | 0.134 | 18 | [0.197 – 0.723] | 0.001 |
| North America | 0.782 | 0.206 | 22 | [0.378 – 1.186] | <.0001 |
| North Europe | 0.039 | 0.197 | 10 | [-0.347 – 0.427] | 0.841 |
| West Europe | 0.211 | 0.273 | 6 | [-0.323 – 0.746] | 0.438 |
| Children | | | | | |
| Asia | 0.104 | 0.371 | 3 | [-0.623 – 0.831] | 0.778 |
| South America | 0.779 | 0.252 | 8 | [0.285 – 1.273] | 0.002 |

The general meta-analysis on food pathways demonstrated that, as a whole, children were more likely to become infected with *Giardia duodenalis* through food consumption (overall OR=1.885; 95% CI: 1.211 – 2.933) than the mixed population (overall OR=1.567; 95% CI: 1.237 – 1.982 in Table 7B). On meta-analysis, the mixed population with giardiasis infection were more likely to have consumed produce (including raw vegetables, fresh fruits, salads and unwashed vegetables; overall OR=1.545; 95% CI: 1.219 – 1.956) or multi-ingredient foods (eaten at cafeteria, supermarket, food vendor, delicatessen shops and restaurants; overall OR=2.190; 95% CI: 1.297 – 3.699) than the controls (Table 7B). No statistical association with giardiasis was found for the food subcategories of beverages (bottled water and unpasteurised juice; p=0.488), dairy foods (raw milk and raw dairy products; p=0.835) and meat (meat from butcher and private kill; p=0.761), probably due to the very few ORs recovered in these subcategories (Table 7B). The trend was similar in the children population: whereas produce was a strong determinant of giardiasis (overall OR=1.925; 95% CI: 1.340 – 2.765 for unwashed raw vegetables, fresh fruits and raw cabbage), meat was not found to be a source of giardiasis (overall OR=0.759; 95% CI: 0.419 – 1.374). After Cook's distance assessment, two potentially-biased OR popped up as influential points, and hence, they were removed (Table 7B). Although, in the children population, there was a strong effect of study size on the measured ORs (p=0.001), the funnel plots revealed an acceptable distribution of observations (Figure 9).

Table 7B. Meta-analyses of food pathways for acquiring giardiasis in the mixed (n=56) and children (n=13) population – no geographical region removed

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | All foods | 0.449 | 0.120 | | [0.213 – 0.684] | <.0001 |
| (13 stud) | Fixed effects | | | | | |
| | Beverage | 0.184[a] | 0.265 | 4 | [-0.336 – 0.705] | 0.488 |
| | Composite | 0.784[ab] | 0.267 | 15 | [0.260 – 1.308] | 0.003 |
| | Dairy | 0.156[a] | 0.755 | 2 | [-1.322 – 1.636] | 0.835 |
| | Meat | 0.269[a] | 0.885 | 2 | [-1.466 – 2.004] | 0.761 |
| | Produce | 0.435[b] | 0.121 | 33 | [0.198 – 0.671] | 0.001 |
| | Before 2000 | | | | | 0.180 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.1067 | QE(df=49) | | QM(df=5) | |
| | $s^2$ (residual) | 0.3897 | p<.0001 | | p=0.001 | |
| | Publication bias | 0.0001 | 0.0001 | | | 0.339 |
| | Points removed | 2 | | | | |
| Children | All foods | 0.634 | 0.225 | | [0.192 – 1.076] | 0.005 |
| (9 stud) | Fixed effects | | | | | |
| | Meat | -0.276[a] | 0.303 | 3 | [-0.869 – 0.318] | 0.363 |
| | Produce | 0.655[b] | 0.185 | 10 | [0.293 – 1.017] | <.0001 |
| | Before 2000 | 0.744 | 0.399 | | [-0.879 – 1.526] | 0.062 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0862 | QE(df=10) | | QM(df=2) | |
| | $s^2$ (residual) | 0.4280 | p=0.035 | | p=0.001 | |
| | Publication bias | -0.0016 | 0.0006 | | | 0.001 |
| | Points removed | 0 | | | | |

The meta-analysis on produce pathways of transmission by geographical region showed that in the mixed population, there was a strong association between consumption of produce and giardiasis in Asia (pooled OR=1.624; p=0.002), whereas in Latin American children, such association was even stronger (pooled OR=2.201; p<.0001 in Table 8A). However, in Europe, consumption of produce did not come out as an important vehicle of transmission of *Giardia duodenalis* (pooled OR=1.206; p=0.366 in Table 8A). For the general meta-analysis by produce class, the Asian and American regions were removed as their pooled ORs were higher than that of Europe. Children who consumed raw or unwashed vegetables in Latin American countries presented 2.192 times (95% CI: 1.465 – 3.277; Table 8B) greater odds of becoming infected with giardiasis than those who did not. Such level of risk was significantly higher than that of people from the European mixed population exposed through the ingestion of raw or unwashed vegetables, which presented an overall OR of 1.283 (95% CI: 0.877 – 1.876 in Table 8B). This value, summarising only European studies, was only slightly lower than the pooled OR of 1.340 (95% CI: 1.110 – 1.620), obtained by meta-analysing studies from all countries (this is, without restriction by geographical region). The corresponding forest plot is shown in Figure 11.

Since only two data points were available for the consumption of fruits, the resulting overall OR of giardiasis due to fruits is very uncertain and, hence, not reliable (pooled OR=0.824; 95% CI: 0.447 – 1.517). In these meta-analyses, no potentially-biased OR was influential. However, it is very likely that exposures related to produce consumption, investigated in small case-control studies, may have remained unpublished due to their inability to find significant or strong association with disease (p=0.003 in the mixed population and p=0.014 in the children population; Table 8B). Corresponding funnel plots are shown in Figure 10.

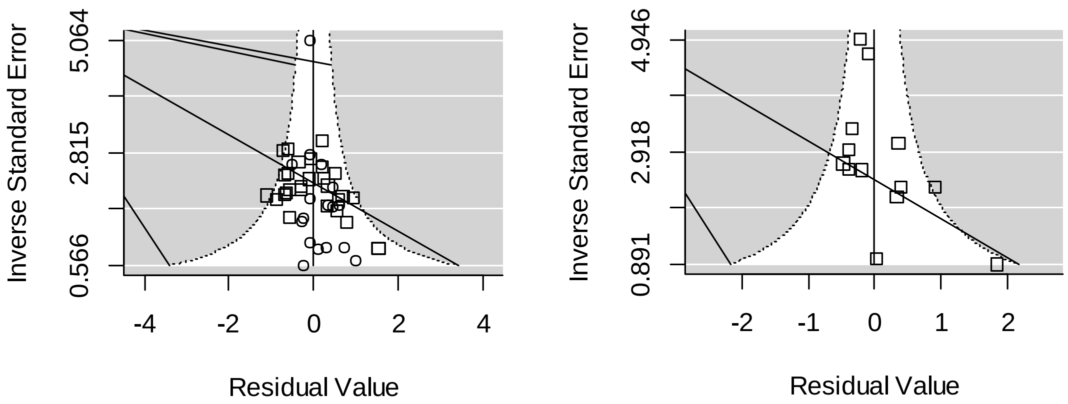



Figure 9. Funnel plots of the meta-analyses of food pathways for acquiring giardiasis in the mixed (left) and children population (right). Circle markers belong to OR measures with potential for bias

Table 8A. Meta-analyses of produce consumption related pathways for acquiring giardiasis by geographical region in the mixed (n=33) and children (n=9) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| Asia | 0.485 | 0.158 | 18 | [0.176 – 0.795] | 0.002 |
| America | 0.611 | 0.434 | 2 | [-0.248 – 1.469] | 0.163 |
| Europe | 0.187 | 0.207 | 13 | [-0.218 – 0.593] | 0.366 |
| Children | | | | | |
| South America | 0.789 | 0.210 | 9 | [0.377 – 1.200] | <.0001 |

Table 8B. Meta-analyses of produce consumption related pathways for acquiring giardiasis in the mixed (n=13) and children (n=10) population – Asian and American region removed from the mixed population

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Mixed | Fixed effects | | | | | |
| (4 stud) | Fruits | -0.194[a] | 0.311 | 2 | [-0.806 – 0.417] | 0.533 |
| | Vegetables | 0.249[a] | 0.194 | 11 | [-0.131 – 0.629] | 0.199 |
| | Before 2000 | | | | | 0.529 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0960 | QE(df=11) | | QM(df=2) | |
| | $s^2$ (residual) | 0.2815 | p=0.004 | | p=0.889 | |
| | Publication bias | -0.0020 | 0.0007 | | | 0.003 |
| | Points removed | 0 | | | | |
| Children | Fixed effects | | | | | |
| (7 stud) | Vegetables | 0.785 | 0.205 | 10 | [0.382 – 1.187] | <.0001 |
| | Before 2000 | | | | | 0.558 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.1436 | QE(df=9) | | | |
| | $s^2$ (residual) | 0.5145 | p=0.026 | | | |
| | Publication bias | -0.0020 | 0.0008 | | | 0.014 |
| | Points removed | 0 | | | | |

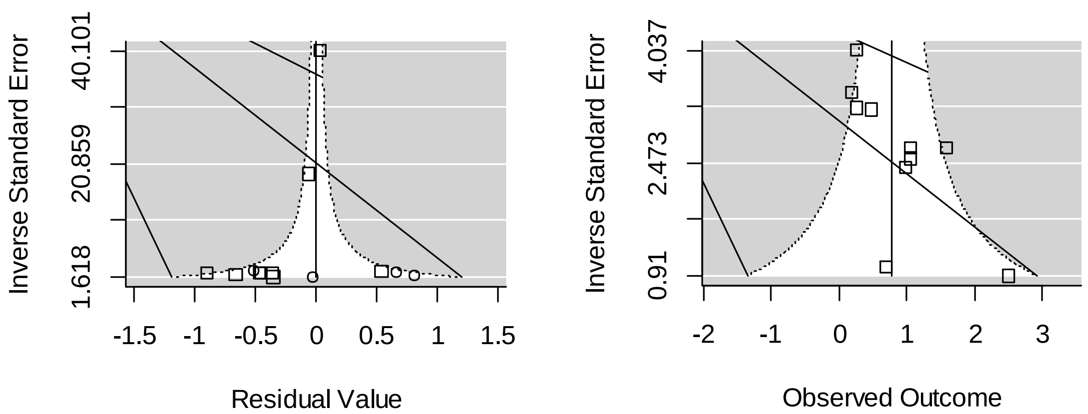



Figure 10. Funnel plots of the meta-analyses of produce consumption pathways for acquiring giardiasis infection in the mixed (left) and children population (right). Circle markers belong to OR measures with potential for bias

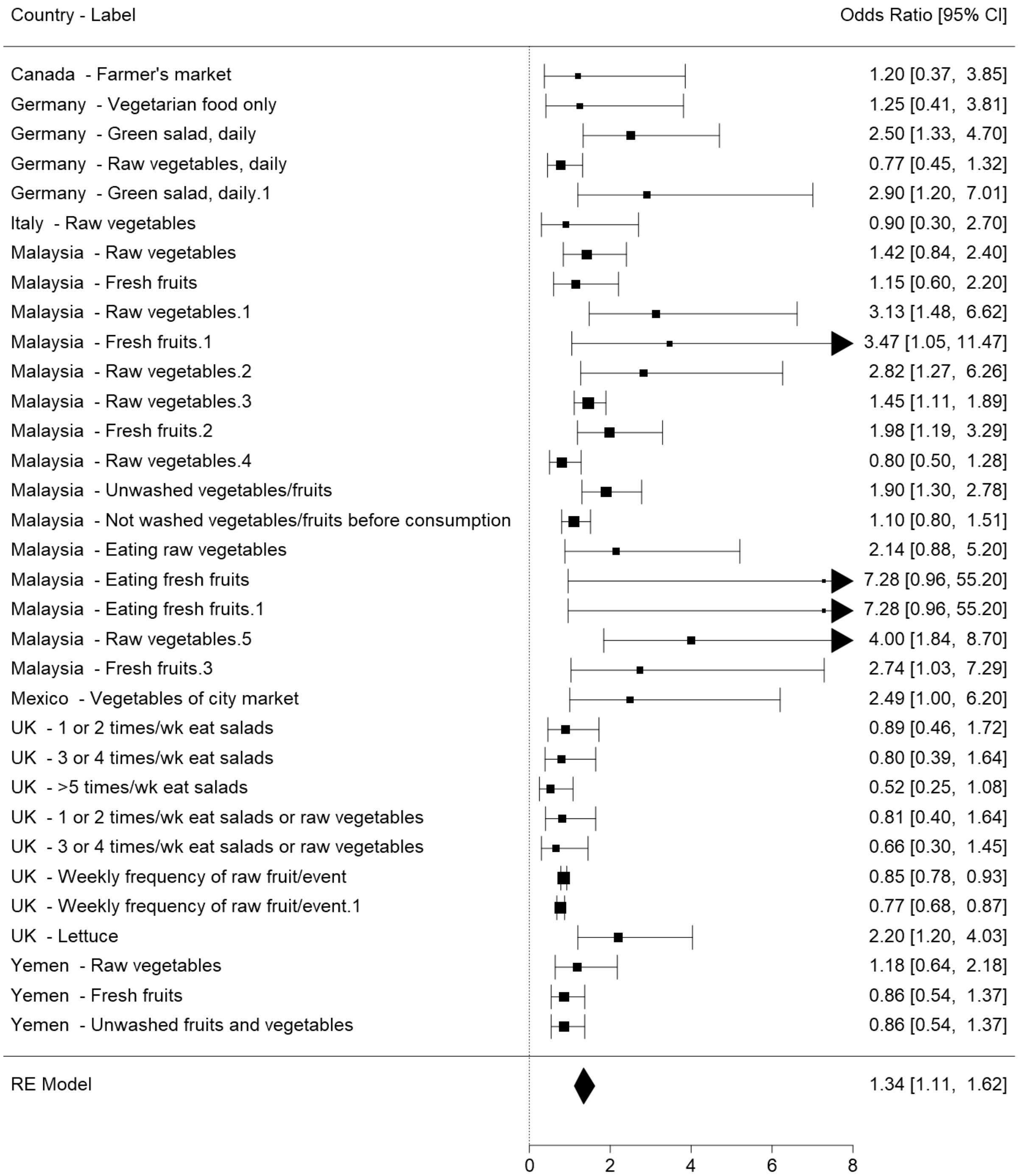



Figure 11. Forest plot of the meta-analysis on the association between giardiasis and consumption of fruits and vegetables in the mixed population from all countries

Composite foods, mostly comprising food prepared outside the home, constituted an important source of giardiasis in the mixed population from North America, from which all data in this subset was extracted (pooled OR=2.389; p<.0001 in Table 9A). The general meta-analysis by composite food class estimated that people with giardiasis were on average 2.210 times (95% CI: 1.508 – 3.238; Table 9B) more likely to have consumed any multi-ingredient dish or fast-food than controls. The level of risk associated to RTE deli foods bore a comparable level of risk (overall OR=2.293; 95% CI: 0.862 – 6.098), although this must be interpreted with caution since it was estimated from only 3 data points (Table 9B). In this meta-analysis, the observations were well dispersed within the funnel plot, therefore clueing the unlikely presence of publication bias (Figure 12), which was also corroborated for the non-significant effect of study size on the OR measures (p=0.295; Table 9B). No potentially-biased OR turned out to be influential in the meta-analysis regression.

Table 9A. Meta-analysis of composite-food related pathways for giardiasis by region for the combined mixed (n=14) and children (n=1) population

| Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|
| Mixed | | | | | |
| North America | 0.871 | 0.179 | 15 | [0.466 – 1.169] | <.0001 |

Table 9B. Meta-analysis of composite-food related pathways for acquiring giardiasis in the combined mixed (n=15) and children (n=1) population – no region was removed

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Both | Fixed effects | | | | | |
| (3 stud) | Dishes&FastFood | 0.793[a] | 0.195 | 13 | [0.411 – 1.175] | <.0001 |
| | RTE deli | 0.830[a] | 0.498 | 3 | [-0.148 – 1.808] | 0.096 |
| | Before 2000 | | | | | 0.937 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.2668 | QE(df=14) | | QM(df=2) | |
| | $s^2$ (residual) | 0.3445 | p=0.309 | | p=0.955 | |
| | Publication bias | 0.0016 | 0.0015 | | | 0.295 |
| | Points removed | 0 | | | | |

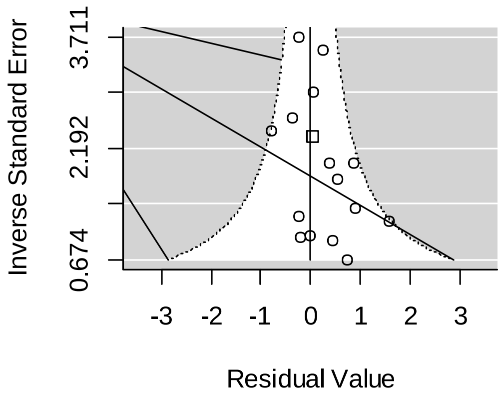



Figure 12. Funnel plot of the meta-analysis of composite food consumption pathways for acquiring giardiasis in the mixed population. Circle markers belong to OR measures with potential for bias

### *3.6.3 Meta-analysis on the risk of giardiasis due to poor handwashing habits*

From the case-control primary studies, many of the exposures investigated poor hygiene habits, in special poor or no handwashing prior to meals and no handwashing after going to toilet. Children who did not wash hands before eating (overall OR=1.573; 95% CI: 1.177 – 2.100) had significantly higher odds of acquiring giardiasis than adults engaging in the same poor habits (overall OR=1.112; 95% CI: 0.838 – 1.477 in Table 10). On meta-analysis, children with giardiasis were more likely to have not washed their hands after going to toilet (overall OR=1.178; 95% CI: 1.030 – 1.347) than control children. Although such level of risk can be thought of being mild, still it is significant (p=0.016; Table 10). This result emphasises that children must be instructed on adopting hygienic habits since very young age. Although the observations within the funnel plots were quite homogeneously spread (Figure 13), the formal assessment of publication bias suggested that probably some OR estimates failing to determine an association between giardiasis and poor handwashing habits before eating remained unpublished (p=0.003; Table 10).

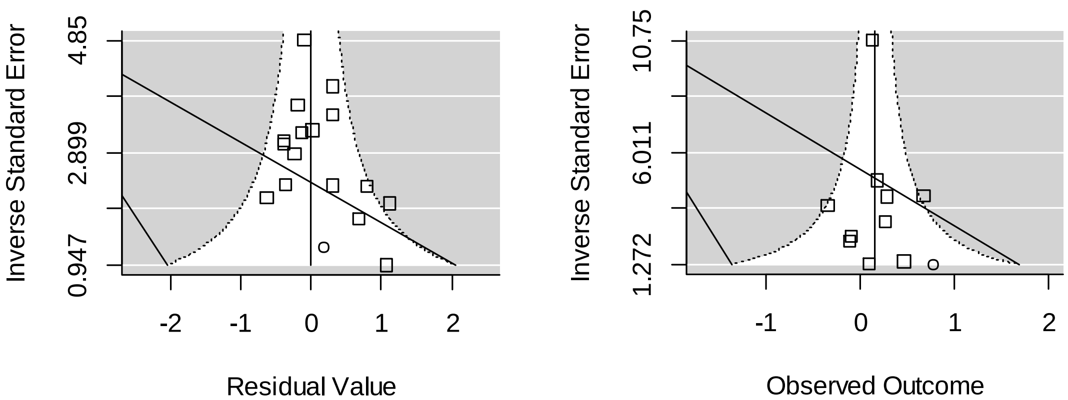



Figure 13. Funnel plots of the meta-analyses of no handwashing before meals (left) and no handwashing after using toilet (right) as risk factors for acquiring giardiasis in the combined mixed and children population. Circle markers belong to OR measures with potential for bias

Table 10. Meta-analyses of no-handwashing before eating and after using toilet as risk factors for the transmission of giardiasis – no geographical region removed

| When? | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Before eating (12 stud) | Fixed effects | | | | | |
| | Children | 0.453[b] | 0.148 | 11 | [0.163 – 0.742] | 0.002 |
| | Mixed | 0.106[a] | 0.145 | 6 | [-0.177 – 0.390] | 0.462 |
| | Before 2000 | | | | | ND |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0115 | QE(df=15) | | QM(df=2) | |
| | $s^2$ (residual) | 0.2757 | p=0.014 | | p=0.007 | |
| | Publication bias | -0.0005 | 0.0002 | | | 0.003 |
| | Points removed | 0 | | | | |
| After toilet (9 stud) | Fixed effects | | | | | |
| | Children | 0.164 | 0.068 | 11 | [0.030 – 0.298] | 0.016 |
| | Before 2000 | | | | | ND |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.0000 | QE(df=10) | | | |
| | $s^2$ (residual) | 0.1130 | p=0.415 | | | |
| | Publication bias | -0.0001 | 0.0001 | | | 0.211 |
| | Points removed | 1 | | | | |

The main results from the meta-analyses on risk factors and pathways of exposure were compiled by means of two forest plots: one forest plot was designed for comparing among the *global* risk categories as potential determinants of giardiasis (Figure 14) and the other forest plot for comparing among the *disaggregated* risk categories and specific pathways of exposure (Figure 15). To visually distinguish the most important routes of transmission, the pooled ORs listed in the forest plots were ranked by mean's decreasing order, and to assess the reliability in the pooled ORs, the number of ORs used in the computations were displayed.

Examining the global categories (Figure 14), the most important risk factors for the transmission of giardiasis in the mixed population were: travel (overall OR=3.16; 95% CI: 2.08 – 4.81), host-specific factors predisposing people to disease (overall OR=3.10; 95% CI: 1.84 – 5.24) and contagion from person to person (overall OR=2.54; 95% CI: 1.52 – 4.25), which bore discernibly higher risk of giardasis than foods as a whole (overall OR=1.57; 95% CI: 1.24 – 1.98) and all animal-related pathways of transmission (overall OR=1.34; 95% CI: 1.12 – 1.61). The global food categories that, on meta-analysis, came up as important vehicles of transmission of giardiasis in the mixed population were: composite foods eaten out (overall OR=2.19; 95% CI: 1.30 – 3.70) and produce (overall OR=1.54; 95% CI: 1.22 – 1.96).

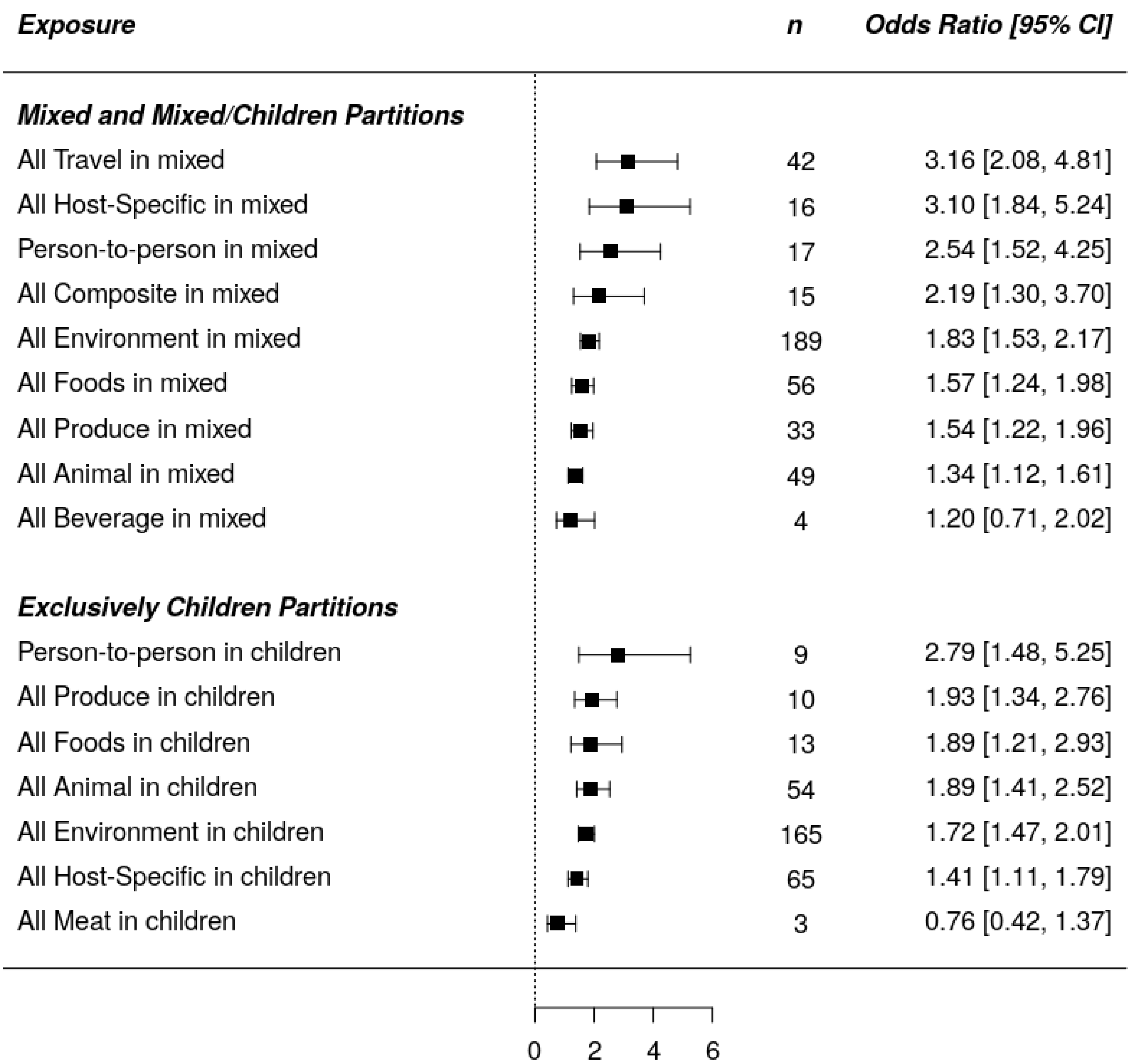



Figure 14. Forest plot summarising overall odds-ratios of acquiring giardiasis for global risk categories, ranked in decreasing order. The global risk categories presented are only those made up of at least n=3 ORs

For the children population (Figure 14), it is important to bear in mind that global risk categories could be ranked only for those factors for which sufficient data were available. In children, person-to-person contagion (overall OR=2.64; 95% CI: 1.79 – 3.90) was the most important determinant of giardiasis while the global food consumption routes (overall OR=1.89; 95% CI: 1.21 – 2.93), animal-related routes (overall OR=1.89; 95% CI: 1.41 – 2.52) and environmental routes (overall OR=1.72; 95% CI: 1.47 – 2.01) bore a comparable level of risk. In children, the host-specific factors (overall OR=1.41; 95% CI: 1.11 – 1.79) were not as predisposing of giardiasis infection as in the mixed population (breastfeeding data was removed for meta-analysing the global host-specific factors in children).

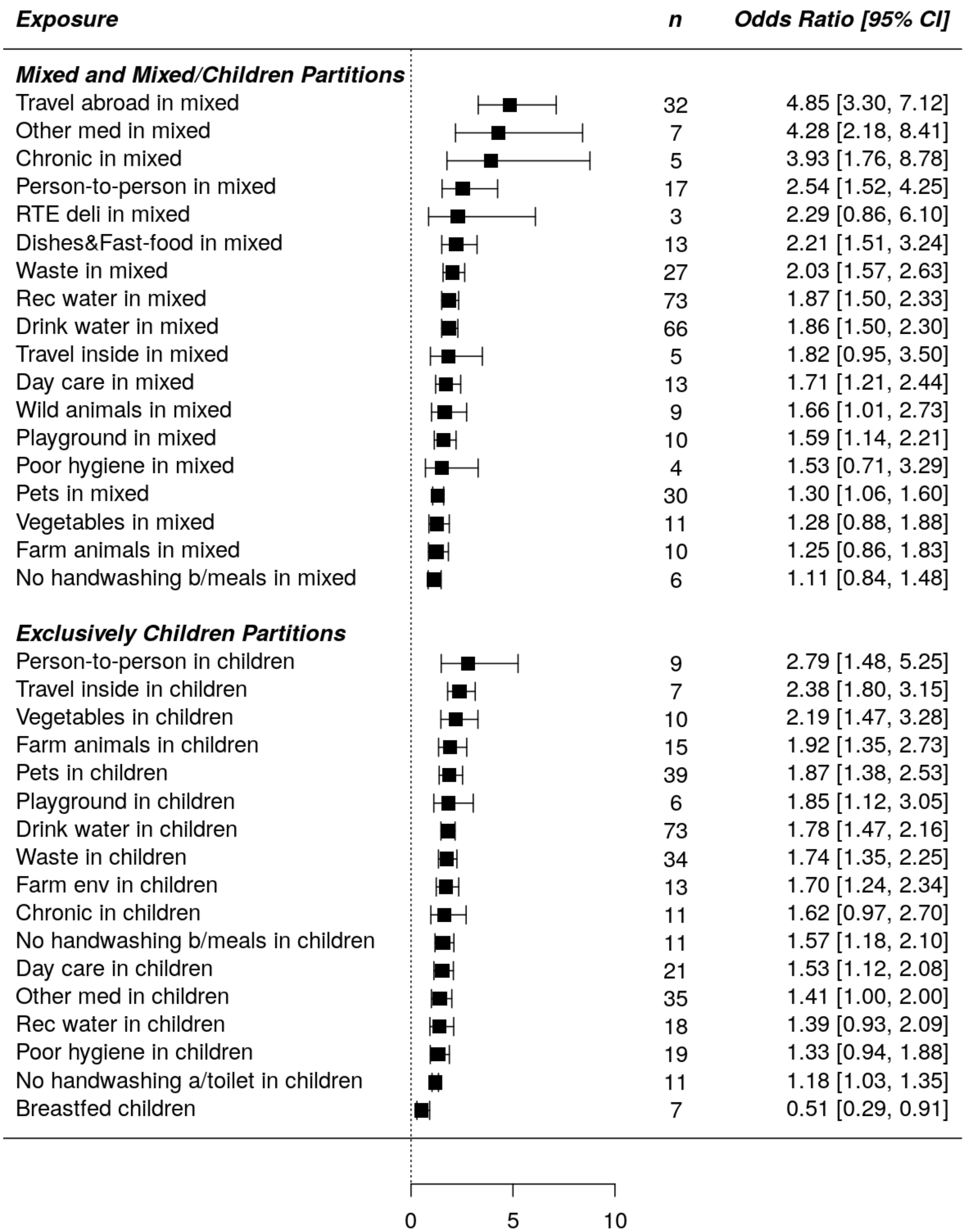



Figure 15. Forest plot summarising overall odds-ratios of acquiring giardiasis for disaggregated risk categories, ranked in decreasing order. The disaggregated risk categories presented are only those made up of at least n=3 ORs

Zooming into the disaggregated risk factors (Figure 15), it became evident that international travel (overall OR=4.85; 95% CI: 3.30 – 7.12), suffering from a gastrointestinal condition (overall OR=4.28; 95% CI: 2.18 – 8.41) and suffering from chronic immunocompromising diseases (overall OR=3.93; 95% CI: 1.76 – 8.78) were the most important determinant factors for acquiring giardiasis in the mixed population. Person-to-person contagion bore a significantly higher association with giardiasis (overall OR=2.54; 95% CI: 1.52 – 4.25) than dishes and fast-food eaten out (overall OR=2.21; 95% CI: 1.51 – 3.24) and environmental routes of transmission such as exposure to human waste/waste water (overall OR=2.03; 95% CI: 1.57 – 2.63), recreational water (overall OR=1.87; 95% CI: 1.50 – 2.33), drink water (overall OR=1.86; 95% CI: 1.50 – 2.30), day care (overall OR=1.71; 95% CI: 1.21 – 2.44) and camping/gardening (overall OR=1.59; 95% CI: 1.14 – 2.21). In the mixed population, the animal related exposures, such as contact with wild animals (overall OR=1.66; 95% CI: 1.01 – 2.73) and contact with pets (overall OR=1.30; 95% CI: 1.06 – 1.60) were not as strong determinants of giardiasis as the environmental routes of exposure to waste water, recreational water and drinking water.

In the children population (Figure 15), among the disaggregated categories for which data were available, the highest associations with giardiasis were posed by person-to-person transmission (overall OR=2.79; 95% CI: 1.48 – 5.25), travel (overall OR=2.38; 95% CI: 1.80 – 3.15) and consumption of fresh/unwashed vegetables (overall OR=2.19; 95% CI: 1.47 – 3.28). Unlike the mixed population, in children, the animal-related routes of transmission of farm animals (overall OR=1.92; 95% CI: 1.35 – 2.73) and pets (overall OR=1.87; 95% CI: 1.38 – 2.53) were at least as important vehicles of giardiasis as the environmental routes of playground (overall OR=1.85; 95% CI: 1.12 – 3.05), drink water (overall OR=1.78; 95% CI: 1.47 – 2.16) and exposure to human waste (overall OR=1.74; 95% CI: 1.35 – 2.25). Other significant pathways of transmission of giardiasis in children were, in decreasing order: living on a farm (overall OR=1.70; 95% CI: 1.24 – 2.34), suffering from chronic disease (overall OR=1.62; 95% CI: 0.97 – 2.70), no handwashing before eating (overall OR=1.57; 95% CI: 1.18 – 2.10), attending day care or school (overall OR=1.53; 95% CI: 1.12 – 2.08), other medical conditions (overall OR=1.41; 95% CI: 1.00 – 2.00), recreational water (overall OR=1.39; 95% CI: 0.93 – 2.09), poor hygienic habits (overall OR=1.33; 95% CI: 0.94 – 1.88) and no handwashing after using toilet (overall OR=1.18; 95% CI: 1.03 – 1.35).

Routes of exposure that on meta-analysis had minor association with giardiasis in the mixed population were: poor hygienic habits (overall OR=1.53; 95% CI: 0.71 – 3.29), fresh/unwashed vegetables (overall OR=1.28; 95% CI: 0.88 – 1.88), contact with farm animals (overall OR=1.25; 95% CI: 0.86 – 1.83) and no handwashing before eating (overall OR=1.11; 95% CI: 0.84 – 1.40). Breastfeeding protected children against acquiring giardiasis since, on meta-analysis, children who were breastfed presented approximately half the odds (95% CI: 0.29 – 0.91) of becoming infected than those who were not.

#### *3.6.4 Meta-analysis on the effect of handling on the risk of transmission of giardiasis by consumption of vegetables*

The only data partition comprising sufficient data that could support the assessment of the effect of handling was that of vegetables. Full estimates and funnel plot of the meta-analysis on the effect of not washing vegetables are shown in the supplementary Table S1 and Figure S1, respectively. There was no data partition with sufficient information on setting; thus, the effect of eating food prepared outside home on the overall association with disease could not be appraised. On meta-analysis, people who admitted having eaten unwashed vegetables had 1.404 (95% CI: 0.954 – 2.065; Table 11) greater probability of acquiring giardiasis infection than those who stated having consumed *simply* fresh vegetables (without having being specific as to whether it was washed or unwashed). Said otherwise, people who ate fresh vegetables and became ill were 1.404 times (95% CI: 0.954 – 2.065) more likely to have consumed them unwashed than those who consumed fresh vegetables but did not get ill. Hence, not washing fresh vegetables before consumption represents a significant risk of giardiasis on its own (p=0.086; Table S1)

Table 11. Meta-analysed effect of not washing vegetables prior to consumption on base OR for giardiasis

| Food class | Estimate | Mean | 2.5$^{th}$ percentile | 97.5$^{th}$ percentile |
|---|---|---|---|---|
| Vegetables | OR(Base) | 1.542 | 1.176 | 2.024 |
| | OR(Unwashed)/OR(Base) | 1.404 | 0.954 | 2.065 |

### *3.6.5 Synthesis of the risks associated to ways of preparation and poor handling of foods*

The forest plot of Figure 16 compiles exposures related to food preparation, which were examined as potential risk factors for sporadic giardiasis in the case-control and cohort studies. Washing food with bleached water, vinegar or soap and water could exert some protective effect against giardiasis, while washing only with water – particularly in world's regions where water is not treated – might increase the risk of transmission of giardiasis.

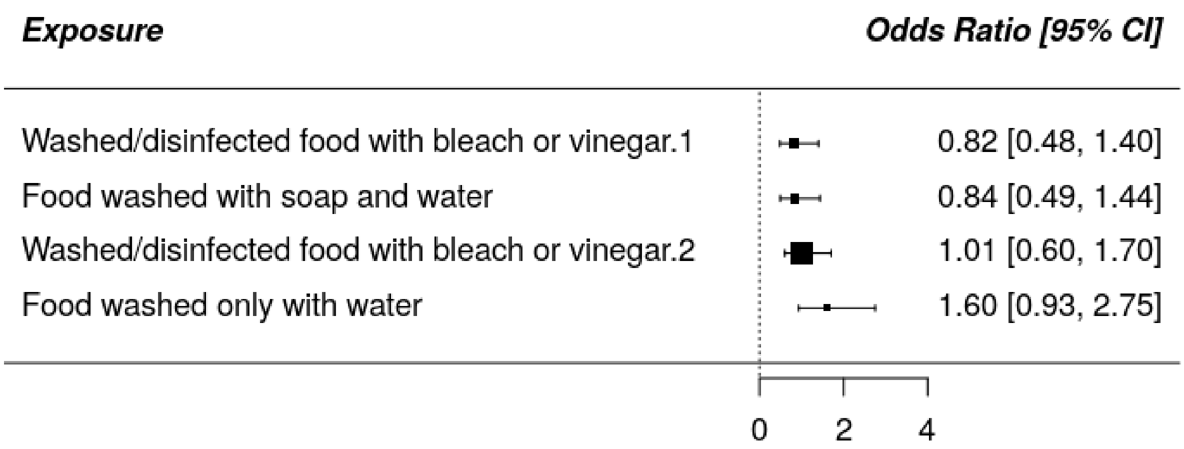



Figure 16. Forest plot of the odds-ratios of acquiring giardiasis associated to food preparation practices in the children population

Most of the food handling poor habits explored in the primary studies related to hand hygiene (Figure 17). Odds-ratios for not washing hands before eating ranged between 0.84 and 4.60 in the children population while they were lower, between 0.98 and 1.50, in the mixed population. Poor hand hygiene prior to food preparation or when feeding children were risky practices affecting children, with ORs between 1.89 and 4.78. Eating with hands was found to have a variable association with giardiasis in the mixed population, with ORs in the range of 0.76 to 1.51 (Figure 17). Likewise, improper food storage and washing food with untreated water could not be proven to be determinant of giardiasis in the children population.

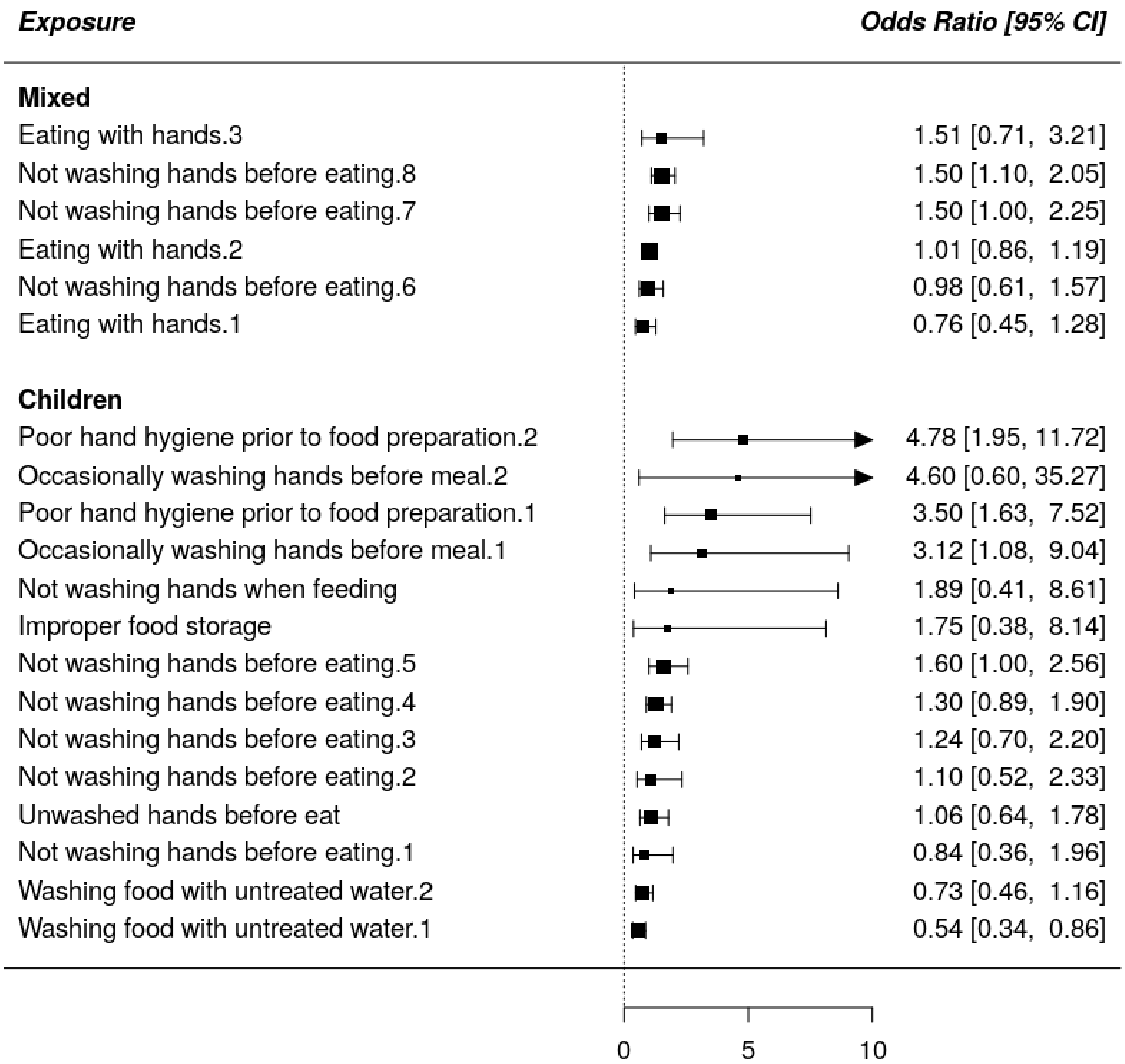



Figure 17. Forest plot of the odds-ratios of acquiring giardiasis associated to poor food handling practices in the mixed and children population

# 4. Conclusions

### 4.6 *Meta-analysis on risk factors for sporadic giardiasis*

- The outcomes from 72 case-control and cohort studies investigating routes of exposure for sporadic giardiasis were meta-analysed, taking into account geographical region and period of study.

- No data was available for food subcategories other than produce and composite food, so the significance of these sources as potential vehicles of transmission of giardiasis could not be appraised.

- The top three determinants of giardiasis in the mixed population are: international travel (OR=4.85) and the host-specific factors of suffering from gastrointestinal disease (OR=4.28) and chronic immunocompromising disease (OR=3.93).

- Person-to-person transmission has a higher association with giardiasis (OR=2.54) than all of the environmental routes assessed, namely, exposure to human waste or lack of waste water system (OR=2.03), recreational water (OR=1.87), drink water (OR=1.86), day care (1.71) and camping/gardening (OR=1.59).

- Consumption of dishes eaten out and fast-food (OR=2.21) can be as strong a risk factor for giardiasis as person-to-person transmission.

- In the mixed population, the animal related exposures, such as contact with wild animals (OR=1.66) and contact with pets (overall OR=1.30) are not as strong determinants of giardiasis as the environmental routes.

- In the children population, the highest associations with giardiasis are posed by person-to-person transmission (OR=2.79), travel (OR=2.38) and consumption of fresh/unwashed vegetables (OR=2.19).

- Unlike the mixed population, in children, the animal-related exposures of contact with farm animals (OR=1.92) and pets (OR=1.87) are at least as important vehicles of *Giardia duodenalis* as the environmental routes of playground (OR=1.85), drink water (OR=1.78) and exposure to human waste (OR=1.74).

- Other important pathways of transmission of giardiasis in children are: living on a farm (OR=1.70), chronic disease (OR=1.62), no handwashing before eating (OR=1.57), attending day care or school (OR=1.53), other medical conditions (OR=1.41) and recreational water (OR=1.39).

- Routes of exposure that have minor (not statistically proven) association with giardiasis in the mixed population are: poor hygiene habits (OR=1.53), consuming fresh or unwashed vegetables (OR=1.28), contact with farm animals (OR=1.25) and not washing hands before eating (OR=1.11).

- Routes of exposure that have minor association with giardiasis in children are poor hygiene habits (OR=1.33) and not washing hands after using toilet (OR=1.18).

- Infants who are breastfed present half the probability of acquiring giardiasis than those who are not.

- People who ate fresh vegetables and became ill were 1.40 times more likely to have consumed them unwashed than those who consumed fresh vegetables but did not get ill.

# 5. References

### 5.6 *Seventy-two case-control and cohort studies assessing risk factors for giardiasis*

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## 6 Supplementary tables and figures of the effects of handling on the transmission of giardiasis

Table S1. Meta-analysis of the effect of setting on the risk of giardiasis by consumption of vegetables in the combined mixed (n=23) and children population (n=10)

| Pop | Parameters | Mean | Standard error | n | 95% CI | Pr > \|t\|, Z |
|---|---|---|---|---|---|---|
| Both | Handl: Base | 0.433 | 0.138 | 24 | [0.162 – 0.705] | 0.002 |
| (18 stud) | Handl: Unwashed | 0.339 | 0.197 | 9 | [-0.047 – 0.725] | 0.086 |
| | Random effects | | | | | |
| | $s^2_u$ (Intercept) | 0.1544 | QE(df=31) | | | |
| | $s^2$ (residual) | 0.4135 | p<.0001 | | | |
| | Publication bias | 0.0000 | 0.0001 | | | 0.655 |
| | Points removed | 0 | | | | |

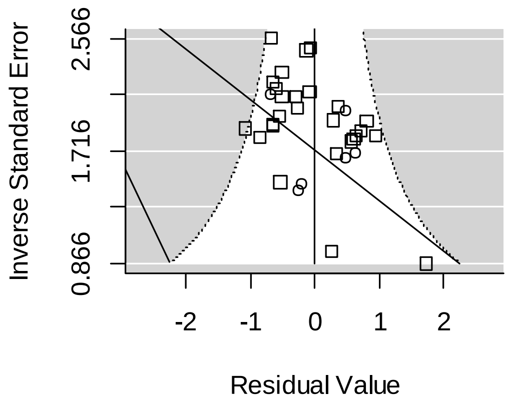


Figure S1. Funnel plot of the meta-analysis of the effect of handling on the risk of giardiasis by consumption of vegetables. Circle markers belong to OR measures with potential for bias