Susceptibility of lymnaeid snails to *Fasciola hepatica* and *Fasciola gigantica* (Digenea: Fasciolidae): a systematic review and meta-analysis

Philile Ignecious Ngcamphalala[1], Ignore Nyagura[1], Mokgadi Pulane Malatji[1] and Samson Mukaratirwa[1,2]

[1] School of Life Sciences, University of KwaZulu-Natal, Durban, Durban, KwaZulu-Natal, South Africa
[2] One Health Centre for Zoonoses and Tropical Veterinary Medicine, Ross University School of Veterinary Medicine, Besseterre, Saint Kitts and Nevis

ABSTRACT

Background: Fasciolosis is a food-borne disease that causes major economic losses, globally. This zoonotic disease is caused by *Fasciola hepatica* and *Fasciola gigantica* species which employ freshwater snails from the family Lymnaeidae as their intermediate hosts. Thus, a key aspect of understanding the epidemiology of the disease lies in understanding the transmission ecology of the parasite. Therefore, this systematic review and meta-analysis were conducted to assess the experimental susceptibility and prevalence of natural infections of *F. hepatica* and *F. gigantica* in lymnaeid snails.

Methods: Relevant peer-reviewed articles published in the past 20 years (2004–2023) were searched and appraised. Prevalence and infection rate estimates were based on 41 studies that met the inclusion criteria.

Results: Five thousand five hundred and seventy-five (5,575) lymnaeid snails were subjected to experimental infections and 44,002 were screened for natural infections. The overall pooled infection rate was higher in experimental infections 50% (95% CI [42–58%]) compared to natural infections of field-collected snails 6% (95% CI [0–22%]). The highest pooled infection rate was recorded in South America at 64% (95% CI [48–78%]) for experimental infections while the lowest was recorded for natural infections at 2% (95% CI [0–6%]) in Europe and 2% (95% CI [0–17%]) in Asia. In experimental studies, *F. gigantica* recorded the highest pooled prevalence at 73% (95% CI [61–84%] compared to *F. hepatica* which recorded 47% (95% CI [38–56%]). For natural infections, however, *F. hepatica* had the highest prevalence (12% (95% CI [0–30%]) while the lowest was noted for naturally infected *F. gigantica* at 2% (95% CI [0–18%]). Based on the snail species, the highest pooled prevalence was recorded for *Pseudosuccinea columella* infected with *F. hepatica* and *F. gigantica* at 47% (95% CI [33–61%]) while the lowest was recorded for *F. hepatica* naturally infected *Galba truncatula* at 4% (95% CI [0–10%]). Natural *Fasciola* spp. infections in intermediate snail hosts decreased in prevalence while experimental infections have increased in prevalence over the past 20 years.

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Corresponding author
Philile Ignecious Ngcamphalala, 212510872@stu.ukzn.ac.za

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Conclusions: While there seems to be a strong intermediate host specificity between the two *Fasciola* spp., experimental infection results showed that *G. truncatula* and *R. natalensis* are susceptible to *F. hepatica* and *F. gigantica*, respectively.

Subjects Parasitology, Global Health, Freshwater Biology
Keywords *Fasciola hepatica*, *Fasciola gigantica*, Intermediate hosts, Lymnaeids, Experimental infections, Natural infections, Prevalence

INTRODUCTION

Fasciolosis is a zoonotic food-borne disease of livestock and wild ruminants, and humans caused by the digenean liver flukes *Fasciola hepatica* Linnaeus, 1758 and *Fasciola gigantica* Cobbold, 1855 (*Kithuka et al., 2002*; *Chen et al., 2013*; *Vázquez et al., 2019a*; *Ahmad et al., 2022*). This parasitic disease causes economic losses in the livestock industry (*Gutierrez et al., 2001*) resulting from reduced productivity, liver condemnation, mortality, and expenditures for anthelmintics (*Kithuka et al., 2002*; *Kleiman et al., 2007*; *Mucheka et al., 2015*). These global economic losses have been estimated to exceed two billion dollars annually (*Spithill, Smooker & Copeman, 1999*; *Medeiros et al., 2014*; *Lalor et al., 2021*).

Fasciolosis has been recorded in more than 70 countries, across all inhabited continents (*Lalor et al., 2021*). *Fasciola hepatica* has been reported in temperate regions of Europe, Asia, Africa, Australia, and Americas (*Mas-Coma, 2004*; *Lalor et al., 2021*; *Ahmad et al., 2022*). Conversely, *F. gigantica* is mostly distributed in tropical and subtropical regions of Africa, Asia (*Mas-Coma, 2004*; *Shoriki et al., 2014*; *Mucheka et al., 2015*; *Lalor et al., 2021*; *Ahmad et al., 2022*), and the Middle East (*Lalor et al., 2021*). Furthermore, the overlapping geographical distribution of both species has been reported in many parts of Africa (*Aleixo et al., 2015*; *Ahmad et al., 2022*; *Nukeri et al., 2022*) and Central Asia where hybrid forms of the parasite have been recorded (*Shoriki et al., 2014*; *Aleixo et al., 2015*).

According to *McKown & Ridley (1995)*, the presence of a compatible snail intermediate host (IH) in fasciolosis endemic areas is crucial to complete the life cycle of the parasite. The transmission of *Fasciola* spp. in a specific geographical region mostly depends on the presence of vector snails from the family Lymnaeidae Rafinesque, 1815 (*Alba et al., 2019*). As *Vinarski (2013)* reported, this family consists of two subfamilies, Radicinae and Lymnaeinae, with about 26 genera collectively. Species from the genera *Galba* (*Cruz-Mendoza et al., 2004*; *Gutierrez et al., 2011*; *Alemu, 2019*), *Lymnaea* (*Kim et al., 2014*; *Alemu, 2019*), *Pseudosuccinea* (*Cucher et al., 2006*; *Alemu, 2019*; *Ngcamphalala, Malatji & Mukaratirwa, 2022*), *Forassia* (*Cruz-Mendoza et al., 2004*; *Alemu, 2019*), *Radix* (*Bargues et al., 2011*; *Imani-Baran et al., 2012*; *Huang et al., 2019*), *Austropeplea* (*Dung et al., 2013*; *Kim et al., 2014*), and *Omphiscola* (*Correa et al., 2017*; *Rondelaud, Vignoles & Dreyfuss, 2022*) act as the IHs for *Fasciola* species. Although approximately 1,200 lymnaeid snail species have been described globally (*Vázquez et al., 2019b*), only 30 species are known as IHs of *Fasciola* spp. (*Alba et al., 2019*; *Vázquez et al., 2019a*). These species are distributed worldwide (*Prepelitchi et al., 2011*; *Vinarski, 2013*) and have been reported to extend from tropical to temperate regions with some occurring at extremely cold latitudes (*Vázquez et al., 2019b*).

Like most trematodes, *Fasciola* spp. show a marked snail host specificity (*Bargues & Mas-Coma, 2005*; *Bargues et al., 2011*). *Fasciola gigantica* is mainly transmitted by snail species from the genus *Radix*, particularly those belonging to *Hubendick*'s *(1951)* superspecies of *Radix auricularia* (Linnaeus, 1758), which includes *R. natalensis* (Krauss, 1848) in Africa, *R. rubiginosa* (Michelin, 1831) in Asia (*Brown, 1994*) and *R. auricularia* in Europe (*Mas-Coma, Bargues & Valero, 2005*). Recently, *Pseudosuccinea columella* has been shown to transmit this species in Africa (*Grabner et al., 2014*, *Carolus et al., 2019*; *Malatji & Mukaratirwa, 2020*). *Fasciola hepatica* is, however, transmitted by snails from various genera, but *Galba* species are the main IHs of this species globally (*Bargues & Mas-Coma, 2005*). According to *Vázquez et al. (2019a)*, research on the compatibility of snail and parasite populations may serve to understand better and predict the transmission of fasciolosis globally. Additionally, a key aspect of understanding the epidemiology of a disease lies in understanding the transmission ecology of the parasite (*Vázquez et al., 2015*). Furthermore, understanding the susceptibility of the IHs to *Fasciola* spp. in a given locality may assist with the development of strategic control programs. Therefore, this study reviewed and analysed the results of peer-reviewed research reporting on the global experimental susceptibility/infectivity and natural infections of *F. hepatica* and *F. gigantica* in lymnaeid snail species in the past 20 years (2004–2023).

METHODS

Search strategy

A systematic literature search was conducted on PubMed, Web of Science, and Google Scholar databases, and a combination of the following search terms and Boolean operators (OR, AND) were used: *Fasciola hepatica* OR *Fasciola gigantica* AND intermediate hosts OR lymnaeids OR Lymnaeidae OR *Pseudosuccinea OR Galba OR Fossaria OR Lymnaea* OR *Omphiscola* OR *Austropeplea* OR *Radix* AND natural infection OR experimental infection OR prevalence OR infectivity. Peer-reviewed articles published in the last 20 years (2004–2023) were retrieved and appraised. Additional articles were identified by cross-referencing selected articles' biographies (snowballing). EndNote reference manager version X8 (Clarivate Analytics, Philadelphia, PA, USA) was used to retrieve and manage full-text articles.

Inclusion and exclusion criteria

The following inclusion criteria were used to select articles for the systematic review and meta-analysis: (i) clearly stated the number of lymnaeid snails screened and/or infected with *F. hepatica* and *F. gigantica*, (ii) identified and reported the *Fasciola* species up to species level, (iii) identified the intermediate host snails up to species level, (iv) reported prevalence based on natural infections or infection rate based on experimental infections, and (v) indicated the detection method used.

Studies were excluded if they reported only on *Fasciola* spp. infection in definitive hosts, identification and distribution of the intermediate hosts without *Fasciola* infections, and articles written in other languages besides English.

Data extraction

Based on the study design, PIN and IN screened the titles and abstracts of articles, and relevant articles were retrieved. Full texts of retrieved articles were thoroughly reviewed and those that did not meet the inclusion criteria were excluded. Microsoft (MS) Excel spreadsheet was used to capture data from the text, tables, and figures for meta-analysis. Data extracted from relevant articles included the author's names, year of publication, continent, country where the study was conducted, *Fasciola* species, snail host, sample size, number of infected host snails, prevalence, and detection method. In cases of differences regarding the inclusion and exclusion criteria, the researchers discussed and reached a favorable conclusion, however, the final decision was made by Dr. MP Malatji.

Quality assessment

The overall quality of the articles for meta-analysis was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (*Guyatt et al., 2008*; *Doi & Thalib, 2008*). The quality of all studies included was assessed by scoring one point for each inclusion criterion that was fulfilled and a 0 was given for each unfulfilled criterion. As a result, each selected study was assigned a score ranging from 0 to 5. Studies with an index score of five were deemed high quality, four as moderate, and those that scored ≤3 were considered low quality and excluded from the analysis (*Doi & Thalib, 2008*). The total quality scores for all included studies ranged from moderate to good quality (Tables S1, S2).

Data analysis

The double arcsine approach was used to transform the prevalence data to avoid overestimating the weight of individual studies (*Barendregt et al., 2013*). This approach utilizes the arcsine transformation twice to the prevalence values to account for the heterogeneity caused by studies with extreme proportions or smaller sample sizes (*Barendregt et al., 2013*). MetaXL add-in for Microsoft Excel (www.epigear.com) was employed to compute a quality effects model to account for the heterogeneity (*Barendregt et al., 2013*). The level of heterogeneity between estimates was evaluated using inverse variance statistic (I^2 index), and the differences were accounted for using Cochrane's Q test (*Barendregt, 2016*). The I^2 index score was interpreted as low heterogeneity if it was <25%, moderate at 50%, and high heterogeneity at >75% (*Higgins et al., 2003*). The estimated prevalence and the 95% confidence interval (CI) of *Fasciola* species infections in lymnaeid snails were demonstrated on forest plots. Subgroup analysis was done to assess heterogeneity and factors that could influence the observed pooled prevalence estimates (PPE); thus, the data was grouped according to the region/continent on which studies were conducted, snail species involved, *Fasciola* species, method of detection, and period covered by the studies and publication bias was evaluated using funnel plots. To identify the sources of heterogeneity, meta-regression was performed with continents, diagnostic tests, and *Fasciola* species and study period fixed as independent factors. The meta-regression was treated as a linear model on the logit-transformed prevalence data. Using Egger's test, the linear regression analysis was conducted to evaluate publication bias.

RESULTS

Search results

Of the 861 articles obtained after going through the search databases and snowballing, 774 articles were excluded because they were either duplicates or deemed ineligible based on the title and/or abstract contents (Fig. 1). Full-texts of 46 studies were retrieved and assessed for eligibility based on the predetermined inclusion criteria and five articles were deemed ineligible. The remaining 41 studies met the inclusion criteria and quality assessment. These were distributed across Europe (13/41; 31.7%), Africa (10/41; 24.4%), South America (9/41; 21.9%), Asia (8/41; 19.5%), and North America (1/41; 2.43%). Of these studies, 46.3% (19/41) reported experimental infection while 53.7% (22/41) reported natural infections.

Overall experimental susceptibility of lymnaeid snails to *Fasciola* spp.

Experimental infections of *Fasciola* species in lymnaeid snails were conducted in Europe (France and Sweden), and in Africa (Egypt). In Asia, experiments were conducted in Iran while in South America experiments were conducted in Argentina, Colombia, and Cuba (Table S1). A total of 5,575 freshwater lymnaeid snails were collected to be subjected to experimental infections with *Fasciola* spp. between 2004 and 2023. Of these freshwater snails, 2,815 (50.45%) were infected with either *F. hepatica* or *F. gigantica*. The lymnaeid snail species involved in the experiments were *Galba (G.) truncatula, G. cubensis, G. cousini, G. viatrix var ventricose, G. neotropica, Pseudosuccinea (P.) columella, Radix (R.) auricularia, R. natalensis, Lymnaea (L.) fuscus, L. palustris*, and *Omphiscola (O.) glabra*. Experimental infection rates ranged from 7.89% in *L. fuscus* infected with *F. hepatica* to 80.37% in *G. cubensis* infected with *F. gigantica* (Table 1). Of the 11 snail species mentioned above, nine were successfully infected with *F. hepatica* and four with *F. gigantica*. Only *P. columella* and *G. truncatula* had records of successful experimental infections with both *Fasciola* species (Table 1). The overall pooled experimental infection rate of lymnaeid snails with *Fasciola* spp. was 50% (95% CI [42–58%] (Fig. S1). High heterogeneity in the results was revealed by the quality effects model (Q = 1,425, $p < 0.001$), with $I^2 = 96\%$ (Fig. S1). The meta-regression model demonstrated a statistically significant overall p-value of 0.05 for experimental investigations, suggesting that the predictors collectively account for the variation in prevalence (Table 2). The R-squared change was 0.119, indicating that 11.9% of the variance in the prevalence data could be explained by the predictors. However, the prevalence level was not significantly impacted by individual factors (Table 2).

Experimental infectivity of lymnaeid snails by Fasciola spp. by continent

The highest pooled infection rate for *Fasciola* spp. (Figs. 2A–2C) was 64% recorded in South America (95% CI [48–78%], Fig. 2C), followed by Africa at 48% (95% CI [35–61%], Fig. 2B) and Europe at 42% (95% CI [28–56%], Fig. 2A). Asia did not qualify for the meta-analysis. Heterogeneity was recorded at $I^2 = 96\%$ for all three reported continents.

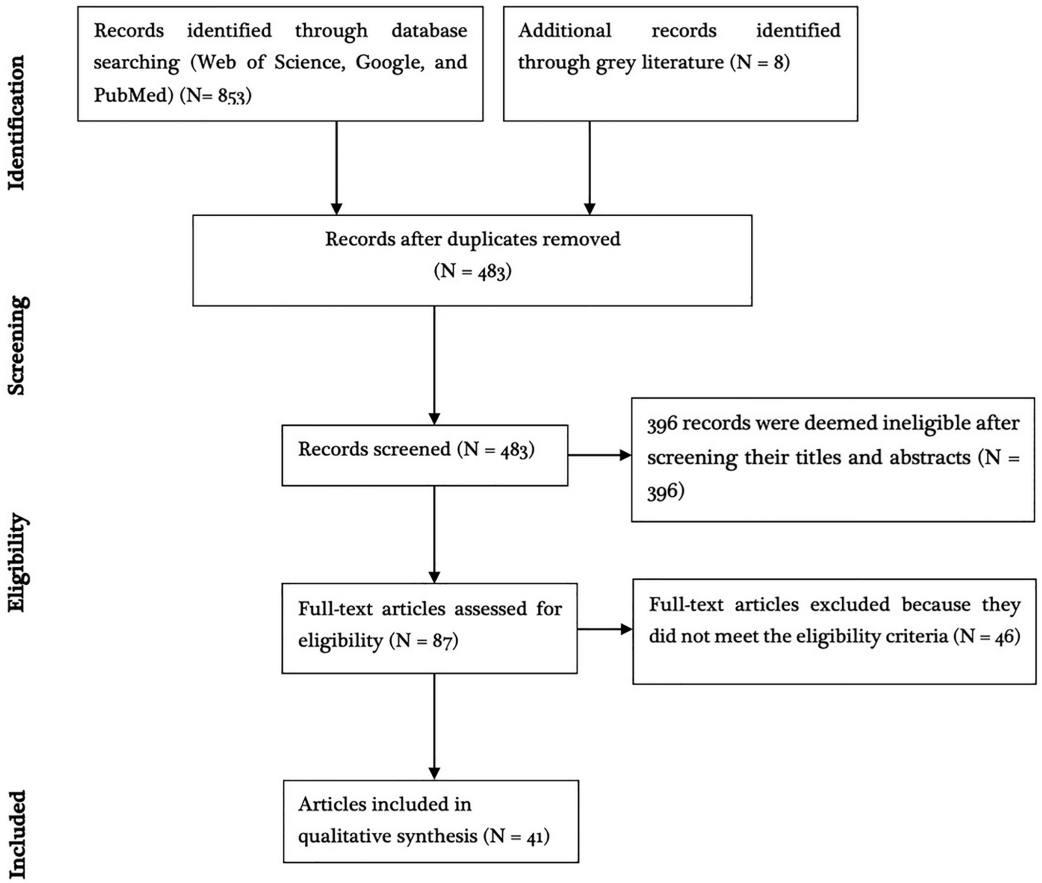

Figure 1 **PRISMA diagram.** Full-size DOI: 10.7717/peerj.18976/fig-1

Table 1 **Frequency of lymnaeid snails experimentally infected with *F. gigantica* and *F. hepatica* in the past 20 years.**

Snail species	No. of studies	No. infected	No. examined	Diagnostic tool (%)		Species of infection		Overall prevalence (%)
				Shedding	Dissection	*Fasciola hepatica*	*Fasciola gigantica*	
Galba (G.) truncatula	10	1,196	2,155	72.03	53.47	1,155	41	55.49
G. cubensis	1	217	270	–	80.37	–	217	80.37
G. cousini	1	34	100	–	34.00	34	–	34.00
G. neotropica	1	50	159	–	31.45	50	–	31.45
Radix (R.) auricularia	1	115	151	76.16	–	–	115	76.16
R. natalensis	2	83	308	–	26.95	83	–	26.95
Pseudosuccinea (P.) columella	8	945	1,963	40.71	52.02	848	97	48.14
Lymnaea (L.) fuscus	1	9	114	–	7.89	9	–	7.89
L. palustris	1	40	119		33.61	40	–	33.61
L. viatrix var. ventricosa	1	73	133	–	54.89	73	–	54.89
Omphiscola (O.) glabra	1	53	103	–	51.46	53	–	51.46
Total	–	**2,815**	**5,575**	**52.74**	**49.81**	**2,386**	**429**	**50.45**

Table 2 Meta-regression of overall and subgroups for individual variables on prevalence of *Fasciola* infections in snail intermediate host in the past 20 years.

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	R^2	95.0% Confidence interval for B	
		B	Std. Error	Beta				Lower bound	Upper bound
Natural infection	Continents	0.124	0.364	0.069	0.341	0.736		−0.622	0.870
	Fasciola sp.	1.616	0.964	0.362	1.676	0.105		−0.362	3.595
	Diagnostic test	−0.411	0.617	−0.116	−0.666	0.511		−1.677	0.855
	period	−1.883	0.639	−0.458	−2.947	0.007		−3.195	−0.572
	Combined effect					0.001	0.474		
Experimental infection	Continents	0.167	0.136	0.234	1.227	0.226		−0.107	0.441
	Fasciola sp.	−0.533	0.476	−0.182	−1.120	0.269		−1.493	0.427
	Diagnostic test	−0.033	0.344	−0.015	−0.097	0.923		−0.727	0.661
	period	0.672	0.332	−0.391	2.021	0.050		0.002	1.343
	Combined effect					0.05	0.194		

Experimental infection rate of lymnaeid snails by Fasciola spp. per snail species

Only three of the 11 recorded lymnaeid snail species qualified for meta-analysis, viz *G. truncatula*, *R. natalensis*, and *P. columella*. The estimated pooled infection rate of lymnaeid snails infected with *F. hepatica and F. gigantica* is illustrated in Figs. 3A–3C. *Galba truncatula* infected with *F. hepatica* (96.57%, 1,155/1,196) and *F. gigantica* (3.43%, 41/1,196) (Table 1) showed a pooled infection rate of 37% (95% CI [17–59%], Fig. 3A), *R. natalensis* with *F. hepatica* with a prevalence of 21% (95% CI [03–48%], Fig. 3C) and *P. columella* with both *F. hepatica* (43.20%, 848/1,963) and *F. gigantica* (4.64%, 91/1,963) (Table 1) with a pooled infection rate of 47% (95% CI [33–61%], Fig. 3B). *Galba truncatula* and *P. columella* showed a heterogeneity of I^2 = 97% while *R. natalensis* demonstrated a heterogeneity of I^2 = 95%.

Experimental infection rate of lymnaeid snails by parasite species

The pooled infection rate of *Fasciola* spp. to lymnaeid snails was high with *F. gigantica* at 73% (95% CI [61–84%] and low in *F. hepatica* at 47% (95% CI [38–55%]. However, heterogeneity was higher for *F. hepatica* (Q = 1,234.09, $p < 0.001$; I^2 = 96%, Fig. 4A) compared to *F. gigantica* (Q = 31.73, $p < 0.001$; I^2 = 87%, Fig. 4B).

Experimental infectivity of lymnaeid snails by Fasciola spp. per method of detection

The pooled experimental infection rate of *Fasciola* snails in lymnaeid snails was higher using cercariae shedding 52% (95% CI [32–72%], Fig. 5A, Table 1) compared to 49% (95% CI [40–59%], Fig. 5B, Table 1) using snail dissection. Heterogeneity was documented as I^2 = 97% and I^2 = 96% for shedding and dissection, respectively.

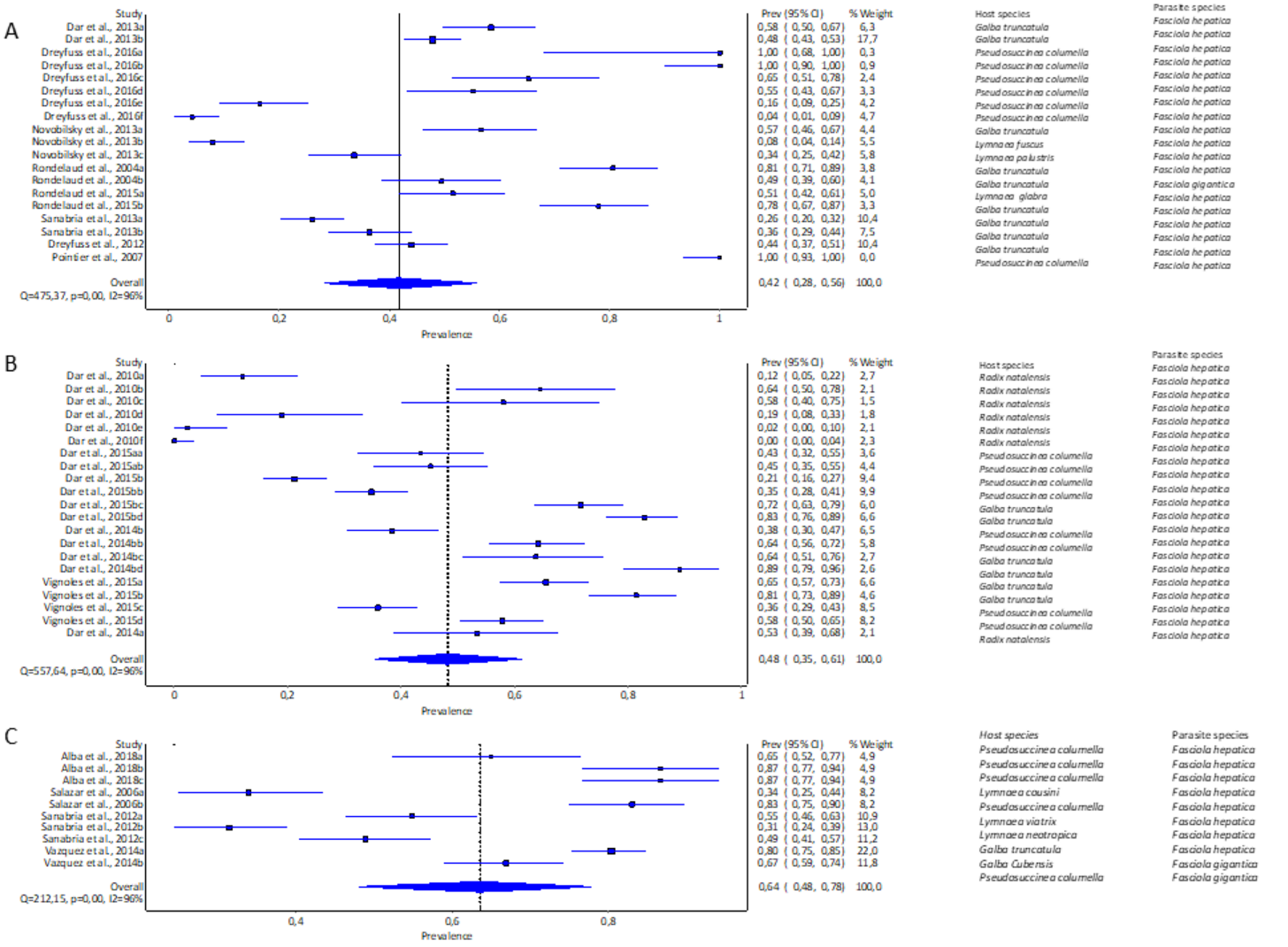

Figure 2 Forest plots of experimental infection rates of *Fasciola hepatica* and *Fasciola gigantica* in lymnaeid snails from (A) Europe, (B) Africa, and (C) South America (*Alba et al., 2018*; *Dar et al., 2010*, *2013*, *2014b*, *2015b*; *Dreyfuss, Vignoles & Rondelaud, 2012*, *2016*; *Novobilský et al., 2013*; *Pointier et al., 2007*; *Rondelaud et al., 2004*, *2015*; *Salazar, Estrada & Velásquez, 2006*; *Sanabria et al., 2012*, *2013*; *Vázquez et al., 2014*; *Vignoles, Dreyfuss & Rondelaud, 2015*). Different letters next to references denote different data generated from the same publication.

Full-size DOI: 10.7717/peerj.18976/fig-2

Experimental infection rate of lymnaeid snails by Fasciola spp. based on years

The estimated pooled infection rate of *Fasciola* spp. experimentally infected snails for 20 years is shown in Fig. S2. The pooled infection rate in the decade 2004–2013 was 41% (95% CI [29–53%], Fig. S2A), which was lower than the 57% (95% CI [47–68%], Fig. S2B) pooled infection rate documented between 2014–2023. Heterogeneity was I^2 = 96% for both periods (Figs. S2A–S2B).

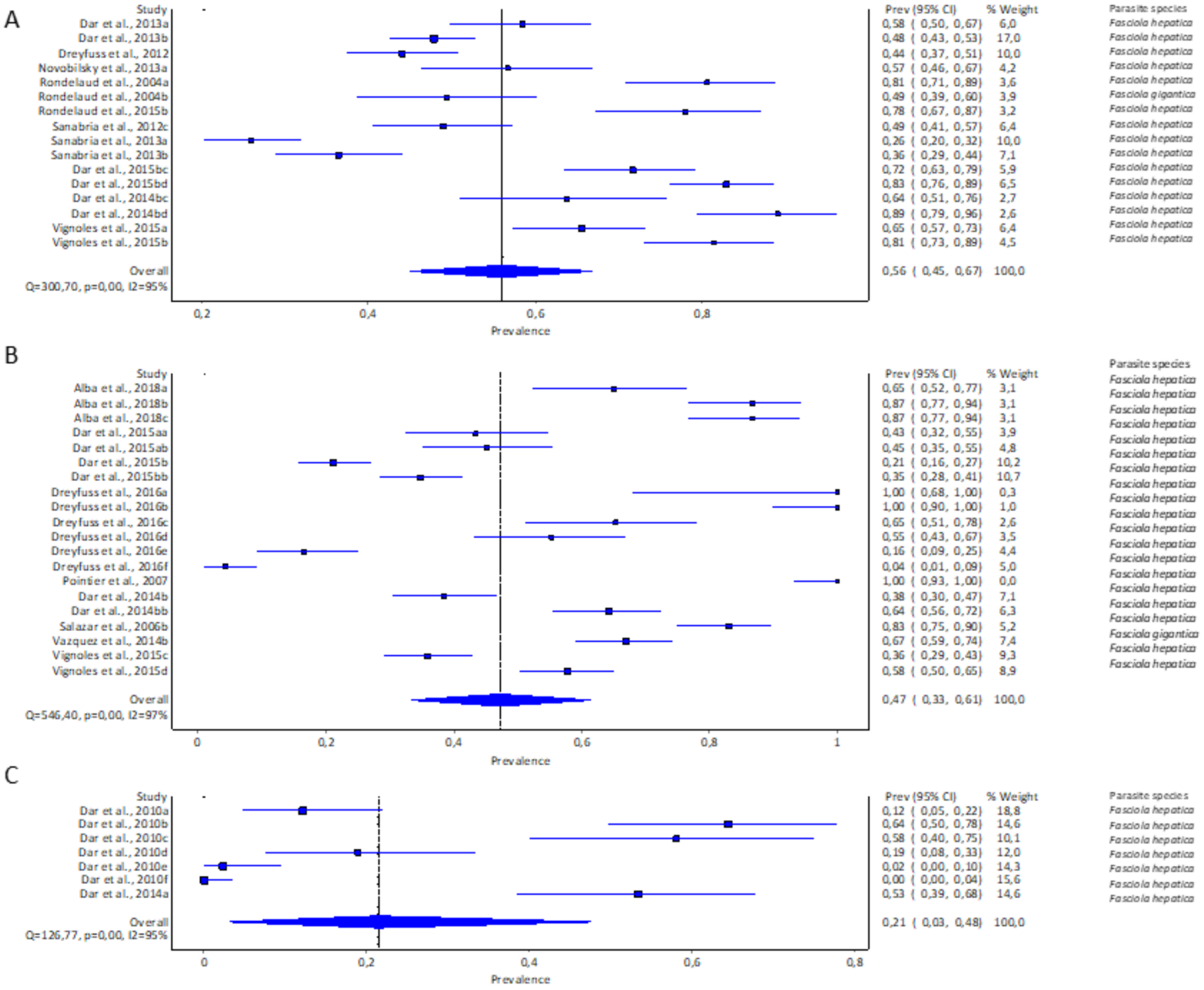

Figure 3 Forest plots of experimental infection rates of *Fasciola* species based on the intermediate hosts: (A) *Galba truncatula*, (B) *Pseudosuccinea columella*, and (C) *Radix natalensis* (*Alba et al., 2018*; *Dar et al., 2010*, *2013*, *2014b*, *2015b*; *Dreyfuss, Vignoles & Rondelaud, 2012*, *2016*; *Novobilský et al., 2013*; *Pointier et al., 2007*; *Rondelaud et al., 2004*, *2015*; *Sanabria et al., 2012*, *2013*; *Salazar, Estrada & Velásquez, 2006*; *Vázquez et al., 2014*; *Vignoles, Dreyfuss & Rondelaud 2015*). Different letters next to references denote different data generated from the same publication. Full-size DOI: 10.7717/peerj.18976/fig-3

Publication bias of studies reporting on experimental infections of Fasciola spp. in lymnaeid snails

Figure S3 shows the funnel plot which is asymmetrical in shape and depicts publication bias which may result from either a small sample size or publication bias within articles.

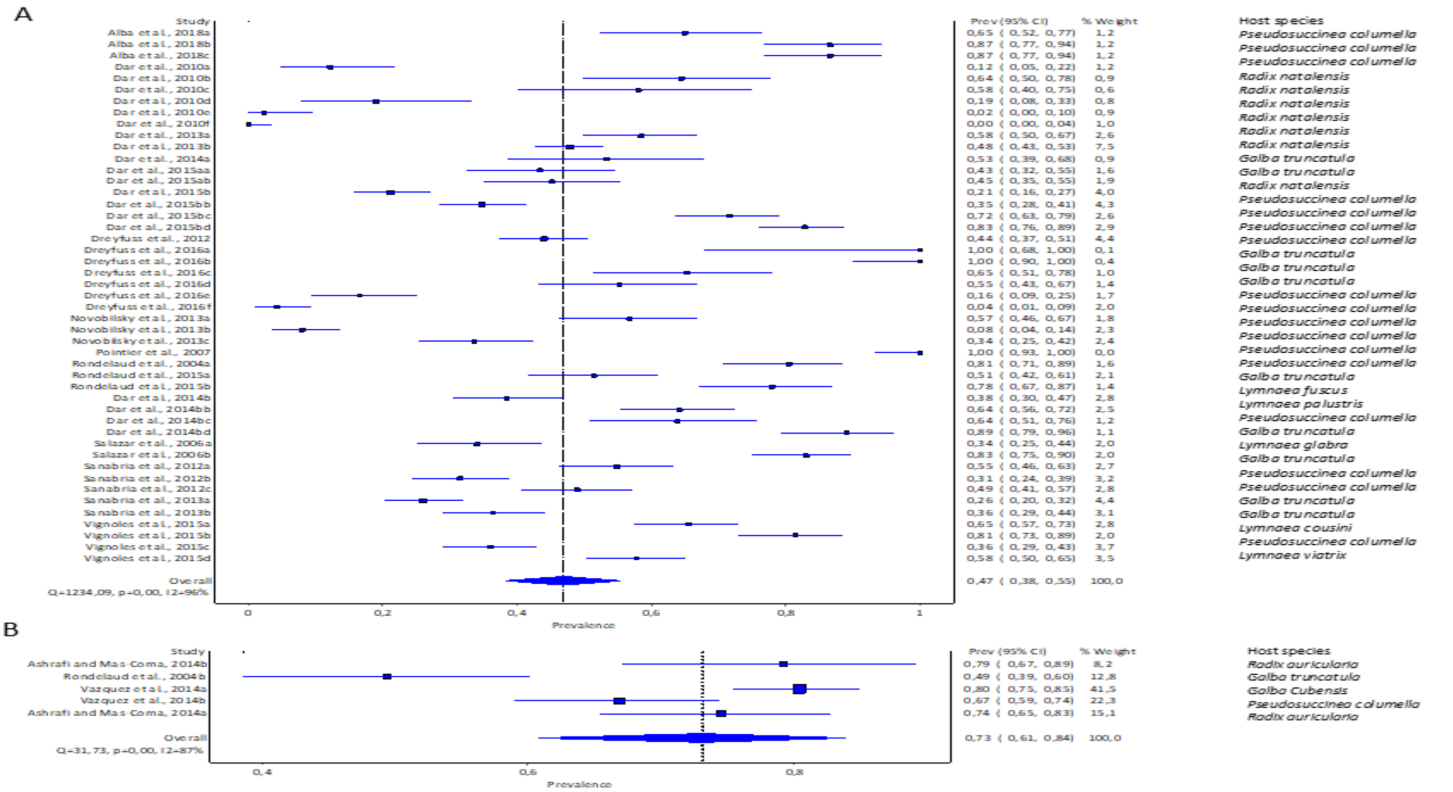

Figure 4 **Forest plots showing experimental infection rates in lymnaeid snails based on *Fasciola* species: (A) *Fasciola hepatica* and (B) *Fasciola gigantica* (*Alba et al., 2018*; *Ashrafi & Mas-Coma, 2014*; *Dar et al., 2010*, *2013*, *2014b*, *2015b*; *Dreyfuss, Vignoles & Rondelaud, 2012*, *2016*; *Novobilský et al., 2013*; *Pointier et al., 2007*; *Rondelaud et al., 2015*, *2004*; *Salazar, Estrada & Velásquez, 2006*; *Sanabria et al., 2012*, *2013*; *Vázquez et al., 2014*; *Vignoles, Dreyfuss & Rondelaud 2015*).** Different letters next to references denote different data generated from the same publication.
Full-size DOI: 10.7717/peerj.18976/fig-4

Natural infections of lymnaeid snails by *Fasciola* spp.

Field prevalence data for lymnaeid snails infected by *Fasciola* spp. was recorded in South America (Colombia, Cuba, Ecuador, and Argentina), North America (Mexico), Africa (Egypt and South Africa), Europe (Ireland, Sweden, Spain, Poland, and France) and Asian (Iran, India, South Korea, China, and Vietnam) (Table S2). Of the 44,002 field-collected lymnaeid snails, 5,656 were positive for *Fasciola* infections with an overall prevalence of 12.85%. Prevalence of lymnaeid snails naturally infected with *F. hepatica* and *F. gigantica* ranged from 0–76.9% in *L. fuscus* and *G. bulimoides*, respectively (Table 3). The 17 infected snail species were *G. truncatula, G. cousini, G. viatrix, G. humilis, G. schirazensis, G. bulimoides, P. columella, R. cucunorica, R. gedrosiana, R. peregra, R. auricularia, R. acuminata, L. palustris, L. ollula, L. fuscus, O. glabra, and Austropeplea (A.) viridis* (Table 3). Only *P. columella* and *G. truncatula*, however, qualified for meta-analysis. The estimated overall pooled prevalence for natural infections in lymnaeid snails was recorded at 6% (95% CI [0–22%]) (Fig. S4). A significantly high heterogeneity was recorded Q = 15,220.37 ($p < 0.001$), with $I^2 = 100\%$ (Fig. S4). The R-squared change was 0.474, and the *p*-value was significant at 0.001 (Table 2). This suggests that the predictors have a greater impact and that the model accounts for 47.4% of the variation in prevalence.

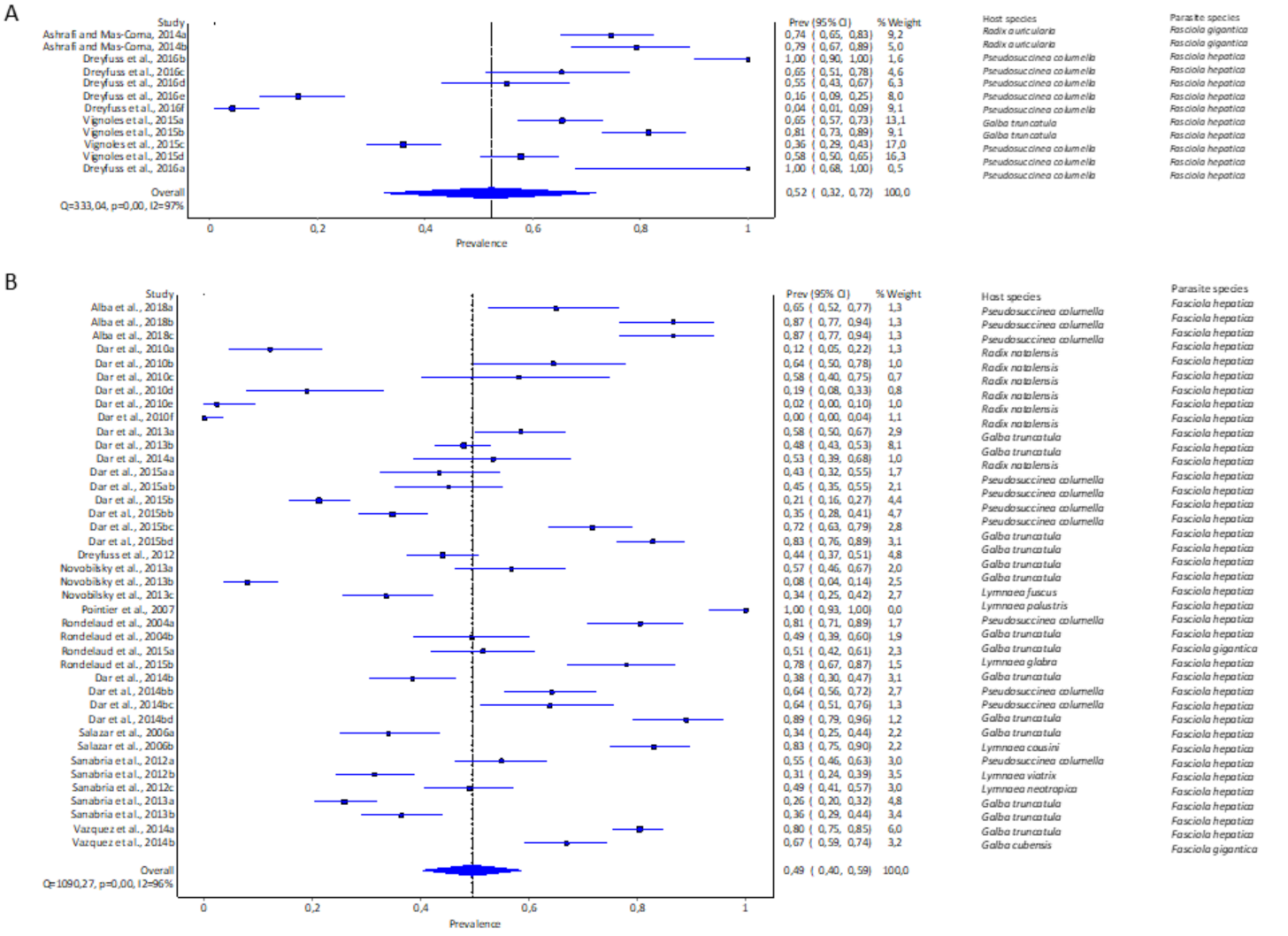

Figure 5 **Forest plots of experimental infection rates of *Fasciola hepatica* and *Fasciola gigantica* based on detection technique (A) shedding and (B) dissection (*Alba et al., 2018*; *Ashrafi & Mas-Coma, 2014*; *Dreyfuss, Vignoles & Rondelaud, 2016*, *2012*; *Dar et al., 2010*, *2013*, *2014b*, *2015b*; *Novobilský et al., 2013*; *Pointier et al., 2007*; *Rondelaud et al., 2004*, *2015*; *Salazar, Estrada & Velásquez, 2006*; *Sanabria et al., 2012*, *2013*; *Vázquez et al., 2014*; *Vignoles, Dreyfuss & Rondelaud 2015*).** Different letters next to references denote different data generated from the same publication. Full-size DOI: 10.7717/peerj.18976/fig-5

Regarding each of the specific factors, the *p*-values for the continents, diagnostic tests, and *Fasciola* species were all greater than 0.05 for natural snail infection by *Fasciola*, indicating no significant effects. Nonetheless, the *p*-value for the time frame was 0.007, suggesting that time had a significant effect on the prevalence of *Fasciola* infection (Table 2).

Prevalence of natural infections of lymnaeid snails by Fasciola spp. per continent

The estimated pooled prevalence estimates for lymnaeid snails naturally infected by *Fasciola* spp. globally is illustrated in Figs. 6A–6C. The prevalence recorded per continent was 2% in Asia (95% CI [0–17], Fig. 6A), 2% in Europe (95% CI [0–6%], Fig. 6B), and 11% in South America (95% CI [0–29%], Fig. 6C). Africa and North America data did not

qualify for meta-analysis. Heterogeneity results were I^2 = 98%, I^2 = 99%, and I^2 = 100% for South America, Europe, and Asia, respectively (Figs. 6A–6C).

Prevalence of natural infections of lymnaeid snails by Fasciola spp. per snail species

Pseudosuccinea columella and *G. truncatula* were the only snail species that qualified for meta-analysis. The average prevalence of natural infections of *P. columella* infected with *F. hepatica* was 17.21% (168/976) and *F. gigantica* was 11.27% (110/976) (Table 3), with an overall pooled prevalence of 26% (95% CI [0–72%], Fig. 7A). *Galba truncatula* infected by *F. hepatica* recorded a pooled prevalence of 4% (95% CI [0–10%], Fig. 7B). Heterogeneity results were documented as I^2 = 99% for both lymnaeid species.

Prevalence of natural infections of lymnaeid snails by Fasciola spp. per parasite species

The estimated pooled infection rates of the individual *Fasciola* spp. naturally infecting lymnaeid snails were reported in Figs. 8A–8B. *Fasciola gigantica* showed a prevalence of 2% (95% CI [0–18%], Fig. 8A) and Fig. 8B shows a prevalence of 12% (95% CI [0–30%] for *F. hepatica* natural infections. The heterogeneity was significantly high for both *F. gigantica* (Q = 1,873.85, $p < 0.001$, I^2 = 100%) and *F. hepatica* (Q = 11,616.37, $p < 0.001$, I^2 = 100%) natural infections (Figs. 8A–8B).

Prevalence of natural infections of lymnaeid snails by Fasciola spp. per method of detection

Detection of natural *Fasciola* spp. infections in lymnaeid snails was based on molecular (PCR), restriction fragment length polymorphism (RFLP), dissection, and cercariae shedding techniques (Table 3, Table S2). Prevalence based on the diagnostic tool utilized raged from 20.72% by dissection to 2.45% by RFLP (Table 3). Molecular and dissection techniques were the only techniques that qualified for meta-analysis (Figs. 9A–9B). Low pooled prevalence was recorded with molecular technique (4%, 95% CI [0–17%] (Fig. 9A) compared to dissection technique at 12% (95% CI [0–40%] (Fig. 9B). Recorded heterogeneity was I^2 = 100% for dissection and I^2 = 99% for molecular technique (Fig. 9B).

Prevalence of natural infections of lymnaeid snails by Fasciola spp. by years

Pooled prevalence for natural *Fasciola* spp. infections in their IHs hosts was 9% (95% CI [0–70%], Fig. S5A) in the decade 2004–2013, which was higher than 3% (95% CI [0–9%] (Fig. S5B) in 2014–2023 (Figs. S5A–S5B). Heterogeneity was I^2 = 100% for 2004–2013 (Fig. S5B) and I^2 = 99% for 2014–2023 (Fig. S5B).

Publication bias of studies reporting on the natural infections of Fasciola spp. in lymnaeid snail spp

Funnel plots showed an asymmetric funnel shape (scattered points) (Fig. S6) indicating the presence of publication bias which may be due to content in the articles or small sample size.

Table 3 Frequency of lymnaeid snails naturally infected with *F. gigantica* and *F. hepatica* in the past 20 years.

Snail species	No. of studies	No. examined	No. infected	Diagnostic tool (%)				Species of infection		Overall prevalence (%)
				Dissection	Molecular	Shedding	RFLP	*Fasciola hepatica*	*Fasciola gigantica*	
Galba (G.) truncatula	7	8,617	408	3.25	12.87	–	–	408	–	4.73
G. bulimoides	1	670	515	76.87	–	–	–	515	–	76.87
G schirazensis	2	1,517	96	–	6.33	–	–	96	–	6.33
G. humilis	1	3,372	2,537	75.24	–	–	–	2,537	–	75.24
G. viatrix	1	68	22	–	61.76	2.94	–	22	–	32.35
G. cousini	1	521	68	13.05	–	–	–	68	–	13.05
Radix (R.) acuminata	2	2,477	250					–	250	10.09
R. auricularia	1	496	12	–	–	–	2.45	–	12	2.45
R. peregra	1	167	62	–	37.13	–	–	62	–	37.13
R. gedrosiana	1	2,543	298	–	11.72	–		–	298	11.72
R. cucunorica	1	409	179	–	43.77	–		179	–	43.77
Pseudosuccinea (P.) columella	4	976	278	–	32.51	17.25	–	168	110	28.48
Lymnaea (L.) fuscus	1	130	0	–	0	–	–	–	–	0
R. ollula	1	15	5	–	33.33	–	–	5	–	33.33
L. palustris	1	668	1	–	0.15	–	–	1	–	0.15
Omphiscola glabra	2	5,980	102	1.26	21.54	–	–	102	–	1.71
Austropeplea (A.) viridis	2	15,376	125	–	0.81	–	–	1	124	0.81
Total	**–**	**44,002**	**5,656**	**20.72**	**5.81**	**15.69**	**2.45**	**4,576**	**1,080**	**12.85**

DISCUSSION

The results showed that the overall pooled prevalence of natural infections of *Fasciola* species in lymnaeids was significantly lower than the infection rate recorded based on experimental infections. This was to be expected as conditions for experimental infections are made optimum and controlled in laboratory settings compared to natural environments where various uncontrolled variables and stressors may hinder successful infection of the intermediate host. Infections under laboratory settings have been shown to be a valuable approach in the investigation of compatibility differences in IHs since they allow for the control and management of variables that can influence the infection outcome (*Sorensen & Minchella, 2001*; *Vázquez et al., 2019a*). These variables include snail shell size (*Dar et al., 2014a*; *Dreyfuss, Vignoles & Rondelaud, 2016*), the infective parasite dose (*Sorensen & Minchella, 2001*; *Pointier et al., 2007*), optimum conditions (optimum temperature, constant light/dark periods, abundant food, pollution free water, dissolved essential minerals) (*de Kock, van Eeden & Pretorius, 1986*; *Dar et al., 2014a*, *2015a*; *Dreyfuss, Vignoles & Rondelaud, 2016*), deliberate exposure to miracidia (*Dreyfuss, Vignoles & Rondelaud, 2016*; *Ashrafi & Mas-Coma, 2014*) amongst others. This

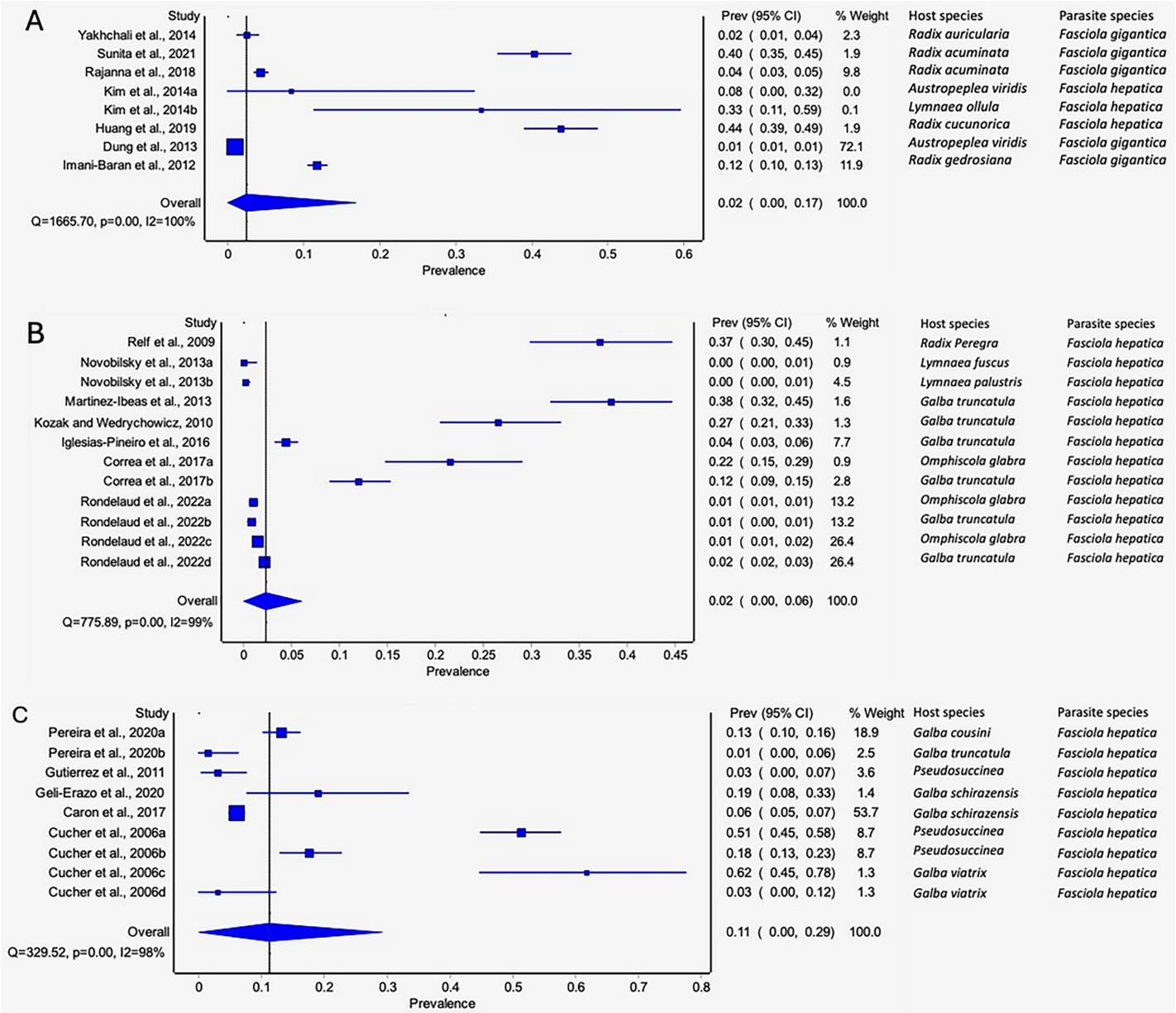

Figure 6 Forest plots of the infection rates of natural infections of *Fasciola* species in lymnaeid snails based on continents; (A) Asia, (B) Europe, and (C) South America (*Caron et al., 2017*; *Correa et al., 2017*; *Cucher et al., 2006*; *Dung et al., 2013*; *Geli-Erazo et al., 2020*; *Gutierrez et al., 2011*; *Huang et al., 2019*; *Iglesias-Piñeiro et al., 2016*; *Imani-Baran et al., 2012*; *Kim et al., 2014*; *Kozak & Wędrychowicz, 2010*; *Martínez-Ibeas et al., 2013*; *Novobilský et al., 2013*; *Pereira, Uribe & Pointier, 2020*; *Rajanna et al., 2018*; *Relf et al., 2009*; *Rondelaud, Vignoles & Dreyfuss, 2022*; *Sunita et al., 2021*; *Yakhchali, Malekzadeh-Viayeh & Imani-Baran, 2014*). Different letters next to references denote different data generated from the same publication. Full-size DOI: 10.7717/peerj.18976/fig-6

control of variables leads to a higher infection rate as the probability of one snail getting infected strictly depends on its suitability as IH and its surrounding ecology (*Vázquez et al., 2015*). *Prepelitchi et al. (2011)* also noted that snail populations are subjected to rigorous ecological constraints due to large environmental temporal fluctuations. Additionally, parasites may die before finding an appropriate IH in a natural environment,

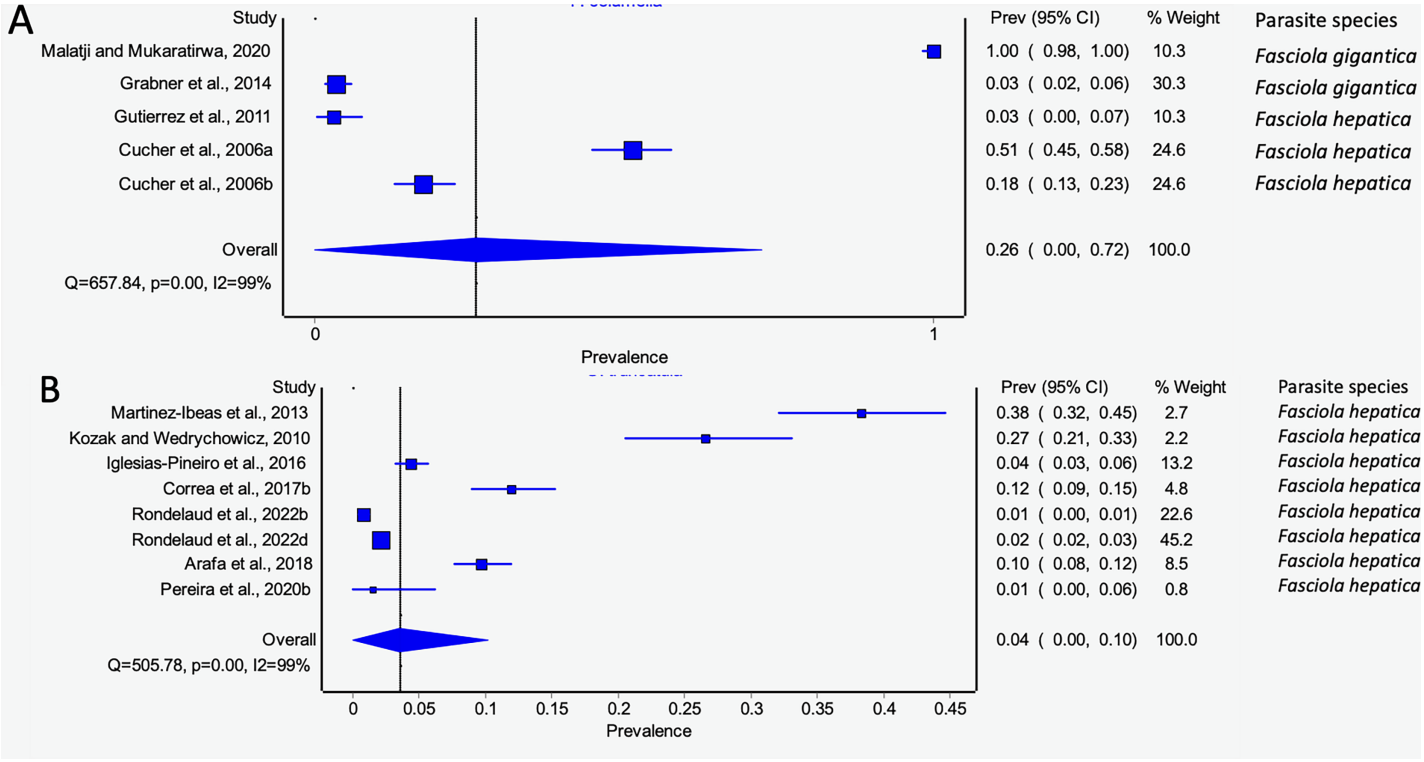

Figure 7 Forest plots of rates of natural infections of *Fasciola* species based on intermediate snail host (A) *Pseudosuccinea columella* and (B) *Galba truncatula* (*Malatji & Mukaratirwa, 2020*; *Grabner et al., 2014*; *Gutierrez et al., 2011*; *Cucher et al., 2006*; *Martínez-Ibeas et al., 2013*; *Kozak & Wędrychowicz, 2010*; *Iglesias-Piñeiro et al., 2016*; *Correa et al., 2017*; *Rondelaud, Vignoles & Dreyfuss, 2022*; *Arafa et al., 2018*; *Pereira, Uribe & Pointier, 2020*). Different letters next to references denote different data generated from the same publication.

Full-size DOI: 10.7717/peerj.18976/fig-7

especially those with a narrow tolerance to specific physicochemical factors (*Vázquez et al., 2015*).

Overall, while the model for natural infection demonstrates stronger explanatory power (R-square = 0.474), the mixed results point to the complexity of factors influencing *Fasciola* infection prevalence in snails, which requires further investigation with more refined models or additional data. *Nyagura et al. (2024)* claimed that when several factors are combined, the variability becomes much more prevalent, suggesting that understanding how these factors interact is essential to comprehending the complexity of epidemiological results. Instead of a single determinant, the results of this study corroborate the idea that the epidemiology of snail-borne parasites is typically driven by a confluence of factors that interact significantly (*Hajipour et al., 2021*).

The wide global distribution of lymnaeid snails is of great concern as the geographic distribution of these *Fasciola* spp. depends on the availability and ecological needs of their respective intermediate host species (*Malatji, Lamb & Mukaratirwa, 2019*). As expected, this review showed a range of lymnaeid snails that were implicated in the transmission of *Fasciola* species in the field and in experimental setting in five of the six inhabited continents. These results are consistent with previous reports as this snail family has been reported in all continents except Antarctica (*Vázquez et al., 2019b*). For both experimental

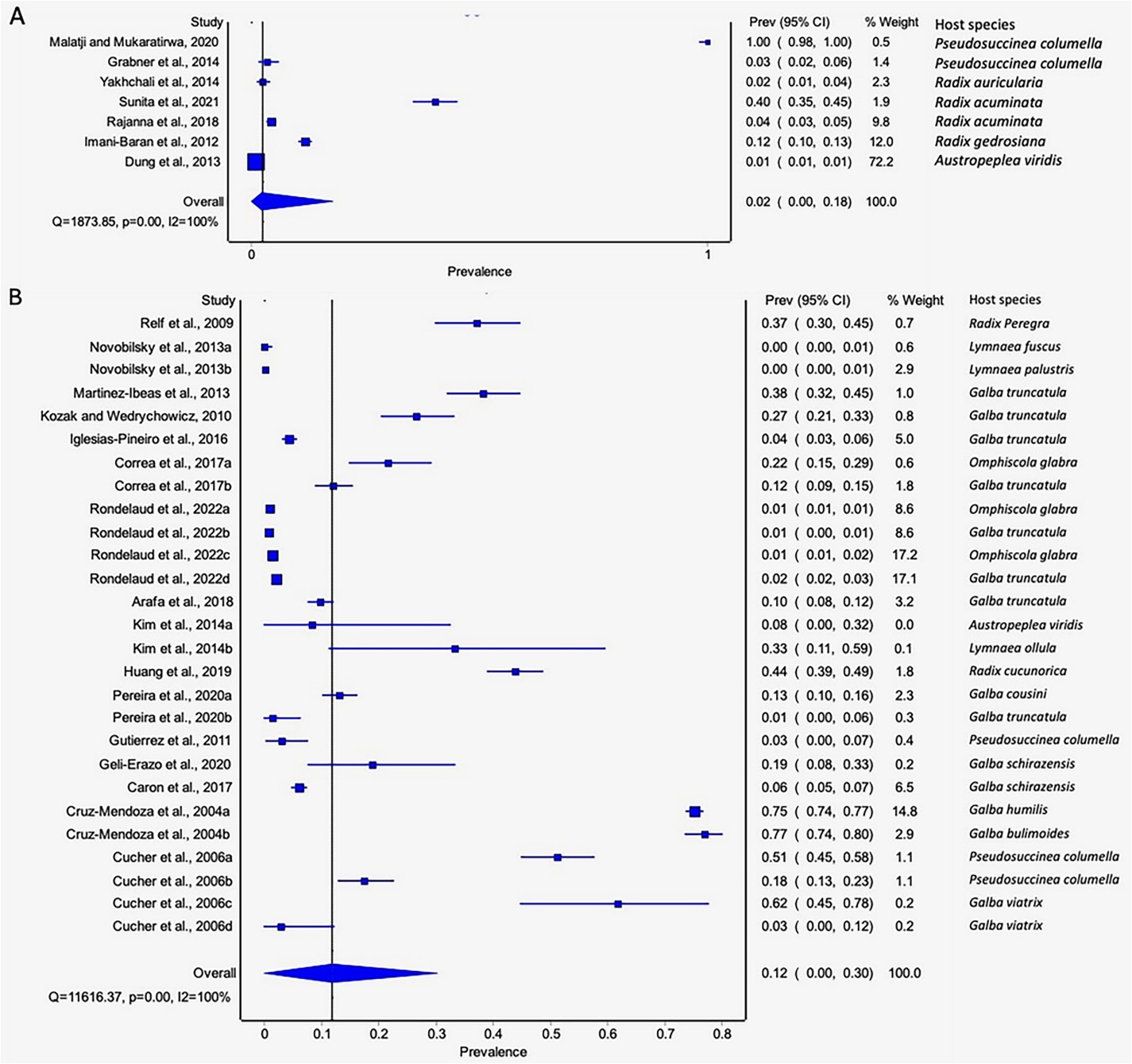

Figure 8 **Forest plots showing the rates of infection of *Fasciola* spp. in naturally infected lymnaeid snails based on species (A) *Fasciola gigantica*, and (B) *Fasciola hepatica* (*Malatji & Mukaratirwa, 2020*; *Grabner et al., 2014*; *Yakhchali, Malekzadeh-Viayeh & Imani-Baran, 2014*; *Sunita et al., 2021*; *Rajanna et al., 2018*; *Imani-Baran et al., 2012*; *Dung et al., 2013*; *Relf et al., 2009*; *Novobilský et al., 2013*; *Martínez-Ibeas et al., 2013*; *Kozak & Wędrychowicz, 2010*; *Iglesias-Piñeiro et al., 2016*; *Correa et al., 2017*; *Rondelaud, Vignoles & Dreyfuss, 2022*; *Arafa et al., 2018*; *Kim et al., 2014*; *Huang et al., 2019*; *Pereira, Uribe & Pointier, 2020*; *Gutierrez et al., 2011*; *Geli-Erazo et al., 2020*; *Caron et al., 2017*; *Cruz-Mendoza et al., 2004*; *Cucher et al., 2006*).** Different letters next to references denote different data generated from the same publication.

Full-size DOI: 10.7717/peerj.18976/fig-8

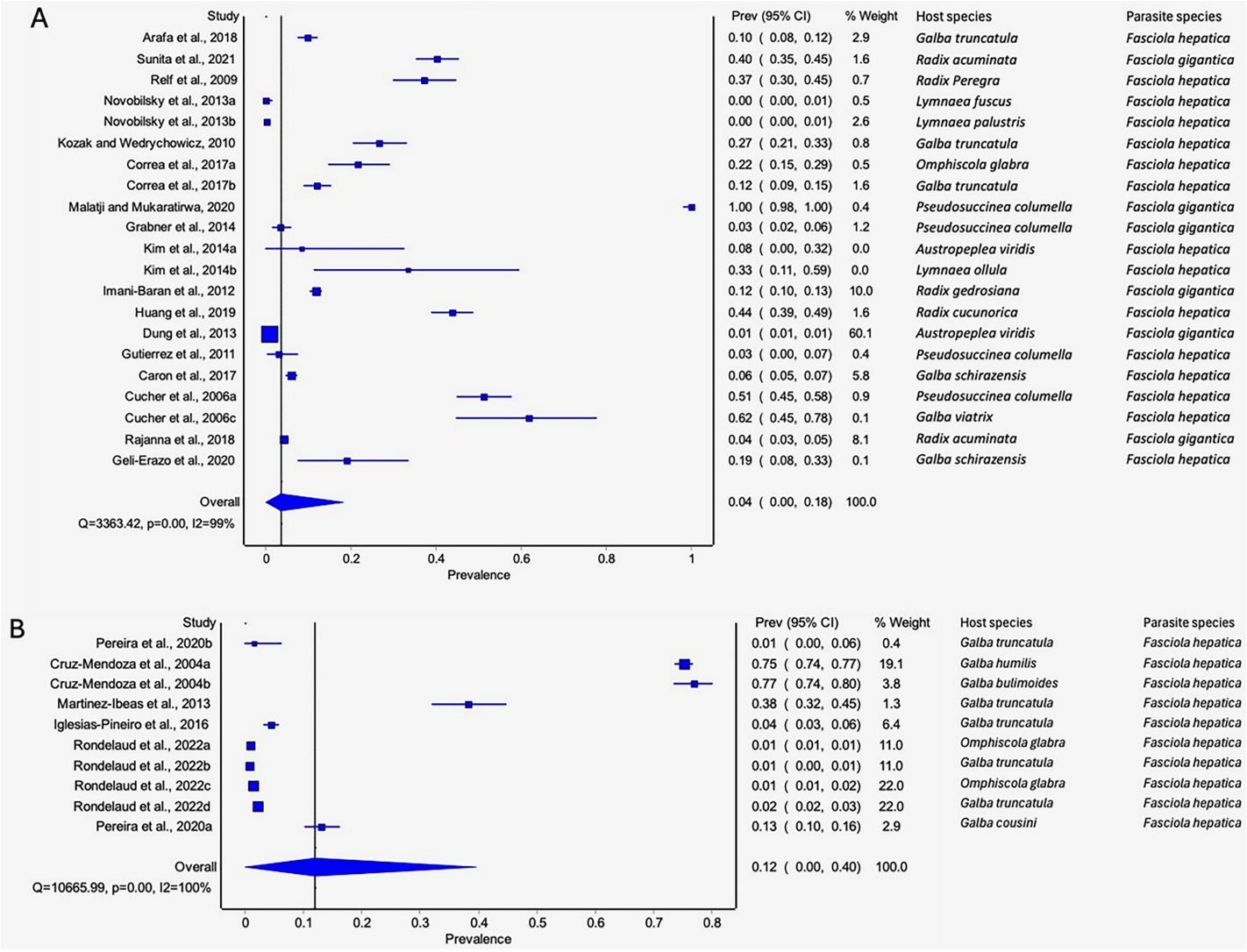

Figure 9 Forest plots showing the rates of natural infections of *Fasciola* species in lymnaeid snails recorded using (A) molecular techniques and (B) dissection (*Arafa et al., 2018*; *Sunita et al., 2021*; *Relf et al., 2009*; *Novobilský et al., 2013*; *Kozak & Wędrychowicz, 2010*; *Correa et al., 2017*; *Malatji & Mukaratirwa, 2020*; *Grabner et al., 2014*; *Kim et al., 2014*; *Imani-Baran et al., 2012*; *Huang et al., 2019*; *Dung et al., 2013*; *Gutierrez et al., 2011*; *Caron et al., 2017*; *Cucher et al., 2006*; *Rajanna et al., 2018*; *Geli-Erazo et al., 2020*; *Pereira, Uribe & Pointier, 2020*; *Cruz-Mendoza et al., 2004*; *Martínez-Ibeas et al., 2013*; *Iglesias-Piñeiro et al., 2016*; *Rondelaud, Vignoles & Dreyfuss, 2022*). Different letters next to references denote different data generated from the same publication. Full-size DOI: 10.7717/peerj.18976/fig-9

infections and natural infections, South America recorded the highest pooled prevalence, and the lowest prevalence was recorded in Europe and Asia. While all three continents recorded multiple snail species involved in the natural transmission of *F. hepatica*, *G. truncatula* and *O. glabra* contributed more to the pooled prevalence. *Mas-Coma, Bargues & Valero (2005)*, implicated the geographical expansion of *G. truncatula* and *P. columella* in the dispersal of *F. hepatica* from Europe to other continents. However, though infections in *G. truncatula* were also noted in South America, other *Galba* species (*G. schirazensis*) proved to contribute more to the pooled prevalence. The latter results may be a misinterpretation however, as *G. schirazensis* had been frequently confused with

G. truncatula. A reason for this may be that *G. schirazensis* and *G. truncatula* can be considered as cryptic species as they are very similar in anatomical variation and shell morphology (*Correa et al., 2011*; *Vázquez et al., 2019a*). Furthermore, South America comparatively recorded the highest infections (76.9%) of *Fasciola* spp. (*Cruz-Mendoza et al., 2004*) compared to Europe (38.3%) (*Martínez-Ibeas et al., 2013*).

Fasciola gigantica recorded a high pooled prevalence in experimental infections, however, in the natural environment, the pooled prevalence was higher with *F. hepatica*. Furthermore, the later species showed a wider geographical expansion, recorded in five continents while *F. gigantica* showed restriction to two continents. Contributing to this may be the wide range of snail species involved in the natural transmission of *F. hepatica*, which explains its geographical expansion compared to *F. gigantica*. Furthermore, *Mas-Coma, Bargues & Valero (2005)* linked the smaller geographical distribution of *F. gigantica* to its IHs having a weaker diffusion capacity.

Our analysis showed that while various snail species have been subjected to experimental infections and assessed for natural infection of *Fasciola* spp., most studies were conducted on *G. truncatula*, *R. natalensis*, and *P. columella*. Our results further showed that the invasive snail, *P. columella*, which recorded a high pooled prevalence was infected by both *F. hepatica* and *F. gigantica* for both experimental and natural infections, despite *G. truncatula* and *R. natalensis* being the main IHs of *F. hepatica* (*Gasnier et al., 2000*; *Cruz-Mendoza et al., 2004*; *Bargues & Mas-Coma, 2005*; *Pointier et al., 2009*; *Kim et al., 2014*; *Beesley et al., 2018*; *Alemu, 2019*) and *F. gigantica* (*Bargues & Mas-Coma, 2005*; *Mas-Coma, Bargues & Valero, 2005*; *Rajanna et al., 2018*; *Alemu, 2019*; *Nyagura, Malatji & Mukaratirwa, 2022*), respectively. The ability of *P. columella* to transmit both *F. hepatica* and *F. gigantica* in a natural environment has been documented in many countries (*Grabner et al., 2014*; *Carolus et al., 2019*; *Alba et al., 2019*; *Malatji & Mukaratirwa, 2020*; *Ngcamphalala, Malatji & Mukaratirwa, 2022*). Furthermore, according to *Mas-Coma, Bargues & Valero (2005)*, *P. columella* has been linked to the secondary transmission of *F. hepatica*. Additionally, *A. viridis* was shown to transmit both *F. hepatica* (*Kim et al., 2014*) and *F. gigantica* (*Dung et al., 2013*) in nature even though the prevalence of both infections was significantly low. However, it has been previously noted that even low prevalence in naturally infected intermediate snails matters (*Vázquez et al., 2015*) as a single miracidium infection can produce approximately 4,000 metacercariae leading to substantial environmental contamination (*Andrews, 1999*; *Nguyen et al., 2012*).

Experimental studies recorded a high pooled prevalence based on cercariae shedding, which is the most used and affordable detection method to assess trematode infection in snails. However, this method tends to underestimate the true prevalence of infection as it mainly detects patent infections, and infections that are still at the prepatent stage are regarded as negatives (*Curtis & Hubbard, 1990*; *Born-Torrijos et al., 2014*; *Tigga et al., 2014*). Whilst microscopic dissection recorded the lowest pooled prevalence in experimental infections, this method however had the highest prevalence in natural infections with molecular detection recording the lowest. Contributing to the lowest prevalence of molecular detection (polymerase chain reaction (PCR) might have been due

to the low number of studies using this technique to detect *Fasciola* spp. infections in IH snails. Furthermore, most studies might have opted not to use molecular techniques due to the costs involved equipment, consumables, and sequencing (*Caron et al., 2010*; *Tigga et al., 2014*), and the lack of skilled personnel to carry out the PCR (*Tigga et al., 2014*). Several authors emphasized investing in PCR as a supplementary/confirmation method as it has been shown to detect *Fasciola* spp. infections (DNA) in IH snails after they had been deemed negative by shedding and/or dissection (*Caron et al., 2017*; *Rajanna et al., 2018*; *Geli-Erazo et al., 2020*; *Malatji & Mukaratirwa, 2020*).

Experimental infections showed a high pooled infection rate of *Fasciola* spp. infections in the most recent decade (2014–2023) which might be attributed to the increased number of experimental studies conducted to better understand the host–parasite interaction and transmission of these important zoonotic parasites. However, prevalence data for natural infections showed a decline in prevalence for the past 20 years, with the decade (2014–2023) recording the lowest pooled prevalence. The natural infection model's significant results for the period ($p = 0.007$) indicate that temporal factors, such as seasonal variation or changes in environmental conditions, may be crucial in influencing prevalence, despite the overall heterogeneity seen across the various factors (continents, diagnostic tests, and *Fasciola* species). This decline in natural infections may be attributed to climate change as the primary determinant of transmission efficiency is the relationship between rainfall and temperature (*Fox et al., 2011*) both of which have either positive or negative effects on the distribution of the intermediate snail hosts and survival of free-living stages of the *Fasciola* spp. parasite (*Madsen & Stauffer, 2022*). Another reason for the decline in natural infections prevalence may be the effectiveness of control strategies targeting infections in definitive hosts and IHs such as the use of anthelmintics and controlling the snail IH using molluscicides (*Madsen & Stauffer, 2022*).

There could be a number of reasons for the continents, diagnostic tests, and *Fasciola* species' lack of significance, such as the fact that these variables may not vary sufficiently between studies or that other unmeasured confounders may obscure their impact. The observed high level of heterogeneity may potentially indicate that the results are being influenced by other factors that were not taken into account in the model, such as methodological or regional variances.

The limitation of this review is that only articles written in English were included to ensure that there was no misrepresentation of methodologies and results in cases where there could be incorrect translations from other languages to English. Additionally, publication bias was detected for both field and experimental studies. Despite using a standardized analysis process, it was difficult to achieve consistent meta-analysis due to the differences in study design, detection, and quantification methods in the different studies. Furthermore, several studies failed to provide complete information on the prevalence of *Fasciola* species amongst freshwater snails, and those that had all the information were not evenly distributed across continents. Hence, meta-analysis could not be conducted for some IH species and some continents. As a result, the prevalence data presented in this

review does not fully represent the prevalence of *Fasciola* spp. infections amongst freshwater snail spp. globally.

CONCLUSIONS

The review highlighted crucial information on the prevalence of *Fasciola* spp. infection in their intermediate snail hosts across the globe. Natural infection results showed a strong intermediate host specificity between the two *Fasciola* spp., where *G. truncatula* and *R. natalensis* are susceptible to *F. hepatica* and *F. gigantica* respectively, whilst *P. columella* is able to transmit both species. This information is important in determining and estimating the species-specific distribution and transmission, which can be used as baseline data for interrupting the life cycle of fasciolosis in a given area.

We therefore, recommend continuous surveillance and monitoring of the dispersal of the lymnaeid snail species involved in the transmission of specific *Fasciola* spp. Additionally, to employ the use of molecular detection methods to supplement classic parasitological methods, to confirm the detection of infection and identity of species. Moreover, focus on developing and using other protocols such as the loop-mediated isothermal amplification (LAMP) and other PCR-based protocols that can detect and identify species without the extra sequencing costs is required.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Philile Ignecious Ngcamphalala performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Ignore Nyagura performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Mokgadi Pulane Malatji conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, resolved disagreements on search strategy, and approved the final draft.

- Samson Mukaratirwa conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a systematic review/meta-analysis.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.18976#supplemental-information.

REFERENCES

Ahmad AA, Ramadan HKA, Hassan WA, Hakami MA, Huseein EAM, Mohamed SAA, Mohamed AA, Elossily NA. 2022. New perspectives for fascioliasis in Upper Egypt's new endemic region: Sociodemographic characteristics and phylogenetic analysis of *Fasciola* in humans, animals, and lymnaeid vectors. *PLOS Neglected Tropical Diseases* **16(12)**:1–12 DOI 10.1371/journal.pntd.0011000.

Alba A, Tetreau G, Chaparro C, Sánchez J, Vázquez AA, Gourbal B. 2019. Natural resistance to *Fasciola hepatica* (Trematoda) in *Pseudosuccinea columella* snails: a review from literature and insights from comparative "omic" analyses. *Developmental and Comparative Immunology* **101**:1–14 DOI 10.1016/j.dci.2019.103463.

Alba A, Vázquez AA, Sánchez J, Duval D, Hernández HM, Sabourin E, Vittecoq M, Hurtrez-Boussés S, Gourbal B. 2018. *Fasciola hepatica-Pseudosuccinea columella* interaction: effect of increasing parasite doses, successive exposures and geographical origin on the infection outcome of susceptible and naturally-resistant snails from Cuba. *Parasite Vectors* **11(1)**:1–9 DOI 10.1186/s13071-018-3155-3.

Aleixo MA, Freitas DF, Dutra LH, Malone J, Martins IVF, Molento MB. 2015. *Fasciola hepatica*: epidemiology, perspectives in the diagnostic and the use of geoprocessing systems for prevalence studies. *Semina: Ciências Agrárias* **36(3)**:1451–1465 DOI 10.5433/1679-0359.2015v36n3p1451.

Alemu B. 2019. Bovine fasciolosis in ethiopia-a review. *Journal of Veterinary and Animal Research* **2(2)**:1–12.

Andrews SJ. 1999. The life cycle of *Fasciola hepatica*. *Fasciolosis* **3**:1–29.

Arafa WM, Hassan AI, Snousi SAM, El-Dakhly KM, Holman PJ, Craig TM, Aboelhadid SM. 2018. *Fasciola hepatica* infections in cattle and the freshwater snail *Galba truncatula* from Dakhla Oasis, Egypt. *Journal of Helminthology* **92(1)**:56–63 DOI 10.1017/S0022149X17000086.

Ashrafi K, Mas-Coma S. 2014. *Fasciola gigantica* transmission in the zoonotic fascioliasis endemic lowlands of Guilan, Iran: experimental assessment. *Veterinary Parasitology* **205(2)**:96–106 DOI 10.1016/j.vetpar.2014.07.017.

Barendregt JJ. 2016. *MetaXL user guide version 5.3*. Australia: EpiGear International Pty Ltd. *Available at www.epigear.com*.

Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. 2013. Meta-analysis of prevalence. *Journal of Epidemiology and Community Health* **67(11)**:974–978 DOI 10.1136/jech-2013-203104.

Bargues MD, Artigas P, Khoubbane M, Flores R, Glöer P, Rojas-Garcia R, Ashrafi K, Falkner G, Mas-Coma S. 2011. Lymnaea schirazensis, an overlooked snail distorting fascioliasis data:

genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. *PLOS ONE* **6(9)**:1–33 DOI 10.1371/journal.pone.0024567.

Bargues MD, Mas-Coma S. 2005. Reviewing lymnaeid vectors of fascioliasis by ribosomal DNA sequence analyses. *Journal of Helminthology* **79(3)**:257–267 DOI 10.1079/JOH2005297.

Beesley NJ, Caminade C, Charlier J, Flynn RJ, Hodgkinson JE, Martinez-Moreno A, Martinez-Valladares M, Perez J, Rinaldi L, Williams DJL. 2018. *Fasciola* and fasciolosis in ruminants in Europe: identifying research needs. *Transboundary and Emerging Diseases* **65**:199–216 DOI 10.1111/tbed.12682.

Born-Torrijos A, Poulin R, Raga JA, Holzer AS. 2014. Estimating trematode prevalence in snail hosts using a single-step duplex PCR: how badly does cercarial shedding underestimate infection rates? *Parasite Vectors* **7**:243–251 DOI 10.1186/1756-3305-7-243.

Brown D. 1994. *Freshwater snails of Africa and their medical importance*. London: Taylor & Francis.

Carolus H, Muzarabani KC, Hammoud C, Schols R, Volckaert FA, Barson M, Huyse TA. 2019. A cascade of biological invasions and parasite spillback in man-made Lake Kariba. *Science of the Total Environment* **659(3)**:1283–1292 DOI 10.1016/j.scitotenv.2018.12.307.

Caron Y, Celi-Erazo M, Hurtrez-Boussès S, Lounnas M, Pointier JP, Saegerman C, Losson B, Benítez-Ortíz W. 2017. Is *Galba schirazensis* (Mollusca, gastropoda) an intermediate host of *Fasciola hepatica* (Trematoda, digenea) in Ecuador? *Parasite* **24(24)**:1–9 DOI 10.1051/parasite/2017026.

Caron Y, Righi S, Lempereur L, Saegerman C, Losson B. 2010. An optimized DNA extraction and multiplex PCR for the detection of *Fasciola* sp. in lymnaeid snails. *Veterinary Parasitology* **178(2)**:93–99 DOI 10.1016/j.vetpar.2010.12.020.

Chen JX, Chen MX, Ai L, Xu XN, Jiao JM, Zhu TJ, Su HY, Zang W, Luo JJ, Guo YH, Lv A, Zhou XN. 2013. An outbreak of human Fascioliasis gigantica in southwest China. *PLOS ONE* **8(8)**:1–10 DOI 10.1371/journal.pone.0071520.

Correa AC, De Meeûs T, Dreyfuss G, Rondelaud D, Hurtrez-Boussès S. 2017. *Galba truncatula* and *Fasciola hepatica*: genetic costructures and interactions with intermediate host dispersal. *Infection Genetics and Evolution* **55**:186–194 DOI 10.1016/j.meegid.2017.09.012.

Correa A, Escobar J, Noya O, Velázquez L, González-Ramírez C, Hurtrez-Boussès S, Pointier J. 2011. Morphological and molecular characterization of Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. *Infection Genetics and Evolution* **11(8)**:1978–1988 DOI 10.1016/j.meegid.2011.09.003.

Cruz-Mendoza I, Figueroa JA, Correa D, Ramos-Martínez E, Lecumberri-López J, Quiroz-Romero H. 2004. Dynamics of *Fasciola hepatica* infection in two species of snails in a rural locality of Mexico. *Veterinary Parasitology* **121(2)**:87–93 DOI 10.1016/j.vetpar.2004.02.006.

Cucher MA, Carnevale S, Prepelitchi L, Labbé JH, Wisnivesky-Colli C. 2006. PCR diagnosis of *Fasciola hepatica* in field-collected *Lymnaea columella* and *Lymnaea viatrix* snails. *Veterinary Parasitology* **137(2)**:74–82 DOI 10.1016/j.vetpar.2005.12.013.

Curtis LA, Hubbard KM. 1990. Trematode infections in a gastropod host misrepresented by observing shed cercariae. *Journal of Experimental Marine Biology and Ecology* **143**:131–137 DOI 10.1016/0022-0981(90)90115-S.

Dar Y, Lounnas M, Teukeng DF, Mouzet R, Courtioux B, Hurtrez-Boussès S, Vignoles P, Dreyfuss G, Rondelaud D. 2013. Variations in local adaptation of allopatric Fasciola hepatica to French Galba truncatula in relation to parasite origin. *Parasitology Research* **112(7)**:2543–2549 DOI 10.1007/s00436-013-3421-4.

Dar Y, Rondelaud D, Vignoles P, Dreyfuss G. 2014b. *Fasciola hepatica*: development of redial generations in experimental infections of *Pseudosuccinea columella*. *Parasitology Research* **113(7)**:2467–2473 DOI 10.1007/s00436-014-3893-x.

Dar Y, Rondelaud D, Vignoles P, Dreyfuss G. 2015b. *Fasciola hepatica*: the redial generations in juvenile *Pseudosuccinea columella*. *Parasitology Research* **114(8)**:2845–2851 DOI 10.1007/s00436-015-4485-0.

Dar Y, Teukeng FD, Vignoles P, Dreyfuss G, Rondelaud D. 2010. *Radix natalensis* (Gastropoda: Lymnaeidae), a potential intermediate host of *Fasciola hepatica* in Egypt. *Parasite* **17(3)**:251–256 DOI 10.1051/parasite/2010173251.

Dar Y, Vignoles P, Rondelaud D, Dreyfuss G. 2014a. *Radix natalensis*: the effect of *Fasciola hepatica* infection on the reproductive activity of the snail. *Parasite* **21(24)**:1–6 DOI 10.1051/parasite/2014026.

Dar Y, Vignoles P, Rondelaud D, Dreyfuss G. 2015a. Role of the lymnaeid snail *Pseudosuccinea columella* in the transmission of the liver fluke *Fasciola hepatica* in Egypt. *Journal of Helminthology* **89(6)**:699–706 DOI 10.1017/S0022149X14000406.

de Kock KN, van Eeden JA, Pretorius SJ. 1986. Effect of laboratory breeding on the population dynamics of successive generations of the freshwater snail *Bulinus tropicus* (Krauss). *South African Journal of Science* **82**:369–372.

Doi SA, Thalib L. 2008. A quality-effects model for meta-analysis. *Epidemiology* **19(1)**:94–100 DOI 10.1017/S0022149X14000406.

Dreyfuss G, Vignoles P, Rondelaud D. 2012. Local adaptation of the trematode Fasciola hepatica to the snail Galba truncatula. *Parasite* **19(3)**:271–275 DOI 10.1051/parasite/2012193271.

Dreyfuss G, Vignoles P, Rondelaud D. 2016. *Pseudosuccinea columella*: experimental co-infections of juvenile and pre-adult snails with the digeneans *Calicophoron daubneyi* and *Fasciola hepatica*. *Journal of Helminthology* **90(6)**:753–759 DOI 10.1017/S0022149X15001078.

Dung BT, Doanh PN, The DT, Loan HT, Losson B, Caron Y. 2013. Morphological and molecular characterization of lymnaeid snails and their potential role in transmission of *Fasciola* spp. Vietnam. *The Korean Journal of Parasitology* **51(6)**:657–662 DOI 10.3347/kjp.2013.51.6.657.

Fox NJ, White PC, McClean CJ, Marion G, Evans A, Hutchings MR. 2011. Predicting impacts of climate change on Fasciola hepatica risk. *PLOS ONE* **6(1)**:1–9 DOI 10.1371/journal.pone.0016126.

Gasnier N, Rondelaud D, Abrous M, Carreras F, Boulard C, Diez-Banos P, Cabaret J. 2000. Allopatric combination of *Fasciola hepatica* and *Lymnaea truncatula* is more efficient than sympatric ones. *International Journal of Parasitology* **30(5)**:573–578 DOI 10.1016/S0020-7519(00)00038-2.

Geli-Erazo M, Alda P, Montenegro-Franco M, Pavon D, Minda-Aluisa E, Calvopiña M, Pointier JP, Hurtrez-Boussès S, Cevallos W, Benítez-Ortíz W, Rodríguez-Hidalgo R. 2020. Prevalence of *Fasciola hepatica* infection in *Galba cousini* and *Galba schirazensis* from an Andean region of Ecuador. *Veterinary Parasitology: Reginal Studies and Reports* **20(4)**:1–7 DOI 10.1016/j.vprsr.2020.100390.

Grabner DS, Mohamed FA, Nachev M, Meabed EM, Sabry AHA, Sures B. 2014. Invasion biology meets parasitology: a case study of parasite spill-back with Egyptian Fasciola gigantica in the invasive snail Pseudosuccinea columella. *PLOS ONE* **9(2)**:1–7 DOI 10.1371/journal.pone.0088537.

Gutierrez A, Vázquez AA, Hevia Y, Sánchez J, Correa AC, Hurtrez-Boussès S, Pointier JP, Théron A. 2011. First report of larval stages of *Fasciola hepatica* in a wild population of

Pseudosuccinea columella from Cuba and the Caribbean. *Journal of Helminthology* **85(1)**:109–111 DOI 10.1017/S0022149X10000350.

Gutierrez A, Yong M, Wong L, Sánchez J. 2001. The combined effect of isolation and *Fasciola hepatica* infection on the life history traits of *Fossaria cubensis*. *Journal of Invertebrate Pathology* **78(20)**:66–71 DOI 10.1017/S0022149X15001078.

Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ. 2008. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* **336(7650)**:924–926 DOI 10.1136/bmj.39489.470347.AD.

Hajipour N, Soltani M, Ketzis J, Hassanzadeh P. 2021. Zoonotic parasitic organisms on vegetables: impact of production system characteristics on presence, prevalence on vegetables in northwestern Iran and washing methods for removal. *Food Microbiology* **95(2)**:103704 DOI 10.1016/j.fm.2020.103704.

Higgins JP, Thompson SG, Deeks JJ, Altman DG. 2003. Measuring inconsistency in meta-analyses. *BMJ* **327(7414)**:557–560 DOI 10.1136/bmj.39489.470347.AD.

Huang SY, Gong JZ, Yang B, Fan YM, Yao N, Wang CR. 2019. Development of a nest-PCR for detection of *Fasciola hepatica* DNA in the intermediate snail host, *Radix cucunorica*, and the prevalence in northwestern China. *Infection, Genetics and Evolution* **75**:1–6 DOI 10.1016/j.meegid.2019.103984.

Hubendick B. 1951. *Recent Lymnaeidae, their variation, morphology, taxonomy, nomenclature, and distribution.* Vol. 3. Kungliga Svenska Vetenskapsakade miens Handlingar, 1–223.

Iglesias-Piñeiro J, González-Warleta M, Castro-Hermida JA, Córdoba M, González-Lanza C, Manga-González Y, Mezo M. 2016. Transmission of *Calicophoron daubneyi* and *Fasciola hepatica* in Galicia (Spain): temporal follow-up in the intermediate and definitive hosts. *Parasite Vectors* **9(1)**:1–14 DOI 10.1186/s13071-016-1892-8.

Imani-Baran A, Yakhchali M, Malekzadeh Viayeh R, Paktarmani R. 2012. Molecular study for detecting the prevalence of *Fasciola gigantica* in field-collected snails of *Radix gedrosiana* (Pulmonata: Lymnaeidae) in northwestern Iran. *Veterinary Parasitology* **189(2–4)**:374–377 DOI 10.1016/j.vetpar.2012.04.027.

Kim HY, Choi IW, Kim YR, Quan JH, Ismail HA, Cha GH, Hong SJ, Lee YH. 2014. *Fasciola hepatica* in snails collected from water-dropwort fields using PCR. *The Korean Journal of Parasitology* **52(6)**:645–652 DOI 10.3347/kjp.2014.52.6.645.

Kithuka JM, Maingi N, Njeruh FM, Ombui JN. 2002. The prevalence and economic importance of bovine fasciolosis in Kenya-an analysis of abattoir data. *Onderstepoort Journal of Veterinary Research* **69**:255–262.

Kleiman F, Pietrokovsky S, Prepelitchi L, Carbajo AE, Wisnivesky-Colli C. 2007. Dynamics of *Fasciola hepatica* transmission in the Andean Patagonian valleys. *Argentina Veterinary Parasitology* **145(4)**:274–286 DOI 10.1016/j.vetpar.2006.12.020.

Kozak M, Wędrychowicz H. 2010. The performance of a PCR assay for field studies on the prevalence of *Fasciola hepatica* infection in *Galba truncatula* intermediate host snails. *Veterinary Parasitology* **168(2)**:25–30 DOI 10.1016/j.vetpar.2009.10.014.

Lalor R, Cwiklinski K, Calvani NED, Dorey A, Hamon S, Corrales JL, Dalton JP, De Marco Verissimo C. 2021. Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that cause the zoonosis Fasciolosis. *Virulence* **12(1)**:2839–2867 DOI 10.1080/21505594.2021.1996520.

Madsen H, Stauffer JR. 2022. Zoonotic trematode infections; their biology, intermediate hosts and control. In: *Parasitic Helminths and Zoonoses-From Basic to Applied Research*. London, UK: IntechOpen DOI 10.5772/intechopen.102434.

Malatji MP, Lamb J, Mukaratirwa S. 2019. Molecular characterization of liver fluke intermediate host lymnaeids (Gastropoda: Pulmonata) snails from selected regions of Okavango Delta of Botswana, KwaZulu-Natal and Mpumalanga provinces of South Africa. *Veterinary Parasitology: Regional Studies and Reports* **17**:1–11 DOI 10.1017/S0022149X19000531.

Malatji MP, Mukaratirwa S. 2020. Molecular detection of natural infection of *Lymnaea* (*Pseudosuccinea*) *columella* (Gastropoda: Lymnaeidae) with *Fasciola gigantica* (Digenea: Fasciolidae) from two provinces of South Africa. *Journal of Helminthology* **94(e38)**:1–6 DOI 10.1017/S0022149X19000129.

Martínez-Ibeas AM, Gonzalez-Warleta M, Martínez-Valladares M, Castro-Hermida JA, González-Lanza C, Minambres B, Ferreras C, Mezo M, Manga-González MY. 2013. Development and validation of a mtDNA multiplex PCR for identification and discrimination of *Calicophoron daubneyi* and *Fasciola hepatica* in the *Galba truncatula* snail. *Veteterinary Parasitology* **195(2)**:57–64.

Mas-Coma S. 2004. Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. *The Southeast Asian Journal of Tropical Medicine and Public Health* **35(1)**:1–11.

Mas-Coma S, Bargues MD, Valero MA. 2005. Fascioliasis and other plant-borne trematode zoonoses. *International Journal of Parasitology* **35(12)**:1255–1278 DOI 10.1016/j.ijpara.2005.07.010.

McKown RD, Ridley RK. 1995. Distribution of fasciolosis in Kansas, with results of experimental snail susceptibility studies. *Veterinary Parasitology* **56(4)**:281–291 DOI 10.1016/0304-4017(94)00688-9.

Medeiros C, Scholte RGC, D'ávila S, Caldeira RL, Carvalho ODS. 2014. Spatial distribution of lymnaeidae (Mollusca, Basommatophora), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematoda, Digenea) in Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* **56(3)**:235–252 DOI 10.1590/S0036-46652014000300010.

Mucheka VT, Lamb JM, Pfukenyi DM, Mukaratirwa S. 2015. DNA sequence analyses reveal co-occurrence of novel haplotypes of *Fasciola gigantica* with *F. hepatica* in South Africa and Zimbabwe. *Veterinary Parasitology* **214(2)**:144–151 DOI 10.1016/j.vetpar.2015.09.024.

Ngcamphalala PI, Malatji MP, Mukaratirwa S. 2022. Geography and ecology of invasive *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) and implications in the transmission of *Fasciola* species (Digenea: Fasciolidae)—a review. *Journal of Helminthology* **96**:1–18 DOI 10.1017/S0022149X21000717.

Nguyen ST, Nguyen DT, Van Nguyen T, Huynh VV, Le DD, Fukuda Y, Nakai Y. 2012. Prevalence of *Fasciola* in cattle and of its intermediate host *Lymnaea* snails in central Vietnam. *Tropical Animal Health and Production* **44**:1847–1853.

Novobilský A, Kašný M, Beran L, Rondelaud D, Höglund J. 2013. *Lymnaea palustris* and *Lymnaea fuscus* are potential but uncommon intermediate hosts of *Fasciola hepatica* in Sweden. *Parasite Vectors* **6**:1–10.

Nukeri S, Malatji MP, Sengupta ME, Vennervald BJ, Stensgaard AS, Chaisi M, Mukaratirwa S. 2022. Potential hybridization of *Fasciola hepatica* and *F. gigantica* in Africa—a scoping review. *Pathogens* **11(11)**:1–25 DOI 10.3390/pathogens11111303.

Nyagura I, Malatji MP, Mukaratirwa S. 2022. Occurrence of *Fasciola* (Digenea: Fasciolidae) species in livestock, wildlife and humans, and the geographical distribution of their intermediate hosts in South Africa a scoping review. *Frontiers in Veterinary Science* **9**:1–11 DOI 10.3389/fvets.2022.935428.

Nyagura I, Malatji MP, Ngcamphalala PI, Mukaratirwa S. 2024. A systematic review and meta-analysis on the prevalence of amphistome infection in domestic ruminants in the sub-Saharan Africa region. *Journal of Helminthology* **98**:1–13 DOI 10.1017/S0022149X24000725.

Pereira AE, Uribe N, Pointier JP. 2020. Lymnaeidae from Santander and bordering departments of Colombia: morphological characterization, molecular identification and natural infection with *Fasciola hepatica*. *Veterinary Parasitology: Regional Studies and Reports* **20(1)**:1–9 DOI 10.1016/j.vprsr.2020.100408.

Pointier JP, Coustau C, Rondelaud D, Theron A. 2007. *Pseudosuccinea columella* (Say 1817) (Gastropoda, Lymnaeidae), snail host of *Fasciola hepatica*: first record for France in the wild. *Parasitology Research* **101(5)**:1389–1392 DOI 10.1007/s00436-007-0656-y.

Pointier JP, Noya O, Alarcon de Noya B, Theron A. 2009. Distribution of Lymnaeidae (Mollusca: Pulmonata), intermediate snail hosts of *Fasciola hepatica* in Venezuela. *Memórias do Instituto Oswaldo Cruz* **104(5)**:790–796 DOI 10.1590/S0074-02762009000500022.

Prepelitchi L, Pietrokovsky S, Kleiman F, Rubel D, Issia L, Moriena R, Racioppi O, Álvarez J, Wisnivesky-Colli C. 2011. Population structure and dynamics of *Lymnaea columella* (Say, 1817) (Gastropoda: Lymnaeidae) in Wetlands of Northeastern Argentina. *Zoological Studies* **50(2)**:164–176.

Rajanna R, Thimmareddy PM, Sengupta PP, Siju SJ, Mamatha GS. 2018. Epidemiological and genetic characterization of larval stages of *Fasciola gigantica* in snail intermediate hosts in Karnataka State, India. *Acta Parasitologica* **63(3)**:609–616 DOI 10.1515/ap-2018-0070.

Relf V, Good B, McCarthy E, De Waal T. 2009. Evidence of *Fasciola hepatica* infection in *Radix peregra* and a mollusc of the family Succineidae in Ireland. *Veterinary Parasitology* **163(2)**:152–155 DOI 10.1016/j.vetpar.2009.04.003.

Rondelaud D, Denève C, Belfaiza M, Mekroud A, Abrous M, Moncef M, Dreyfuss G. 2004. Variability in the prevalence of infection and cercarial production in *Galba truncatula* raised on a high-quality diet. *Parasitology Research* **92(3)**:242–245 DOI 10.1007/s00436-003-1025-0.

Rondelaud D, Teukeng FD, Vignoles P, Dreyfuss G. 2015. *Lymnaea glabra*: progressive increase in susceptibility to Fasciola hepatica through successive generations of experimentally infected snails. *Journal of Helminthology* **89(4)**:398–403 DOI 10.1017/S0022149X14000169.

Rondelaud D, Vignoles P, Dreyfuss G. 2022. Changes in the populations of two Lymnaeidae and their infection by *Fasciola hepatica* and/or *Calicophoron daubneyi* over the past 30 years in central France. *Animals* **12(24)**:1–12 DOI 10.3390/ani12243566.

Salazar L, Estrada VE, Velásquez LE. 2006. Effect of the exposure to *Fasciola hepatica* (Trematoda: Digenea) on life history traits of *Lymnaea cousini* and *Lymnaea columella* (Gastropoda: Lymnaeidae). *Experimental Parasitology* **114(2)**:77–83 DOI 10.1016/j.exppara.2006.02.013.

Sanabria R, Mouzet R, Courtioux B, Vignoles P, Rondelaud D, Dreyfuss G, Cabaret J, Romero J. 2012. Intermediate snail hosts of *French Fasciola hepatica*: *Lymnaea neotropica* and *Lymnaea viatrix* are better hosts than local *Galba truncatula*. *Parasitology Research* **111(5)**:2011–2016 DOI 10.1007/s00436-012-3049-9.

Sanabria R, Mouzet R, Courtioux B, Vignoles P, Rondelaud D, Dreyfuss G, Cabaret J, Romero J. 2013. Comparative strategies and success of sympatric and allopatric *Fasciola hepatica* infecting *Galba truncatula* of different susceptibilities. *Parasitology Research* **112(6)**:2255–2259 DOI 10.1007/s00436-013-3385-4.

Shoriki T, Ichikawa-Seki M, Devkota B, Rana HB, Devkota SP, Humagain SK, Itagaki T. 2014. Molecular phylogenetic identification of *Fasciola* flukes in Nepal. *Parasitology International* **63(6)**:758–762 DOI 10.1016/j.parint.2014.07.001.

Sorensen R, Minchella D. 2001. Snail-trematode life history interactions: past trends and future directions. *Parasitology* **123**:3–18 DOI 10.1017/S0031182001007843.

Spithill TW, Smooker PM, Copeman B. 1999. Fasciola gigantica: epidemiology, control, immunology and molecular biology. In: Dalton JP, ed. *Fasciolosis*. Wallingford, UK: CABI publishing, 1–30.

Sunita K, Mas-Coma S, Bargues MD, Khan MA, Habib M, Mustafa S, Husain SA. 2021. Buffalo infection by *Fasciola gigantica* transmitted by *Radix acuminata* in Uttar Pradesh, India: a molecular tool to improve snail vector epidemiology assessments and control surveillance. *Acta Parasitology* **66(4)**:1396–1405 DOI 10.1007/s11686-021-00414-3.

Tigga MN, Bauri RK, Deb AR, Kullu SS. 2014. Prevalence of snail's intermediate host infected with different trematodes cercariae in and around Ranchi. *Veterinary World* **7(8)**:630–634 DOI 10.14202/vetworld.2014.630-634.

Vignoles P, Dreyfuss G, Rondelaud D. 2015. *Fasciola hepatica*: comparative metacercarial productions in experimentally-infected *Galba truncatula* and *Pseudosuccinea columella*. *Parasite* **15**:1–7 DOI 10.1051/parasite/2015015.

Vinarski MV. 2013. One, two, or several? How many lymnaeid genera are there? *Ruthenica Russian Malacology Journal* **23(1)**:41–58.

Vázquez AA, Alda P, Lounnas M, Sabourin E, Alba A, Pointier JP, Hurtrez-Boussès S. 2019b. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: Digenea): a worldwide review. *CABI Reviews* **13**:1–15 DOI 10.1079/PAVSNNR201813062.

Vázquez AA, de Vargas M, Alba A, Sanchez J, Alda P, Sabourin E, Vittecoq M, Alarcón-Elbal PM, Pointier JP, Hurtrez-Boussès S. 2019a. Reviewing *Fasciola hepatica* transmission in the West Indies and novel perceptions from experimental infections of sympatric vs. allopatric snail/fluke combinations. *Veterinary Parasitology* **275**:1–8 DOI 10.1016/j.vetpar.2019.108955.

Vázquez AA, Sánchez J, Alba A, Pointier JP, Hurtrez-Boussès S. 2015. Natural prevalence in Cuban populations of the lymnaeid snail *Galba cubensis* infected with the liver fluke *Fasciola hepatica*: small values do matter. *Parasitology Research* **114(11)**:4205–4210 DOI 10.1007/s00436-015-4653-2.

Vázquez AA, Sánchez J, Pointier JP, Théron A, Hurtrez-Boussès S. 2014. *Fasciola hepatica* in Cuba: compatibility of different isolates with two intermediate snail hosts, *Galba cubensis* and *Pseudosuccinea columella*. *Journal of Helminthology* **88(4)**:434–440 DOI 10.1017/S0022149X13000382.

Yakhchali M, Malekzadeh-Viayeh R, Imani-Baran A. 2014. PCR-RFLP analysis of 28 SrDNA for specification of *Fasciola gigantica* (Cobbold, 1855) in the infected *Lymnaea auricularia* (Linnaeus, 1785) snails from Northwestern Iran. *Iranian Journal of Parasitology* **9(3)**:358–364.