



RESEARCH ARTICLE

Systematic Evidence of the Association of Zoonotic Metacercaria of Trematodes with Seafood

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ABSTRACT

Zoonotic food-borne trematodes are transmitted to humans via aquatic vegetables, fish, crabs and crayfish as part of the freshwater ecosystem. Though both parasites may be delivered by the same food vehicle, the infective metacercariae are found in different biological food vehicles making global comparisons and food-safety surveillance difficult. A systematic evidence synthesis was performed as an integrative approach and included: indexed biomedical literature, systematic reviews, meta-analyses, primary prevalence surveys and authoritative WHO and EFSA reports. Evidence was grouped into three prespecified pathways as follows: (1) *Fasciola* spp. on aquatic vegetation; (2) *Clonorchis* spp., *Opisthorchis* spp. and *Paragonimus* spp. on freshwater crustaceans. Pooled estimates were not recalculated, but primary surveys were compared based on food vehicle, host species, production system and diagnostic method. Quantitative evidence was most well developed for fish-borne trematodes. The prevalence of *C. sinensis* metacercariae in fish was estimated from 71 studies and found to be 30.5%, with figures of 35.1, 29.7 and 8.4% in China, Korea and Vietnam, respectively. Overall prevalence of zoonotic trematodes in individual Vietnamese and Lao surveys ranged from about 40 to 69%. The prevalence of these second intermediate hosts in China was 52.07% for *P. westermani* and 31.10% for *P. skrjabini*. *Fasciola* metacercariae of edible aquatic plants were poorly documented: only *F. hepatica* DNA was found in 2 of 500 samples of water-dropwort in Korea and low but persistent metacercarial contamination of natural beds of watercress was found in France over a long period. The presence of zoonotic metacercariae in freshwater food systems is widespread; however, there is unevenness among the evidence both taxonomically and geographically. Estimates based on fish, crustacean and plant should not be combined into a single prevalence estimate without stratification. For credible global surveillance, food-unit sampling, species confirmation, viability assessment and metadata for the supply chain must be harmonized.

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INTRODUCTION

Foodborne trematodes are complex zoonotic agents with a complex life cycle involving freshwater snails, aquatic food organisms, plants, mammalian hosts, and human beings, which are neglected (Chai and Jung, 2022; Shamsi, 2025). Clonorchiasis, opisthorchiasis, fascioliasis and paragonimiasis are the four most important foodborne trematode infections recognized by the World Health Organization (Tidman *et al.*, 2023; Maher and Shaapan, 2025). Transmission of these parasites occurs by ingestion of raw or improperly processed food that contains viable metacercaria (Maher

and Shaapan, 2025) (freshwater fish for *Clonorchis* and *Opisthorchis*, freshwater crustaceans for *Paragonimus*, aquatic vegetables for *Fasciola*) (Tan and Machrumnizar, 2023; Štrbac *et al.*, 2024).

The environmentally resistant stage between the aquatic phase and definitive host is the metacercaria, which is most often infective (Tan and Machrumnizar, 2023; Javaid *et al.*, 2025). In fish-borne systems, the snails excrete the cercariae into the water and then into the susceptible fish, where they penetrate the fish and encyst within the tissues, fins, scales, gills, or musculature. Humans and reservoir hosts become infected by eating raw, fermented, pickled, lightly salted

or inadequately cooked fish (Tan and Machrumnizar, 2023). The metacercariae of paragonimiasis develop in freshwater crabs or crayfish (Tan and Machrumnizar, 2023). Fasciola cercariae are released from lymnaeid snails and encyst mainly on the free surfaces of water or aquatic plants and infection is obtained by ingesting contaminated plants or water (Biswas, 2026).

The public-health significance is not just in the field of acute parasitic diseases. Chronic infection by *C. sinensis* and *Opisthorchis viverrini* has been shown to lead to chronic hepatobiliary inflammation, and both diseases have been listed as risk factors for cholangiocarcinoma (Huang *et al.*, 2024). The infection with *Paragonimus* can present like pulmonary tuberculosis or malignancy and can be ectopic (Sharma *et al.*, 2024). Fascioliasis causes acute hepatic migration, chronic biliary disease, and has significant production losses in ruminants (Shoulah *et al.*, 2024). Zoonotic intestinal flukes, such as *Haplorchis*, *Metagonimus*, *Centrocestus*, *Procerovum* and *Echinochasmus*, are often found in fish, even in areas with little-known human disease. (Scholz *et al.*, 2024)

Globalisation of aquatic-food trade, growth of freshwater aquaculture, urban demand for raw-fish dishes and multiple other factors change exposure to aquatic foods (Luo *et al.*, 2026; Yutong *et al.*, 2026). Meanwhile, surveillance is also inconsistent. Some studies report any metacercarial cyst, some identify parasite morphology, some use molecular methods (Qin *et al.*, 2026), others report prevalence per fish, and some report intensity per gram and/or per host or per farm (Abd-Elrahman *et al.*, 2023; Maraganga, 2023; Mahdy *et al.*, 2024; Mata-Marcano *et al.*, 2025). The food-chain stage is also significant: infection in wild fish, aquaculture ponds, wholesale and retail products and infection in food chain products (Gabriël *et al.*, 2022)

There are some helpful, but limited, insights from existing reviews (Keiser and Utzinger, 2004; Hung *et al.*, 2013; Acosta-Pérez *et al.*, 2022; Tidman *et al.*, 2023; Shamsi *et al.*, 2025). But these reviews aren't one integrated food-vehicle framework. The present manuscript thus combines the most appropriate systematic and primary information for aquatic food with metacercariae.

The goals were to outline the main zoonotic trematodes transmitted via waterborne foods; analyze published prevalence rates for fish, crustaceans and aquatic vegetables; review the influence of host, geographic area, food production system and laboratory diagnosis; identify evidence appropriate for quantitative synthesis; and suggest priorities for coordinated One Health surveillance and control of food chains.

MATERIALS AND METHODS

Review question and design: This work was designed as a structured integrative evidence synthesis, not a de novo prevalence meta-analysis or a PRISMA-compliant systematic review. It combined an overview of relevant systematic and scoping reviews with a targeted examination of primary food-level studies. The review question was: what occurrence of zoonotic trematode metacercariae has been reported in freshwater foods

intended for human consumption? The unit of interest was an aquatic food sample rather than an infected human, livestock reservoir or first intermediate snail host (Fig. 3).

Eligibility framework: Eligible evidence comprised cross-sectional surveys, longitudinal surveillance, market surveys, food-chain investigations and baseline data from intervention studies involving freshwater or brackish fish, edible freshwater crustaceans or edible aquatic plants evaluated for zoonotic trematodes. Systematic reviews and meta-analyses were retained to identify published pooled estimates and regional evidence gaps. Human, livestock and snail studies were used only for biological or burden context and were not treated as food-prevalence observations. Reports without an interpretable food denominator were excluded from quantitative description (Gurevitch *et al.*, 2018; Lemire *et al.*, 2023).

Identifying information sources and evidence: Information sources included PubMed/MEDLINE, Europe PMC, reference lists of eligible reviews and primary studies, and WHO and EFSA materials for transmission and food-safety context. The literature-coverage period ended on 31 July 2026. A reproducible retrospective database search applying this cutoff was rerun and verified on 25 September 2026. No geographical or language restriction was imposed. The searches identified 261 PubMed/MEDLINE and 435 Europe PMC records (696 total). After removal of 93 duplicate records by PMID, DOI or normalized title, 603 unique records underwent title/abstract screening. Of these, 473 were excluded because the title or abstract did not retain the required parasite, food vehicle, metacercarial/trematode and occurrence concepts. The remaining 130 reports were assessed against the food-level denominator, zoonotic relevance, diagnostic interpretability and evidence-scope criteria; 114 were excluded and 16 sources were retained (8 reviews and 8 primary studies; Fig. 3).

Data extraction and classification: The variables extracted were Country, sampling year, food vehicle, host species, wild or cultured origin, water body, supply-chain point, sample size, number positive, parasite identification, diagnostic method, prevalence, intensity and abundance. All estimates were categorized into three (3) biological strata: Fasciola (plant-associated); liver or intestinal fluke of fish (fish-associated); and crustacean-associated Paragonimus. The molecular-positive samples were differentiated from microscopy-confirm intact metacercariae, as parasite DNA does not provide information on parasite viability.

Meta-analytic estimates and confidence intervals were reported in the literature without being re-computed. Prevalence in primary studies was described. Only the estimates with the same combination of parasites and food were directly compared as some did not have the same combination of the two. Human prevalence, animal reservoir prevalence and prevalence of snails and food contamination were not combined. If a future de novo meta-analysis is performed, random effects generalized linear mixed model on the logit scale are suggested, as are study-level random effects and multilevel or robust

handling of multiple host-species or site estimates from the same article.

Quality and certainty considerations: Primary food-level studies were appraised using domains adapted from the Joanna Briggs Institute analytical cross-sectional checklist: sampling-frame relevance, sampling method, sample and setting description, parasite identification validity, outcome measurement consistency, denominator clarity and management of confounding or clustering. Overall confidence was categorized as higher, moderate or lower for descriptive synthesis; it was not interpreted as certainty of causal effects. Reviews were judged on search transparency, eligibility criteria, duplicate handling, appraisal methods and appropriateness of synthesis. Caution was applied to convenience samples, morphologically ambiguous metacercariae, overlapping datasets, composite outcomes, molecular-only detection and incomplete supply-chain metadata. The study-level limitations and confidence judgments are summarized in Table 3.

RESULTS

Evidence profile: The mapped evidence base comprised eight systematic or scoping reviews and eight selected primary food-level studies (Tables 2 and 3). Review-level evidence was concentrated in East and Southeast Asia, especially China, Korea, Vietnam and Lao PDR. Primary studies also represented France and Japan, while a review of *Paragonimus* covered sub-Saharan Africa. Sample sizes ranged from 59 watercress beds and 447–922 crabs to 500 plant samples and 846–1,543 fish; methods included artificial or pepsin digestion with microscopy, morphology with molecular confirmation, and PCR/sequencing of plant washes. The evidence was strongest for fish-borne trematodes and crustacean-borne *Paragonimus*, whereas denominator-based evidence for *Fasciola* contamination of edible aquatic plants remained sparse. These sources were selected to illustrate distinct parasite-food and diagnostic pathways; they do not constitute a complete census of global studies. The documented evidence-selection pathway is summarized in Fig. 3.

Table 1: The framework of eligibility designed for the evidence organization

Domain	Excluded	Included
Outcome	Qualitative occurrence without denominator for quantitative synthesis	Number examined and number positive; intensity or abundance where available
Parasites	Non-zoonotic parasites, protozoa, cestodes and nematodes in the primary synthesis	Confirmed or plausibly zoonotic trematodes at the metacercarial stage
Population/food vehicle	Humans, livestock, wildlife or snails without paired food sampling	Freshwater or brackish fish, crabs, crayfish, edible aquatic plants
Detection	Unvalidated visual inspection alone	Digestion, dissection, compression, microscopy, experimental confirmation, PCR or sequencing
Geography/language	None solely on geography	Global, without intended geographical restriction
Study type	Single clinical case without food denominator; laboratory infection only	Field survey, market survey, surveillance, outbreak trace-back, systematic review

Table 2: Review-level evidence informing the synthesis of structure

Primary scope	Evidence base	Key contribution	Review
<i>C. sinensis</i> in fish	71 studies; China, Korea, Vietnam	Pooled prevalence 30.5%	(Zhang <i>et al.</i> , 2020)
<i>Paragonimus</i> in China	271 publications; 172 on second intermediate hosts	<i>P. westermani</i> 52.07%; <i>P. skrjabini</i> 31.10%	(Liu <i>et al.</i> , 2024)
<i>Fasciola</i> in Southeast Asia	100 included records	No eligible metacercariae-on-plant study	(Hoang Quang <i>et al.</i> , 2024)
Four WHO-targeted trematodiasis	115 studies	Large reporting and surveillance gaps	(Tidman <i>et al.</i> , 2023)
Human fascioliasis globally	Human prevalence studies	Burden context; not food prevalence	(Rosas-Hostos Infantes <i>et al.</i> , 2023)
Zoonotic parasites in tilapia	Multiple parasite groups	Overall pooled prevalence 14%	(Acosta-Pérez <i>et al.</i> , 2022)
Freshwater fish zoonotic helminths in Europe	Six principal helminths	Evidence of European fish-borne risk	(Menconi <i>et al.</i> , 2023)
Human foodborne trematodiasis burden	181 quantitative studies	665,352 estimated DALYs for reference year	

Table 3: Selected primary food-level studies with extractable prevalence or intensity information.

Food vehicle	Sampling/detection	Principal observation	Study	Appraisal and principal limitation
France; watercress	59 beds; surveillance	15-year 2.6–6.3 metacercariae per contaminated bed	(Dreyfuss <i>et al.</i> , 2005)	Moderate: long surveillance period and direct observation; bed-level selection and nonstandard intensity limit generalization.
Korea; water dropwort	500 plant samples; PCR/sequencing	2/500 <i>F. hepatica</i> positive (0.4%)	DNA- (Choi <i>et al.</i> , 2015)	Moderate: clear denominator and sequencing; molecular detection does not establish intact or viable metacercariae.
Vietnam; cultured fish	Grow-out and nursery fish; digestion	44.6% and respectively	43.6%, (Chi <i>et al.</i> , 2008)	Moderate: digestion and defined production stages; composite parasite outcome and clustered fish require caution.
Vietnam; cultured/wild fish	1,543 fish; digestion	64.3% cultured; 68.9% wild	(Phan <i>et al.</i> , 2010)	Moderate: large denominator and cultured/wild comparison; heterogeneous hosts and locations complicate direct comparison.
Vietnam; river/pond/reservoir fish	846 fish; pepsin digestion	56.8%, 46.7%, 29.3%	(Phan <i>et al.</i> , 2016)	Moderate: defined habitats and digestion; host composition differs among habitat groups.
Lao PDR; freshwater fish	1,046 fish; artificial digestion	<i>O. viverrini</i> 37.9%; several heterophyids common	(Chi <i>et al.</i> , 2008)	Moderate: large multispecies sample and artificial digestion; multiple host-parasite estimates are not independent.
Japan; crabs	922 crabs; morphology, ITS2	177 positive for <i>P. ohirai</i> (19.2%)	(Sugiyama <i>et al.</i> , 2004)	Moderate: large crab sample with taxonomic confirmation; focal sampling limits geographical inference.
China; freshwater crabs	447 crabs; molecular confirmation	186 infected (41.6%)	(Shu <i>et al.</i> , 2021)	Moderate to higher: clear denominator plus molecular confirmation; sampling remained geographically focal.

Table 4: Principal evidence gaps and recommended research responses.

Domain	Current limitation	Recommended response
Plant carriers	Few denominator-based surveys; viability rarely assessed	Multicountry market and farm surveys using microscopy plus PCR
Fish taxonomy	Common use of composite “FZT” outcome	Species-specific identification and host-species denominators
Crustaceans	Highly focal studies and uneven regional coverage	Spatially stratified sampling with crab/crayfish species identification
Diagnostics	Variable digestion, dissection and molecular methods	Interlaboratory comparison and recovery-efficiency validation
Food-chain metadata	Harvest and processing history often missing	Standard traceability fields from harvest to retail
Viability	Presence does not always equal infectivity	Validated viability markers and processing-challenge studies
Statistics	Multiple estimates per study treated as independent	Multilevel meta-analysis or robust variance estimation
Geography	Strong concentration in Asian endemic regions	Targeted surveillance in Africa, Europe and Latin America

Fish-borne liver and intestinal flukes: The overall prevalence of *C. sinensis* metacercariae among fish was 30.5%, with the prevalence of *C. sinensis* metacercariae in fish ranging from 35.1% in China to 29.7% in Korea to 8.4% in Vietnam (Fig. 1). The overall prevalence of fish-borne trematodes ranged from 29.3 to 68.9%, which differed significantly by trematode species, fish species, production system, and habitat (Table 3).

Crustacean-borne *Paragonimus*: In Chinese second intermediate hosts, the published pooled prevalence was 52.07% (95% CI 43.56–60.52%) for *P. westermani* and 31.10% (95% CI 24.77–37.80%) for *P. skrjabini* (Fig. 2). The primary crab survey was conducted in selected areas, where 19.2% and 41.6% of the primary crabs were found to be positive in Japan and southern China, respectively (Table 3).

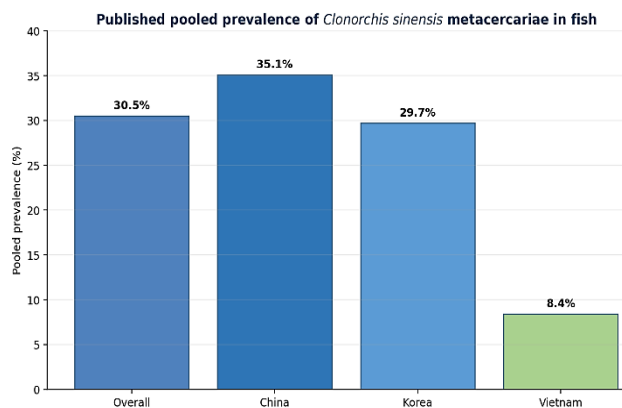


Fig. 1: Published pooled prevalence of *Clonorchis sinensis* metacercariae in fish. Values reproduced from Zhang et al. (2020). bars represent published estimates and were not recalculated in the present synthesis.

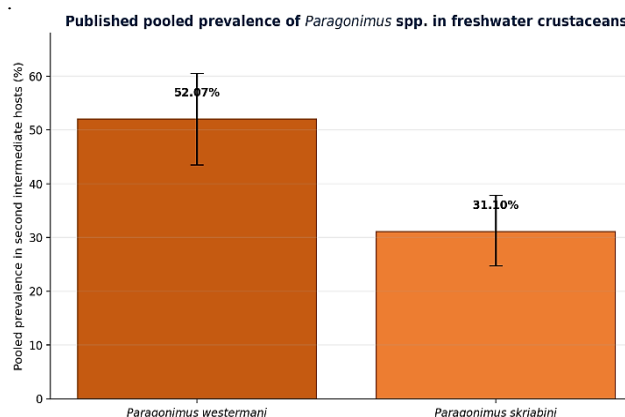


Fig. 2: Published pooled prevalence according to publications of *Paragonimus* species in second intermediate hosts in China. Error bars show published 95% confidence intervals reported by Liu et al. (2024). Estimates were reproduced and were not recalculated in the present synthesis.

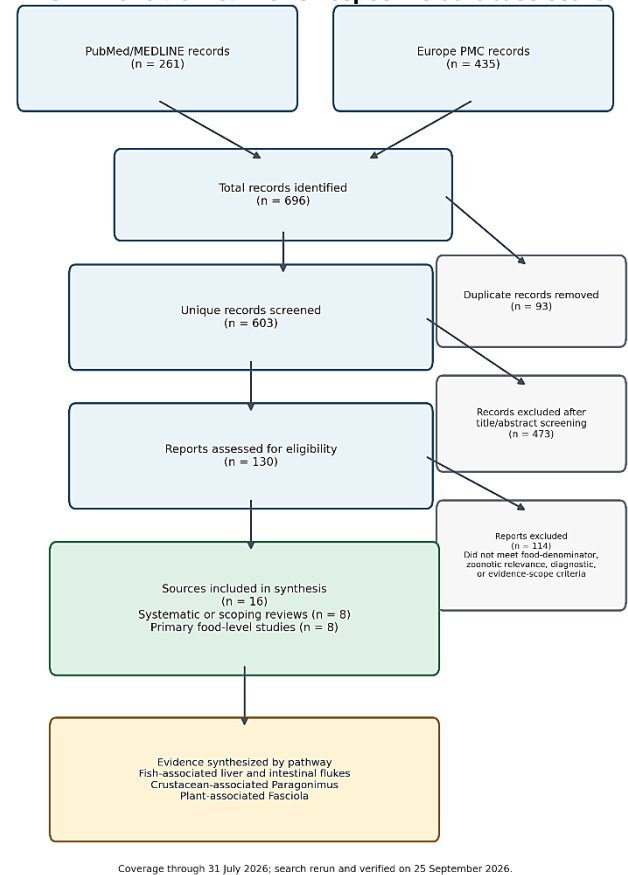
PRISMA 2020 flow of the retrospective database search

Fig. 3: PRISMA 2020 flow diagram for the retrospective PubMed/MEDLINE and Europe PMC search.

The contamination of aquatic vegetables by the parasite *Fasciola*. 3.4 Contamination of aquatic vegetables with the parasite *Fasciola*.

Evidence for the *Fasciola* plant pathway was sparse and based on non-equivalent outcomes. The Southeast Asian review found substantial evidence of infection in humans, livestock and snails but no eligible food-level study demonstrating viable metacercariae on plant carriers. Choi et al. (2015) detected *F. hepatica* DNA in 2 of 500 Korean water-dropwort samples; this establishes molecular contamination only, not the presence or infectivity of intact metacercariae. The French watercress evidence provides ecological and outbreak linkage, but it does not supply a comparable market-prevalence denominator.

Methodological heterogeneity: Substantial heterogeneity occurred in sampling unit, host and parasite identification, recovery or molecular method, outcome definition, habitat and supply-chain stage. Accordingly, published pooled estimates, individual survey prevalence, intensity and

molecular detection are presented as separate evidence types. The data do not support a common worldwide average or direct inference from food contamination to the probability of human infection.

DISCUSSION

Helminths have always been a great threat to human and animal health globally (Rasheed and Aljohani, 2024; Hossain *et al.*, 2025). The evidence indicates that zoonotic trematode metacercariae are relevant hazards in freshwater food chains, but it does not justify a single global prevalence estimate. Most quantitative evidence originated in East and Southeast Asia, and the included studies differed in food vehicle, parasite taxon, denominator and diagnostic method (Table 4). The conclusions must therefore be read as a structured map of pathway-specific evidence rather than a geographically representative global survey (Nyindo and Lukumbagire, 2016; Wang *et al.*, 2022) but fails to justify the calculation of a single coherent “global seafood prevalence”. This interpretation is in line with the scoping review conducted globally by the group: this identified significant gaps in reporting and the review of the burden by Fürst *et al.* (2012) focused on disease burden, not on contaminated food units. The most defensible quantitative benchmarks in the present synthesis were parasite–vehicle specific: *Clonorchis sinensis* in fish (Zhang *et al.*, 2020), *Paragonimus* species in freshwater crustaceans (Liu *et al.*, 2024), and separate fish–plant observations for *Fasciola* (Dreyfuss *et al.*, 2005; Choi *et al.*, 2015). These outcomes include a variety of distinct denominators, diagnostic interpretations, and exposure routes, and should not be combined into a single summary proportion.

Meta-analytic evidence provided by Zhang *et al.* (2020) was presented as the strongest source of information, which refers to fish-borne liver flukes. The pooled prevalence of *C. sinensis* metacercariae in fish was 30.5%, although this varied significantly between China (35.1%), Korea (29.7%) and Vietnam (8.4%). The subgroup patterns reported by Zhang, with summer sampling and higher estimates of contamination in river fish, suggest that the pooled value is an average of the different transmission systems and not a standard probability of contamination. Therefore, it must be evaluated in conjunction with host species, season, habitat and diagnostic method, and in future re-analysis with a prediction interval where possible.

The studies selected from Vietnam provide an explanation as to why an outcome by composite fish-borne trematode is different from the outcome by liver-fluke (Chi *et al.*, 2008). The prevalence of heterophyid and echinostomatid metacercariae was also high in the present study, similar to that reported by (Chi *et al.*, 2008) in grow-out fish (44.6%) and nursery fingerlings (43.6%). Similarly, high overall prevalence was reported in both cultured fish (64.3%) and pond fish (68.9%) by Phan *et al.* (2010), where *Haplorchis pumilio* was common and *C. sinensis* was only found in one pond fish. In a subsequent habitat comparison, Phan *et al.* (2016) reported 56.8% prevalence in river fish; 46.7% in pond fish and 29.3% in reservoir fish, while *C. sinensis* was found only in reservoir and river fish and not pond fish. All these findings indicate

that a high “any zoonotic trematode” prevalence is likely mainly due to intestinal flukes and should not be equated with a clonorchiasis risk prevalence.

The Vietnamese observations also raise a red flag for such an assumption as a cultured fish is always safer than a wild one. The similar prevalence of cultured and wild fish in Phan *et al.* (2016), along with the similar infection rates found in nursery and grow-out systems by Chi *et al.* (2008), indicate that aquaculture ponds might sustain infection if infected snails, reservoir hosts, contamination from feces, or fish self-recruiting are present. The effect of the production system is parasite specific, as *C. sinensis* was not found in pond fish in the latter study by Phan. The dichotomy of wild versus cultured should therefore be considered a moderator for each of the strata of parasite–host–ecosystem and not a risk classification.

The Lao PDR study by Chai *et al.* (2019) also illustrates the value of species resolved data. Of the 1046 fish sampled for 69 species, *O. viverrini* was present at 37.9% and reported prevalence was 45.4% for *H. taichui*, 45.1% for *H. yokogawai*, 18.1% for *C. formosanus* and 8.8% for *H. pumilio*. Some host–parasite combinations had hundreds of metacercaria per infected fish. This discovery brings an aspect of exposure that cannot be accounted for by prevalence – two populations of fish may be equally exposed but may provide very different infective doses.

In addition, the systematic review conducted by (Acosta-Pérez *et al.*, 2022) and the European review by (Menconi *et al.*, 2023) expand the fish-borne evidence beyond the major Asian liver-fluke datasets. The tilapia review, however, was a compilation of several groups of zoonotic parasites, while Menconi *et al.* 2023 compiled multiple groups of freshwater fish-borne helminths, including some that are of importance to Europe. The presence of these adds to a global surveillance approach and stresses the need to not pool taxonomically and biologically dissimilar parasites. The association of quantitative studies in East and Southeast Asia is a pattern of evidence distribution and not evidence that there is no evidence of transmission elsewhere.

Liu *et al.* (2024) obtained the pooled prevalence estimates of *Paragonimus westermani* (52.07%) and *P. skrjabini* (31.10%) in Chinese second intermediate hosts. Ecological modelling is supported by the difference between the two species, and by the reported associations with altitude, temperature and precipitation. Local variation is even higher for the primary crab studies. Sugiyama *et al.* (2004) reported the presence of *P. ohirai* in 19.2% of 922 brackish-water crabs collected in Japan; prevalence measured at each site was between 5% and 89%, and intensity ranged from 1 to 190 metacercariae per infected crab. In China, infection was molecularly confirmed in 41.6% of a total of 447 freshwater crabs from southern China (Shu *et al.*, 2021). Therefore, the regional figures in this paper are of value for regional comparison but may be very different for the consumers depending on the species of crab, the location and the preparation.

Rabone *et al.* (2021) also observed the sub-Saharan African region, which showed a persistent, but geographically variable presence of *Paragonimus* transmission, with high host effects and focal effects. The average prevalence (0–100%) was about 19.9% for *P. africanus* and about 25.1% for *P. uterobilateralis*, but

sometimes was as high as 100% and on other occasions as low as zero across locations and genera of freshwater crabs. This evidence, when complemented with the evidence presented in Liu *et al.* (2024), Sugiyama *et al.* (2004) and Shu *et al.* (2021), justifies the use of spatially stratified sampling and not national convenience sampling. It also allows for reporting of crustacean species, tissue examined, homogenization/digestion procedure, molecular target, and the denominator (site-level). Crustacean surveillance is a valuable One Health early-warning tool as they convert these localized enzootic foci into human exposure when consumed raw, pickled or not cooked thoroughly.

The Fasciola evidence is a problem of scarcity of data and non-equivalent outcomes. There was significant evidence for Fasciola infection in humans, livestock and snails in Southeast Asia, but no suitable study directly demonstrating the presence of metacercariae on plant carriers. Major evidence for Fasciola infection in humans, livestock and snails in SE Asia, no eligible studies showing the presence of metacercariae on plant carriers. In a survey of 500 water-dropwort samples from Korea, Choi *et al.* (2015) found *F. hepatica* DNA in 2 (0.4%) but molecular positivity is not a proof of the presence or viability of intact metacercariae. In direct monitoring of 59 natural beds of French watercress over 15 years, however, Dreyfuss *et al.* (2005) reported low, but regular, burdens of 2.6-6.3 intact metacercariae per contaminated bed. These studies cannot be combined statistically as the one is plant-sample DNA positive and the other, metacercarial burden/ contaminated bed.

Biological and epidemiological evidence about the watercress pathway is provided by the French investigations. Rondelaud *et al.* (2005) associated contamination and upstream migration of lymnaeid snails, and more recent studies on cercarial dispersal highlighted the potential for disproportionate contamination of certain areas within watercress beds (Rondelaud *et al.*, 2020). Mailles *et al.* (2006) then followed up 18 human cases of fascioliasis to the raw watercress that was commercially sold from a producer that was not protected against lymnaeid snails. When combined, surveillance, ecological and outbreak investigations indicate that there is a potential for clustered disease even if the product is contaminated with low frequency but the metacercaria is widely distributed and consumed uncooked. However, the intensity per serving, the viability, the consumption frequency and reach of distribution must be added to prevalence in order to have a risk characterization.

Included studies were also different in methods used to recover metacercariae from fish (digestion-based) and in accounting for the parasite DNA in plant washes and the molecular confirmation of crustacean identification by Shu *et al.* (2021). Chi *et al.* (2008), (Phan *et al.*, 2010) Phan *et al.* (2016) and Chai *et al.* (2019), whereas Choi *et al.* (2015) measured parasite DNA in plant washes and Shu *et al.* (2021) added molecular confirmation to crustacean identification. Recovery efficiency should be reported in future studies and whenever possible, a representative sample should be recovered and confirmed by morphology and ITS or mitochondrial DNA sequence.

International trade strengthens the implications of the studies included as it can be shipped to a distance from the transmission ecosystem. The outbreak in France shows the

importance of producer-level traceability and the prevalence of crabs at the focal point reported by Sugiyama. From this, one might expect that COO is the only factor that matters, but as demonstrated by (Sugiyama *et al.*, 2004) and (Shu *et al.*, 2021), this might not be the case. Traceability fields should contain harvest water body, species, wild or cultured, producer/batch, processing history, storage temperature and intended mode of consumption. Small metacercariae are likely to be missed by gross visual screening and surveillance should focus on raw, fermented, pickled and lightly cooked freshwater products.

Control implications from the evidence included varies by food vehicle. The Vietnamese fish studies (Chi *et al.*, 2008; Phan *et al.*, 2010; Phan *et al.*, 2016) advocate for pond sanitation, to prevent fecal access and control snail and reservoir-host access. The crab studies (Sugiyama *et al.*, 2004; Shu *et al.*, 2021) can be used to help with focal environmental surveillance and consumer guidance for proper cooking of crabs. The French watercress (Dreyfuss *et al.*, 2005; Rondelaud *et al.*, 2005; Mailles *et al.*, 2006; Rondelaud *et al.*, 2020) studies confirm the need to protect watercress beds from infected snails and contaminated upstream habitats. Once the processing steps are reached, the guidance for parasites and products needs to be validated with time, temperature and product. These interventions must be done in conjunction with culturally relevant education that minimizes exposure while not stigmatizing traditional foods.

In summary, there are included systematic reviews and primary studies that are broadly in favor of One Health interpretation. Transmission in food refers to interactions between definitive hosts, snail intermediate hosts, aquatic ecology, agricultural or aquaculture practices, climate and food processing and human dietary habits (Gabriël *et al.*, 2022; Tidman *et al.*, 2023). Coordinated surveillance should thus integrate measurements of food-level metacercariae with animal-reservoir and snail and human-case data and maintain separate analyses of the latter. This would benefit early detection of transmission hotspot sites and would prevent denominator errors of collapsing the human, animal, snail and food infection data into a single epidemiological outcome.

Conclusions: Zoonotic trematode metacercariae continue to pose a threat in freshwater food chains all over the world. The most fully developed evidence is for *C. sinensis*, *Opisthorchis* and for zoonotic intestinal flukes of fish and *Paragonimus* for freshwater crustaceans. Pooled prevalence of *C. sinensis*, ranging up to about one-third of the examined fish, occurs throughout China, Korea and Vietnam and of the major species of *Paragonimus*, up to 30–50% of second intermediate hosts in China. Rapid measurement of Fasciola on edible aquatic plants is less advanced, although there is definite epidemiological proof that infected watercress and related vegetables act as vectors of infection in man. A single “global seafood prevalence” would mask important differences in parasite biology, food vehicle, diagnostic method and consumer exposure. For future surveillance and meta-analysis, different parasite–food strata should be used, denominators should be host- and site-specific, intensity as well as prevalence should be reported, and molecular

contamination from viable metacercariae should be differentiated. Safe aquatic-food production and trade is maintained by coordinated One Health surveillance, adequate traceability, validated food safety processing, environmental sanitation and culturally relevant food safety education, which ensure a decrease in transmission.

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