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Diagnostic tools for the detection of taeniid eggs in different environmental matrices: A systematic review.

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ABSTRACT

The cestode family Taeniidae consists of the genera *Echinococcus* and *Taenia*, both of which include zoonotic tapeworms of serious public health importance. Various environmental matrices have been identified from which parasite transmission to animals and humans can occur, and many techniques for detecting taeniid eggs in different environments have been developed. However, the majority lack appropriate validation, and standardized egg isolation procedures are absent. This hampers interstudy comparisons and poses a challenge for future researchers when deciding which technique to implement for assessing taeniid egg contamination in a particular matrix. Therefore, the aim of this systematic review was to present an overview of the detection methods for taeniid eggs in the environment, to discuss and compare them, and to provide recommendations for future studies. In total, 1814 publications were retrieved from scientific databases, and, ultimately, data were systematically reviewed from 90 papers. The results provide an overview of numerous diagnostic tests for taeniid egg detection in (or on) water, food, soil, insects, objects, and air. These tools could be categorized as either conventional (light microscopy), molecular, or immunodetection tools. The relatively cheap microscopy techniques often lack sensitivity and are unable to identify a taeniid egg at the genus level. Nevertheless, several records ascribed a genus, or even species, to taeniid eggs that had been detected by light microscopy. Molecular and immunodetection tools offer better specificity, but still rely on the preceding egg recovery steps that also affect overall sensitivity. Finally, the majority of the methods lacked any attempt at performance evaluation and standardization, especially at the earlier stages of the analysis (e.g., sampling strategy, storage conditions, egg recovery), and viability was rarely addressed. As such, our review highlights the need for standardized, validated detection tools, that not only assess the extent of environmental contamination, but also the egg genus or species, and address viability.

1. Introduction

The cestode family Taeniidae is composed of two valid genera, *Echinococcus* and *Taenia*, both including zoonotic tapeworms of

Abbreviations: IMH, intermediate host; LOD, limit of detection; FD, formalin-detergent; s.g., specific gravity; ddPCR, digital droplet PCR; *rrnL*, large subunit gene of rRNA; *cox-1*, cytochrome C oxidase subunit I; *nad*, NADH dehydrogenase subunit.

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serious public health importance (Nakao et al., 2013). In the case of *Echinococcus*, humans can acquire alveolar and cystic echinococcosis after ingestion of *Echinococcus multilocularis* and *Echinococcus granulosus* eggs, respectively, with poor prognosis if managed inadequately (Moro and Schantz, 2009; Thompson, 2017). Also, some *Taenia* species (i.e., *Taenia solium*) may cause serious infection (e.g., neurocysticercosis) in humans if eggs are ingested (Garcia and Del Brutto, 2000).

With an estimated global burden of 2.79 million and 871,000 disability-adjusted life-years (DALYs) each year for cysticercosis and echinococcosis, respectively (Torgerson et al., 2015), *Taenia solium*, *E. granulosus,* and *E. multilocularis* are the top-three ranked parasites in the World Health Organization/Food and Agriculture Organization's list of foodborne parasites with the greatest global impact (FAO/WHO, 2014). Furthermore, *Taenia saginata*, *T. solium*, and *E. granulosus* also cause significant economic losses to farmers and the meat sector, as the value of condemned carcasses is about 50–100% lower (Jansen et al., 2018; Yildiz, 2019). Although substantial progress has been made in the development of sensitive and specific tools for detection of these parasites in people, meat, animal feces, and blood (both antigen and antibody), detection of parasite eggs in the environment, which is also critical for control, still remains a challenging task.

Studies suggest that infected dogs and foxes may excrete 42–114 eggs per *Echinococcus* worm daily, while a human tapeworm carrier can disseminate as many as half a million eggs per day. Although the egg release per *Echinococcus* spp. worm is lower than that of *Taenia* spp., the number of worms per infected host is much higher, with up to 100,000 *Echinococcus* worms in an infected canid but usually 1 (up to 10) *Taenia* worm in infected humans (Alvarez Rojas et al., 2018). As such, environmental contamination is extensive, with three matrices being the main vehicles for environmental transmission of taeniids to their intermediate hosts (IMHs); specifically, contaminated water, food, and soil. These matrices could be good indicators for estimating the level of environmental contamination, infection levels in animal and people that may contaminate these environments, and the risks of human and animal infection (Huerta et al., 2008) (Fig. 1).

Fruits and vegetables may easily become contaminated with taeniid eggs in various ways along the production chain, and thereby become a source of parasitic infection to people, especially when consumed raw and not properly washed. Contamination of fresh

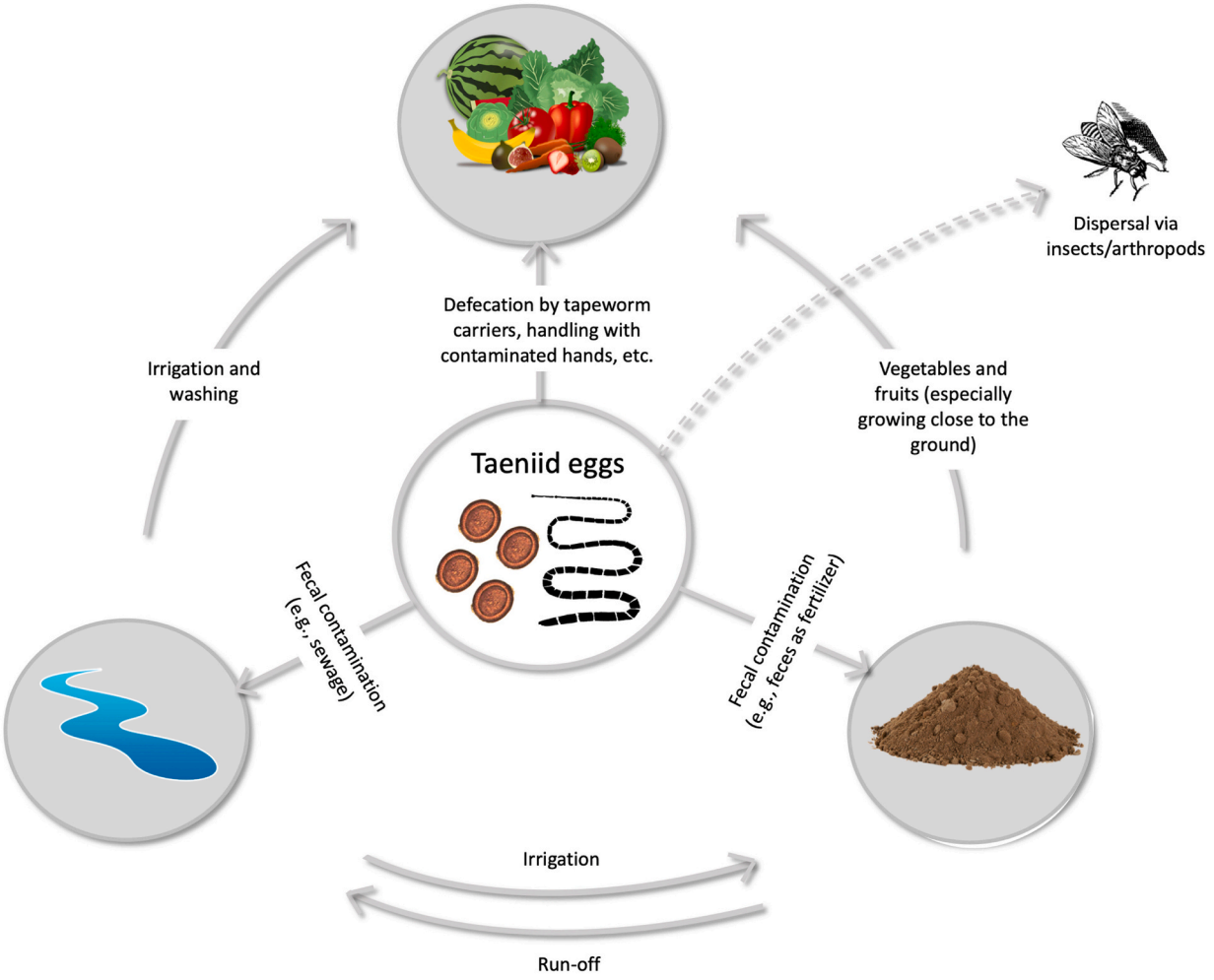

Fig. 1. Overview of environmental matrices from where taeniid transmission can occur to an appropriate or aberrant intermediate host.

produce with *T. solium* eggs in human feces may occur by infected food handlers with parasite eggs on their hands (during harvesting or transport), whereas *Echinococcus* species eggs can contaminate fresh produce via feces of infected dogs or foxes. In addition, in rural villages where open defecation, and/or use of feces as fertilizer, may be common, there is the potential for contamination of vegetables and fruit; washing or irrigating such fresh produce with contaminated water may also result in contamination. Similarly, animal IMHs may become infected by ingestion of vegetables or fruit that have been contaminated this way (Fallah et al., 2012).

Water is another matrix that can act as a transmission vehicle, either indirectly as described above, or by direct ingestion of contaminated water. In addition to irrigation of crops with contaminated water, pastures for livestock may also be contaminated via irrigation water (Fallah et al., 2012; Dudlová et al., 2015). Eggs shed by human or canid tapeworm carriers may contaminate drinking water sources or irrigation water, either by direct defecation into water supplies, run off from contaminated land, or contamination by wastewater.

A final matrix forming a major risk in the dispersal of intestinal parasites among humans and animals, is soil, which can become contaminated with taeniid eggs directly from fecal matter from infected hosts, from irrigation with contaminated water (e.g., land disposal of sewage effluent), or from flooding with contaminated water, including wastewater, particularly during extreme weather events such as excessive rainfall or snowmelt (Jansen et al., 2021). Furthermore, contaminated soil constitutes an important source from which taeniid eggs can be transported directly into water sources, onto vegetables, or onto hands (Aghaindum et al., 2019).

Soil, water, and food/feed are thus the main environmental matrices for taeniid transmission to humans and animals. In addition, infection of people with eggs attached to objects such as doorknobs and tables should not be underestimated, particularly in resource-poor countries where basic sanitation may be inadequate. This is especially important among school-age children who may have greater contact with contaminated objects during play and may not have adequate hygienic habits (Pereira et al., 2016). Insects, such as flies and beetles, have also been linked to transmission of infection. These scavengers feed on, and breed prolifically in, human/animal excrement for which they may travel several kilometers. This, in addition to their sticky feet pads and body hairs that could be laden with potential pathogenic particles, make them the ideal natural transmitters of disease-causing organisms. In fact, it has even been postulated that taeniid eggs can be dispersed up to a distance of 60 km or over 30,000 ha via birds and insects (Torgerson et al., 1995).

In conclusion, various environmental matrices have been identified from which parasite transmission to both animals and humans can occur. As the ingestion of just a few taeniid eggs from contaminated environmental matrices can be responsible for severe disease in humans, and ingestion by the animal IMH maintains the life cycle, assessing the level of environmental contamination is important, not least for determining where interventions should be focused. In the last 40 years, interest in environmental parasitology has increased considerably, with reappraisal of conventional isolation and detection methods. While indispensable for our understanding of environmental transmission routes, these tools often lack sensitivity and specificity. With the upsurge in immunological and molecular techniques, this drawback has somewhat decreased, although the need for more expensive equipment, consumables, and a more sophisticated laboratory, is a drawback.

Environmental samples are also characterized by having different surface structures and sizes in the case of food products, insects, and objects, and a range of turbidities, viscosities, and organic content in the case of water and soil. Furthermore, significant quantities have to be examined to obtain a representative overview of the extent to which a particular environment is contaminated. This is particularly so when eggs are dispersed throughout an extensive area (e.g., a river) and collecting and analyzing several samples of a relatively large volume are needed to increase the likelihood of detection.

Many different techniques for detecting (and quantifying) taeniid eggs in different environmental matrices have been developed and applied. However, many of them lack appropriate validation and standardized procedures for egg isolation from different matrices with regard to sampling strategy and preparation, elution materials, centrifugation settings, detection tools, etc. are often absent. This not only makes interstudy comparisons difficult/impossible, but also poses a challenge for future researchers when deciding which technique to implement for assessing a certain environmental matrix for contamination with taeniid eggs. The latter is especially important in relation to evaluating the impact of a control intervention.

This review aims to: (1) present an overview of all the various tools described for detecting *Taenia* and *Echinococcus* spp. in different environmental matrices; (2) describe and discuss the performances (recovery efficiency, limit of detection (LOD), sensitivity and specificity) of these tools; (3) identify both advantages, as well as gaps, in the detection methods; and (4) provide recommendations for further environmental studies.

2. Methodology

2.1. Review question and search syntax

A systematic search of published literature on 'Diagnostic tools for the detection of taeniid parasites in different environmental matrices' was conducted by implementing the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines (Moher et al., 2015). A completed PRISMA checklist is included in Supplementary Table A to this article. To identify the primary data, a search syntax of Boolean operators (AND, OR, *) and key words referring to the detection/diagnosis of eggs from *Taenia* spp. and *Echinococcus* spp. was developed in agreement of four researchers and systematically applied in three electronic search engines (PubMed, Web of Science, and Scopus). Searches were limited to English language and studies published from January 1986 to September 2020. Hence, only peer-reviewed and indexed literature was retrieved using the following search syntax: "egg*" AND "diagnos*" OR "detect*" AND "*Taenia*" OR "taeniid" OR "taeniasis" OR "taeniosis" OR "taeniidae" AND "*Echinococcus*" OR "echinococcosis".

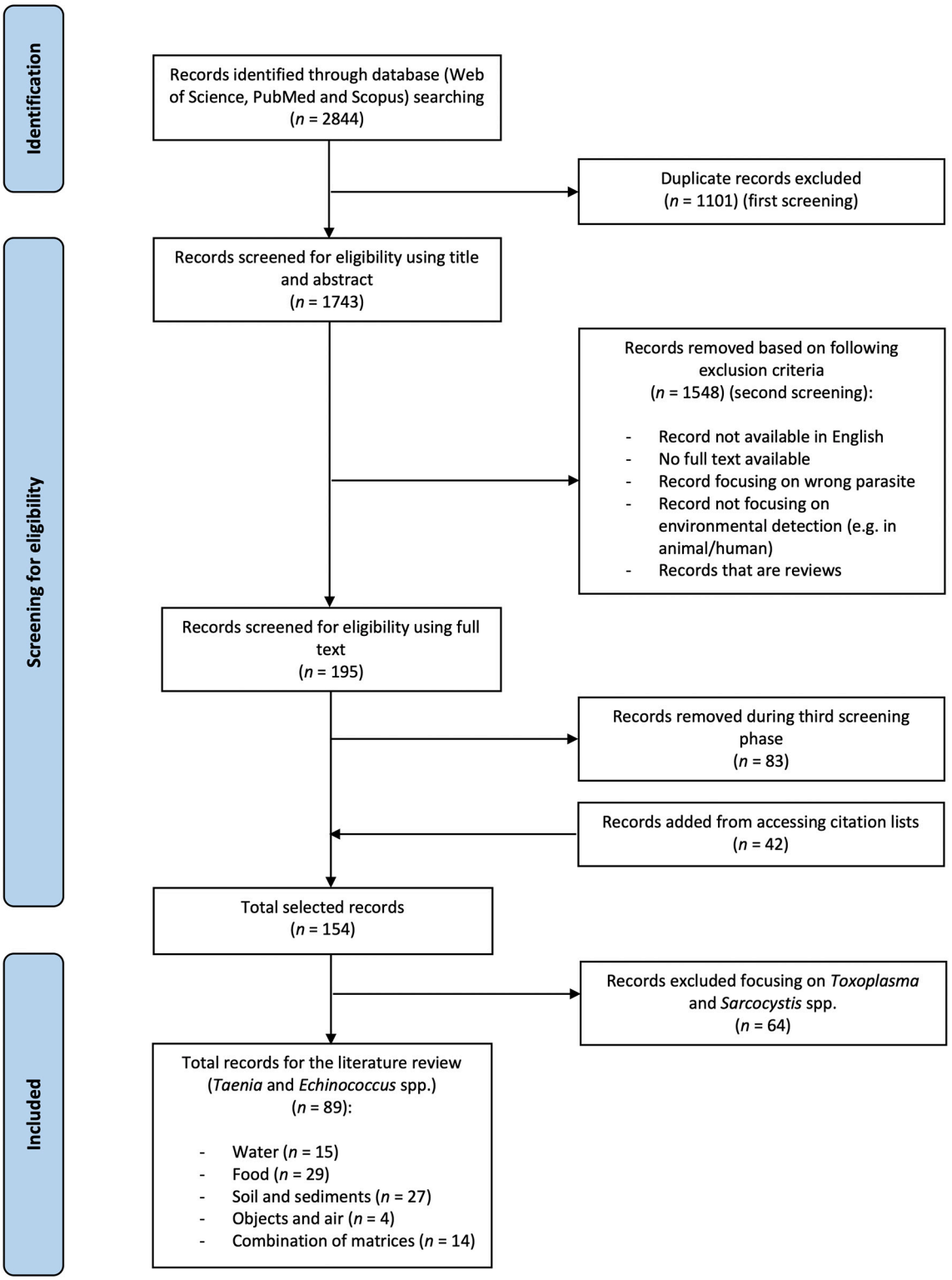

Fig. 2. PRISMA (Preferred Reporting Items for Systematic review and Meta-Analysis) flowchart diagram of the paper selection for the review.

Table 1
Overview of techniques for the detection of taeniid eggs in water samples.

Method	Type of water	Parasite species	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
Sedimentation with microscopy	Drinking water	*Echinococcus/Taenia* spp.	Qualitative	N.M.	Analysis on day of collection	Min. 2	N.M.	Mexico	Diaz-Camacho et al. 1991[a]
	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	N.M.	4	≥ 3 h	Thailand	Wongworapat et al., 2001
Sedimentation with NaOH and microscopy	Well water and rainwater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	N.M.	6–10	≤ 24 h	Vietnam	Noda et al., 2009
Sedimentation by centrifugation, $ZnSO_4$ flotation (s.g. 1.3) and microscopy	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	N.M.	7	≤ 24 h	Morocco	Bouhoum et al., 2000
						7	28d	South Africa	Amoah et al., 2018
Filtration, $ZnSO_4$ flotation (s.g. 1.3) and microscopy	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	Se: 80–90% Sp: 99%	N.M.	4	≤ 12 h	Mexico	Jimenez et al., 2016
Modified Bailenger technique based on sedimentation, ethyl acetate and $ZnSO_4$ flotation (s.g. 1.3) with microscopy	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	N.M.	13	5-6 h	Egypt	Stott et al., 1997
	Effluent wastewater				N.M.	13	5-6 h	Colombia	Madera et al., 2002
	Wastewater				N.M.	13	5-6 h	Iran	Mahvi and Kia, 2006
	Wastewater				Stored on ice and processed within 3 h	13	5-6 h	Pakistan	Ensink et al., 2007
	Wastewater				N.M.	13	5-6 h	Tunisia	Ben Ayed et al., 2009
	Wastewater				At RT for 24 h before analysis	13	≤ 24 h	Tunisia	Khouja et al., 2010[a]
	Surface water				In sealed containers at RT protected from light until analysis	13	5-6 h	Argentina	Souto et al., 2016
	Wastewater				At 4 °C until analysis	13	≤ 24 h	Ethiopia	Woldetsadik et al., 2017
Modified Bailenger technique based on sedimentation, ethyl acetate and saturated sodium chloride (s.g. 1.2) flotation with microscopy	Pond water	*Echinococcus/Taenia* spp.	Quantitative	N.M.	In polyethylene containers at room temperature until analysis	10	≤ 24 h	Argentina	Sánchez Thevenet et al. 2019[a]
Sedimentation, saturated saccharose flotation (s.g. 1.30), centrifugation and microscopy	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	N.M.	5	N.M.	Slovakia	Dudlová et al., 2015
Sedimentation, $MgSO_4$ flotation (s.g. unspecified) with microscopy and Trypan Blue staining for viability	Wastewater	*Echinococcus/Taenia* spp.	Quantitative	N.M.	Stored on ice until analysis	6	N.M.	Bolivia	Verbyla et al. (2013a)[a]
	Wastewater				Stored on ice until analysis	6	N.M.	Bolivia	Verbyla et al. (2013b)
Immunofluorescence test with 4E5 monoclonal antibody	Water from waterholes	*Echinococcus* spp.	Quantitative	N.M.	N.M.	9	7-8 h	Kenya	Craig et al., 1988
Filtration with nested end-point PCR (12S rRNA gene) and real-time PCR (*rrnL* gene)	Surface and ground water	*E. multilocularis*	Quantitative	LOD: 10 eggs/L	At 4 °C until analysis	12	≤ 36 h	Poland	Lass et al., 2019
Filtration with nested end-point PCR (12S rRNA gene), real-time PCR (*rrnL* gene) and LAMP (*nad5* gene)	Wastewater	*E. multilocularis*	Quantitative	LOD: 20 eggs/L	In polypropylene containers	8	≤ 36 h	China	Lass et al., 2020
HDP2 multiplex-PCR	N.M.	*Taenia solium, T. saginata* and *E. granulosus*	Qualitative	Se: <10 pg DNA	N.A.: potential tool for use in the future			Mexico	González et al., 2002

N.M. = not mentioned, N.A. = not applicable, s.g. = specific gravity, Se = sensitivity, Sp = specificity, RT = Room temperature, PCR = Polymerase Chain Reaction, LOD = limit of detection, *rrnL* = large subunit of rRNA, *nad5* = NADH-dehydrogenase subunit 5.

[a] Wrongfully claimed identification at the genus/species level.

Table 2
Overview of detection techniques for taeniid eggs in food.

Method	Parasite species	Food type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
Washing (water), sedimentation, centrifugation and microscopy	*Echinococcus/Taenia* spp.	Fresh leafy greens (celery, watercress, and leek)	Qualitative	N.M.	N.M.	2	N.M.	Iraq	Hadi, 2011[a]
	Echinococcus/Taenia spp.	Fresh vegetables (leek, lettuce, cress, onion, etc.)	Quantitative	N.M.	N.M.	5	9-11 h	Iran	Yakhchali and Ahmadiashtiani, 2004
Washing (DW), sedimentation and microscopy	*Echinococcus/Taenia* spp.	Iceberg lettuce	Qualitative	N.M.	Transported in polyethylene bags in thermal bags for immediate analysis	5	26 h	Brazil	Neto et al., 2012
		Fresh vegetables (potato, onion, spinach, garlic, carrot, etc.)			N.M.	3	N.M.	Pakistan	Eraky et al., 2014[a]
		Fresh vegetables (lettuce, beetroot, radish, etc.) and fruits (apple and grape)			N.M.	4	1-2 h	Iraq	Shakir et al., 2019[a]
Washing (formalin), filtration and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetables (potato, onion, spinach, garlic, carrot, etc.)	Qualitative	N.M.	N.M.	3	1 h	Pakistan	Khan et al., 2017[a]
Washing (0.85% NaCl), sedimentation, centrifugation and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetable (cucumber, lettuce, cress and tomato)	Qualitative	N.M.	N.M.	4	12-13 h	Libya	Abougrain et al., 2010
		Fresh vegetables (lettuce, onion, tomato, etc.)			N.M.	4	$\leq$ 16 h	Ethiopia	Tonjo, 2013[a]
		Fresh vegetables (lettuce, cress, radish, etc.)			N.M.	5	$\leq$ 16 h	Iran	Olyaei and Hajivandii, 2013
		Fresh vegetables (spinach, lettuce, onions, etc.)			In sterile nylon bags	5	26 h	Turkey	Adanir and Tasci, 2013
		Vegetables from supermarket (lettuce, tomato, cucumber, etc.)			In sterile plastic bags	4	$\leq$ 16 h	Jordan	Ismail, 2016
Washing (0.90% NaCl), sedimentation, centrifugation and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetables (cress, leek, radish, etc.)	Qualitative	N.M.	N.M.	4	$\leq$ 16 h	Iran	Fallah et al., 2012
Washing (0.95% NaCl), sedimentation,	*Echinococcus/Taenia* spp.		Qualitative	N.M.	In plastic bags	4	25-26 h	Iran	Daryani et al., 2008[a]

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Table 2 (*continued*)

Method	Parasite species	Food type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
centrifugation and microscopy		Fresh vegetables (cress, lettuce, leek, radish, etc.)							
		Leafy greens (lettuce, spinach, leek, etc.)			N.M.	4	≤ 16 h	Saudi-Arabia	Al-Megrin, 2010
		Fresh vegetables (cress, lettuce, leek, radish, etc.)			N.M.	4	25-26 h	Iran	Saki et al., 2013
		Leafy greens (parsley, coriander, lettuce, etc.)			N.M.	5	8-9 h	Yemen	Al-Sanabania et al., 2016
		Vegetables from supermarket (spinach, leek, radish, etc.)			N.M.	4	6-7 h	Iran	Asadpour et al., 2016
		Leafy vegetables (celery, lettuce, cress, etc.)			N.M.	4	≤ 16 h	Iraq	Khalil, 2019[a]
Formalin-ether sedimentation technique	*Echinococcus/Taenia* spp.	Pre-washed vegetables (onions, pumpkin, carrot, etc.)	Quantitative	N.M.	Wrapped in polyethylene bags	9	9-10 h	Nigeria	Adenusi et al., 2015
		Fresh vegetables (radish, spinach, parsley, lettuce, green onion, etc.)	Qualitative		N.M.	5	10-11 h	Iran	Rostami et al., 2016
Sonication with detergent, sedimentation and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetables (lettuce, tomatoes, carrots, parsley, etc.)	Quantitative	N.M.	N.M.	4	1-2 h	Turkey	Kozan et al., 2005
		Fresh vegetables (lettuce, onion, cabbage, etc.)			At RT until analysis	3	1-2 h	Lao PDR	Maipanich et al., 2011
Washing (DW), shaking with NaCl and $ZnSO_4$ (s.g. unspecified) and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetables (potato, onion, spinach, garlic, carrot, etc.)	Qualitative	N.M.	N.M.	4	1-2 h	Pakistan	Eraky et al., 2014[a] and Khan et al., 2017[a]
Washing (water), filtration, sedimentation, saturated $NaNO_3$ (s.g. unspecified) flotation and microscopy	*Echinococcus/Taenia* spp.	Copra-meal (feed supplement)	Qualitative	N.M.	N.M.	5–6	≤ 16 h	Australia	Jenkins et al., 2013
Washing (DW), sedimentation, saturated salt flotation (s.g. 1.2), centrifugation and microscopy	*Echinococcus/Taenia* spp.	Fresh vegetables (eggplant, pumpkin, spinach, etc.)	Qualitative	N.M.	N.M.	5	1-2 h	Nigeria	Opara and Udoidung, 2003
Washing (0.95% NaCl), sedimentation, saturated sucrose (s.g. 1.21),	*Echinococcus/Taenia* spp.	Fresh vegetables (spinach, lettuce, carrots, etc.)	Qualitative	N.M.	Transported in polyethylene bags	7–8	14–16 h	Nigeria	Maikai et al., 2012

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Table 2 (*continued*)

Method	Parasite species	Food type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
centrifugation and microscopy					for immediate analysis				
	Echinococcus/Taenia spp.	Fresh vegetables (lettuce, carrot, mushroom, union, etc.)	Qualitative	N.M.	Transported at 9–10 °C for immediate analysis	6–7	16-18 h	Iran	Fallah et al., 2016
Modified Bailenger technique (adapted to food) based on washing, sedimentation, ethyl acetate and $ZnSO_4$ (s.g. 1.3) flotation with microscopy	*Echinococcus/Taenia* spp.	Leafy greens, cauliflower, and vegetables with smooth surface	Quantitative	N.M.	Stored on ice and processed within 3 h	9	≤ 16 h	Pakistan	Ensink et al., 2007
		Lettuce			At 4 °C until analysis	9	≤ 16 h	Ethiopia	Woldetsadik et al., 2017
Washing (0.9% NaCl), sedimentation, centrifugation, sucrose flotation (s.g. 1.21), microscopy and end-point PCR (12S rRNA gene)	*Echinococcus/Taenia* spp. (non-molecular part) *E. granulosus* (PCR)	Fresh vegetables (chard, celery, lettuce, etc.)	Quantitative (non-molecular part) and semi-quantitative (PCR)	N.M.	N.M.	10	≤ 36 h	Tunisia	M'Rad et al., 2020
Washing (Tween 20), filtration, Calcium Fluor white staining, microscopy and SYBR green real-time qPCR (*nad1* gene)	*E. multilocularis*	Bilberreis and lingonberries	Semi-quantitative	LOD: 50 eggs/ 250 g berries	At −20 °C until analysis	11	≤ 24 h	Finland and Estonia	Malkamäki et al., 2019a
Washing (Tween 80), sedimentation, centrifugation, $ZnCl_2$ (s.g. 1.4) flotation and nested end-point PCR (12S rRNA gene)	*E. multilocularis*	Berries, mushrooms, and vegetables (lettuce, beets, celery, etc.)	Semi-quantitative	LOD: 100 eggs/ 400 g	In disposable bags	12	≤ 48 h	Poland	Lass et al., 2015
		Berries, mushrooms, and vegetables (lettuce, beets, celery, etc.)						Poland	Lass et al., 2017
Shaking (Alconox®), centrifugation and multiplex real-time qPCR	*E. multilocularis* (12S rRNA), (*Toxoplasma gondii* and *Cyclospora cayetanensis*)	Raspberries and blueberries	Quantitative	LOD: 5 eggs/30 g	N.M.	11	≤ 24 h	N.M.	Temesgen et al., 2019
Washing (water and Tween 20), sieving system, centrifugation and multiplex end-point qPCR	*E. multilocularis* (*nad2*) (and *T. gondii*)	Fresh lettuce	Quantitative	LOD: 1–2 eggs/ 300 g	N.M.	10	≤ 36 h	Switzerland	Guggisberg et al., 2020
Washing (water), filtration, centrifugation, microscope and multiplex end-point qPCR	*E. multilocularis* (*nad1*), *E. granulosus, Taenia* spp. and *Mesocestoides* spp. (12S rRNA)	Vegetables (lettuce, broccoli, leek, beetroot, etc.) and fruits (apple and pear)	Quantitative	N.M.	N.M.	11	N.M.	Switzerland	Federer et al., 2016
			Quantitative		N.M.	16	16 h	Canada	Frey et al., 2019

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Table 2 (*continued*)

Method	Parasite species	Food type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
Washing (Alconox ®, glycine or sodium pyrophosphate), filtration and real-time qPCR with MCA	*E. multilocularis* (*nad1*), *E. granulosus* (12S rRNA) and *Taenia* spp. (12S rRNA)	Leafy greens (romaine lettuce) and berries (strawberries)		LOD: 5 eggs/35 g lettuce or 55 g berries					

s.g. = specific gravity, PCR = Polymerase chain reaction, DW = distilled water, LOD = limit of detection, *nad1* or *2* = NADH-dehydrogenase subunit 1 or 2, N.A. = not applicable, N.M. = not mentioned, RT = room temperature, MCA = melting curve analysis.

[a] Wrongfully claimed identification at the genus/species level.

Table 3
Overview of detection techniques for taeniid eggs in soil samples.

Method	Parasite species	Soil type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
Centrifugation and microscopy (Kato-Katz technique)	*Echinococcus* spp./ *Taenia* spp.	Mud	Quantitative	N.M.	Stored in sterile polyethylene bottles in refrigerator for 24-48 h	3	48 h	Cameroon	Aghaindum et al., 2019[a]
Sedimentation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Mud	Quantitative	N.M.	Stored in sterile polyethylene bottles in refrigerator for 24-48 h	5	48 h	Cameroon	Aghaindum et al., 2019[a]
Dilution (NaCl), sedimentation and microscopy	*Echinococcus*/ *Taenia* spp.	Soil	Qualitative	N.M.	N.M.	2	N.M.	Uzbekistan	Yong et al., 2019
Dilution (Na_3 $(PO_4)_3$), sedimentation, filtration, centrifugation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Soil and toilet sediment	Qualitative	N.M.	N.M.	6	≥ 7d	Russia	Slepchenko et al., 2019[a]
Dilution (water), filtration and microscopy	*Echinococcus* spp./ *Taenia* spp.	Sand, turf and soil	Qualitative	N.M.	N.M.	7	≤ 48 h	Brazil	Tiyo et al., 2008, Moura et al., 2010[a]
Dilution (water), filtration, sedimentation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Sand, turf and soil	Qualitative	N.M.	N.M.	4	≤ 24 h	Brazil	Tiyo et al., 2008, Moura et al., 2010[a]
Filtration, centrifugation and formalin-ether sedimentation technique	*Echinococcus* spp./ *Taenia* spp.	Topsoil	Qualitative	N.M.	In polyethylene bags at 4 °C until analysis	9	≤ 30 h	Argentina	Sánchez-Thevenet et al., 2004
		Soil	Quantitative		In refrigerator until analysis within 72 h	15	≤ 36 h	Mexico	Huerta et al., 2008[a]
		Mud	Quantitative		Stored in sterile polyethylene bottles in refrigerator for 24-48 h	5	48 h	Cameroon	Aghaindum et al., 2019[a]
Dilution (water or saturated salt with s.g. 1.2), filtration, sedimentation, saturated salt (s.g. 1.2) flotation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Soil	Qualitative	N.M.	N.M.	7	1-2 h	Nigeria	Opara and Udoidung, 2003[a]
		Sandsoil and soil				5	1-2 h	Turkey	Aydın, 2020
		Soil and dusts				6	1-2 h	Iran	Gholami et al., 2020[a]
$ZnSO_4$ flotation (s.g. 1.42), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Sand and turf	Qualitative	N.M.	N.M.	Min. 2	N.M.	Brazil	Tiyo et al., 2008
Saturated sugar flotation (s.g. 1.24), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Sand and turf	Qualitative	N.M.	N.M.	Min. 2	N.M.	Brazil	Tiyo et al., 2008
Saturated salt (s.g. 1.2) flotation and microscopy	*Echinococcus*/ *Taenia* spp.	Soil	Qualitative	N.M.	N.M.	2	N.M.	Uzbekistan	Yong et al., 2019
Filtration, $ZnSO_4$ flotation (1.18), centrifugation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Soil	Quantitative	N.M.	In refrigerator until analysis within 72 h	18	≤ 30 h	Mexico	Huerta et al., 2008[a]
Sedimentation, flotation with 10% formalin and $ZnSO_4$ (s.g. 1.18), centrifugation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Mud	Quantitative	N.M.	Stored in sterile polyethylene bottles in refrigerator for 24-48 h	4	48 h	Cameroon	Aghaindum et al., 2019[a]
Flotation with 10% formalin and $ZnSO_4$ (s.g. 1.42), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Soil	Qualitative	N.M.	Analysis the same day	4	1-2 h	Mexico	Díaz Camacho et al., 1991
		Soil	Qualitative	N.M.	N.M.	4	1-2 h	Brazil	Moura et al., 2010[a]
Dilution (NH_4HCO_3), sedimentation, centrifugation, $ZnSO_4$ flotation (s.g. 1.27) and microscopy	*Echinococcus* spp./ *Taenia* spp.	Sludge	Quantitative	N.M.	N.M.	6	1-2 h		Bouhoum et al., 2000[a]
		Sludge	Qualitative			7	≤ 16 h		El Hayany et al., 2018
			Quantitative			6	1-2 h		El Fels et al., 2019

(*continued on next page*)

Table 3 (*continued*)

Method	Parasite species	Soil type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
		Sewage sludge							
Modified Bailenger technique based on washing (NH_4HCO_3), sedimentation, ethyl acetate and $ZnSO_4$ flotation (s.g. 1.28) with microscopy	*Echinococcus*/ *Taenia* spp.	Sludge	Quantitative	N.M.	At RT for 24 h before analysis	10	≤ 16 h	Tunisia	Khouja et al., 2010
Filtration, Sheather's (sucrose) flotation (s.g. 1.30), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Topsoil	Qualitative	N.M.	In polyethylene bags at 4 °C until analysis	9	≤ 30 h	Argentina	Sánchez-Thevenet et al., 2004
				N.M.		6	3-4 h	Saudi Arabia	Shathele and El Hassan, 2009
Filtration, sedimentation, sucrose flotation (s.g. 1.27), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Soil	Quantitative	N.M.	At 48 °C until analysis	7	1-2 h	Nigeria	Maikai et al., 2012
Sheather's (sucrose) flotation (s.g. 1.30), centrifugation and microscopy	*Echinococcus*/ *Taenia* spp.	Silt	Quantitative	RE: 2.5%	At 4 °C until analysis	4	1-2 h	Canada	Scandrett and Gajadhar, 2004
Filtration, centrifugation, formalin-ether sedimentation, $ZnSO_4$ (s.g. 1.40) flotation and microscopy	*Echinococcus*/ *Taenia* spp.	Sludge	Quantitative	RE: 6.7–20.9%	N.M.	11–15	4-5 h	The U.K.	Satchwell, 1986
Dilution (sodium hypochlorite), centrifugation, sucrose flotation (s.g. 1.2) and microscopy	*Echinococcus*/ *Taenia* spp.	Soil	Qualitative	N.M.	At RT until analysis	5	3-4 h	Lao PDR	Maipanich et al., 2011
Dilution (water or Tween 80), filtration, centrifugation, sucrose flotation (1.27) and microscopy	*Echinococcus*/ *Taenia* spp.	Soil from ferry boats	Qualitative	N.M.	N.M.	8	1-2 h	Japan	Matsudo et al., 2003[a]
	Echinococcus/ *Taenia* spp.	Soil		LOD: 10 eggs/g					Matsuo and Kamiya, 2005
Dilution (NaOH), filtration, centrifugation, washing, saturated $NaNO_3$ flotation (s.g. unspecified) and microscopy	*Echinococcus*/ *Taenia* spp.	Topsoil and sand	Qualitative	N.M.	In polyethylene bags at RT until analysis within 1w	7	2-3 h	Bulgaria	Cvetkova et al., 2018
Sedimentation, centrifugation, $ZnSO_4$ flotation (s.g. 1.2), saccharose (s.g. 1.30) sedimentation and microscopy	*Echinococcus* spp./ *Taenia* spp.	Sludge	Qualitative	N.M.	N.M.	5	N.M.	Slovakia	Dudlová et al., 2015[a]
Sedimentation, $MgSO_4$ flotation (s.g. unspecified) with microscopy	*Echinococcus* spp./ *Taenia* spp.	Sludge	Quantitative	N.M.	Stored on ice until analysis	6	N.M.	Bolivia	Verbyla et al. (2013a)[a] Verbyla et al. (2013b)[a]
Immunofluorescence test with monoclonal 4E5 antibody	*Echinococcus* spp.	Soil and sand	Quantitative	N.M.	N.M.	9	7-8 h	Kenya	Craig et al., 1988
Sieving, drying, dilution (PBS + 0.3% Tween 20), microscopy, and Western blot	*E. granulosus*	Topsoil and sludge	Qualitative	N.M.	In polyethylene bags at 4 °C until analysis	N.M.	N.M.	Argentina	Sánchez-Thevenet et al., 2019
Double sieving, $ZnCl_2$ flotation (s.g. 1.45) and ddPCR (*cox*-1)	*Taenia solium*	Soil	Quantitative	N.M.	N.M.	5	N.M.	Tanzania	Maganira et al., 2020
Washing (PBS + Tween 20), $ZnCl_2$ flotation (s.g. 1.45) and dd PCR (*cox*-1)	*T. solium*	Clay, silt, sand and loam soil	Quantitative	Se: 13–36%	N.M.	11	≤ 48 h	Tanzania	Maganira et al., 2019
Washing (0.2% Tween 20), sieving centrifugation $ZnCl_2$ flotation (s.g. 1.42) and real-time qPCR (*rrnL*)	*E. multilocularis*	Soil	Quantitative	LOD: 1 egg/10 g	Stored at −80 °C until analysis	11	≤ 24 h	France	Umhang et al., 2017
Sieving, $ZnCl_2$ flotation (s.g. 1.4) and nested PCR (12S rRNA)	*E. multilocularis*	Soil	Qualitative	LOD flotation: 100 eggs/40 g	Dried at RT for 2–3 days	13	≤ 4d	Poland	Szostakowska et al., 2014

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Table 3 (*continued*)

Method	Parasite species	Soil type	Qualitative/ Quantitative	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
				LOD PCR: 1 egg/40 g					
Sieving, saturated $NaNO_3$ flotation (s.g. 1.35) and real-time qPCR (12S rRNA)	*E. granulosus*	Soil	Qualitative	LOD: 2 eggs/5 g	Stored at −80 °C until analysis	10	≤ 24 h	Kazakhstan	Shaikenov et al., 2004
HDP2 multiplex-PCR	*T. solium, T. saginata* and *E. granulosus*	N.A.	Qualitative	Se: <10 pg DNA	N.A.: potential tool for use in the future			Mexico	González et al., 2002

PCR = Polymerase Chain Reaction, ddPCR = digital droplet PCR, DW = distilled water, *cox*-1 = cytochrome oxidase 1, *rrnL* = large subunit of rRNA, N.A. = not applicable, N.M. = not mentioned, RT = room temperature, s.g. = specific gravity, Se = sensitivity, LOD = limit of detection.

[a] Wrongfully claimed identification at the genus/species level.

2.2. Study selection

Relevant records for the literature review were obtained through three screening phases. A first screening removed duplicates after merging the results from the three search engines in the reference management software EndNote X9 (Clarivate Analytics, 2019). The second screening phase then evaluated titles and abstracts regarding the scope of the study question by use of Rayyan (http://rayyan.qcri.org), a web application developed specifically to expedite the initial screening for reviews in a semi-automatic way (Ouzzani et al., 2016). This was performed blinded and independently by two authors with focus on the following exclusion criteria: (i) record not available in English, (ii) record with no available full text, (iii) record focusing on the wrong parasite, (iv) record not focusing on environmental detection (e.g., detection in the animal/human hosts, focus on treatment, etc.), and finally, (v) record is a review. Likewise, a final screening phase was carried out, now applied to the full text. Lastly, records overlooked by the search syntax were identified by accessing the reference lists of each selected record and their fitness regarding the scope of the review was evaluated by reading the full text. The final selected records were subsequently categorized per environmental matrix (water, soil and sediments, food, insects, and other) in order to extract the following data: parasite genus (and species), country of study, implemented diagnostic tool(s), sample size, sample storage method, equipment and supply needs, qualitative or quantitative test, performance of the diagnostic tool(s), number of steps to results, centrifugation settings, filter/sieve apertures, prevalence outcome, viability determination, and finally, technical advantages and disadvantages.

3. Results and discussion

The literature search identified a total of 1814 records (511, 546, and 757 through PubMed, Web of Science, and Scopus, respectively). During the first screening, 601 duplicates were removed and 1093 were removed after a second screening of titles and abstracts with Rayyan. An additional 51 records did not fit the scope of the review after reading the full text (third screening), while 21 extra records were added from accessing the citation lists of each previous selected record. A total of 89 89 records were consequently retained for the systematic literature review (Fig. 2). Of these, 15 discussed the detection of *Taenia* spp. and/or *Echinococcus* spp. eggs in water, 29 in food, 27 in soil and sediments, four in other matrices (objects, air, etc.), and, finally, 14 in a combination of the previously mentioned matrices. We do not believe that we have omitted any critical papers by restricting our search from January 1986, as a preliminary search found no relevant literature before this date. Although we are aware that at least 44 articles were not completely evaluated due to our restriction to English language (12 in Chinese, 10 in Spanish, 8 in French, 6 in Turkish, 4 in Czech, 2 in Dutch, 1 in Russian, and 1 in Japanese), examination of the English abstracts of these articles indicates that they were unlikely to be relevant to this review.

The results are presented first by analytical methods per matrix for analysis (water, food, soil and sediments, insects, and diverse objects), before more generic topics are discussed regarding standardization and the advantages and disadvantages of the available methods, along with viability assessment and effects of season.

3.1. Tools for the detection of Taenia and/or Echinococcus spp. in water

A considerable number (22) of records addressing the detection of taeniid eggs in water was retrieved by the systematic search, out of which 19 were based on examination by microscopy and three on molecular detection. An overview of all technical details (e.g., sieve apertures, centrifugation settings, sample volume, specific gravity of the flotation solution, sedimentation time, etc.) is provided in Table 1 and Supplementary Table B.

3.1.1. Sample processing

Before applying any detection tool to water samples, large-volume processing was often achieved by a pre-filtration step on-site through a successive series of sieves using a vacuum device. These sieves were then washed with Tween 20 or 80, with or without ethylenediaminetetraacetic acid (EDTA), to capture any remaining eggs (Mahvi and Kia, 2006; Verbyla et al., 2013a; Lass et al., 2019). This left a smaller volume to be transported and investigated further. However, the influence of such sample preparation on egg loss has not been evaluated.

Once in the lab, a combination of sedimentation and/or centrifugation and/or flotation aiming at egg concentration was performed. In one record, the pre-filtered water was again submitted to a series of stainless-steel sieves using water with 0.05% Tween 20 added and the sediment retained on the sieve used for final detection (see section 3.1.2.) (Craig et al., 1986). Three records simply left the water to sediment, with or without centrifugation, prior to applying the detection methods (Diaz Camacho et al., 1991; Wongworapat et al., 2001; Noda et al., 2009). In one of these records, sodium hydroxide was added for the sedimentation (Noda et al., 2009). Eleven records incorporated a zinc sulfate flotation step before final microscopic examination. Zinc sulfate flotation has been widely used in routine procedures for detecting intestinal helminths and protozoa and is based on the separation of parasitic elements from debris through differences in specific gravity (s.g.). Mostly, the sample is either filtered, left to settle, or centrifuged for sedimentation, after which the supernatant is discarded and a saturated zinc sulfate solution added (Jimenez et al., 2016). The flotation samples are generally aliquoted and centrifuged with a cover slip on top, or directly examined under the microscope without centrifugation (Bouhoum et al., 2000). Alternatively, the supernatant is poured through a sieve, after which the contents of the sieve are washed and centrifuged for final examination of the sediment (Amoah et al., 2018). Finally, the majority of the records implementing zinc sulfate flotation, analyze their water samples for the presence of helminth eggs by following the modified Bailenger method according to Ayres and Mara (1996) (Table 1).

A final four records describe the use of a flotation step, but with either saturated salt or magnesium sulfate instead of zinc sulfate. In a paper from Argentina, almost the same procedure as the modified Bailenger method was applied, with the exception of zinc sulfate being replaced by a saturated sodium chloride solution (Sánchez-Thevenet et al., 2019). Notably, a flotation solution with a s.g. of only 1.2 was applied and is unsuitable (< 1.27) for good recovery of taeniid eggs (David and Lindquist, 1982). Dudlová et al. (2015), on the other hand, isolated taeniid eggs from water samples by a sedimentation-flotation method of Cherepanov (1982) using saturated saccharose. Briefly, the water was allowed to settle, the supernatant was decanted, and saturated saccharose (s.g. 1.30) was added. Following centrifugation, the top layer of the supernatant was examined under the microscope (see section 3.1.2.). Lastly, Verbyla et al. (2013a) implemented the Mexican Test Method for the Determination of Helminth eggs in Water Samples, with the exception that magnesium sulfate was used for flotation instead of zinc sulfate (Madero, 1999). This method entails a sedimentation overnight, decanting of the supernatant, followed by a magnesium sulfate flotation step. Thereafter, the supernatant was recovered, diluted in at least 1 L distillated water, and left for another sedimentation overnight. Lastly, a two-phase separation was obtained by adding a sulfuric acid-ethanol solution and ether. The sediment was kept for parasite egg detection (Verbyla et al., 2013a, 2013b).

3.1.2. Detection by microscopy and viability assessment

After sample processing, 19 records describe taeniid egg detection by microscopy examination (Table 1). Although conventional microscopy is reasonably simplistic and budget-friendly, it nevertheless requires appropriately trained and experienced staff that are able to discriminate eggs from other debris, and cannot be used to distinguish between eggs of *Taenia* and *Echinococcus* spp., let alone further differentiation to species level. This also implies that no distinction can be made between zoonotic and non-zoonotic species (e.g., *T. hydatigena*) within these genera. Notably, however, in four of the 19 abovementioned papers, it was claimed that their recovered eggs belonged to *Echinococcus* or *Taenia* spp., or even *T. solium* and *T. saginata,* despite having no apparent evidence for this genus/species-level identification (Diaz Camacho et al., 1991; Khouja et al., 2010; Verbyla et al., 2013a; Sánchez-Thevenet et al., 2019). Additionally, in water samples with a low number of helminth eggs, detection may be difficult, resulting in a very time-consuming process. As this is impractical, particularly for larger sample sizes, Jimenez et al. (2016) developed a digital imaging system that claimed to identify seven species of helminth eggs, including *T. saginata*, rapidly and reliably. This was the sole record of all 19 microscope-based records that determined sensitivity and specificity (i.e., 80 and 99% respectively). However, these values refer to the method from the microscopy onwards and hence do not include the egg recovery and concentration steps. Furthermore, as eggs of *T. saginata* were the only taeniid included in the digital imaging system, this tool would be very unlikely to be able to distinguish between eggs within the Taeniidae family. This could be why no further studies retrieved in the present systematic review made use of this digital imaging system.

An alternative method for microscopic identification to genus level, is the indirect immunofluorescence antibody test (IFAT) that has been used on eggs retrieved from soil and water samples by applying an anti-*Echinococcus* oncosphere monoclonal antibody (4E5), as described by Craig et al. (1986). After sample processing through a series of sieves, sediment retained on the sieve was subsequently treated with hatching solutions by incubation with 1% pepsin/HCl and artificial intestinal fluid, washed in phosphate buffered saline (PBS) and centrifuged. A murine monoclonal antibody, 4D5, raised against oncospheres was then added to the oncosphere-enriched air-dried samples on the slides and detected with a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse antibody. No information was, however, given about the LOD, sensitivity, or specificity.

Finally, detection of taeniid eggs via microscopy does not necessarily mean the eggs are still viable and thus pose a risk to human health or animal infection. Only Verbyla et al. (2013a, 2013b) attempted to determine whether the helminth eggs were viable by using trypan blue exclusion. Although this dye only stains non-intact, and therefore non-viable, cells, it does not necessarily mean that all non-stained eggs are viable. This was the only study of taeniid eggs in water samples where the viability of eggs was investigated.

3.1.3. Molecular detection

To overcome the issue of genus and species identification in conventional microscopy, and potentially to improve both sensitivity and specificity, several molecular tools for the detection of taeniid eggs in water have been developed and described in an additional three records. For the analysis of *E. multilocularis* in water, Lass and colleagues made use of three molecular detection methods (a nested end-point PCR targeting the mitochondrial 12S rRNA gene, a real-time PCR (probe-based) targeting the gene for the large subunit of rRNA (*rrnL*), and a LAMP assay based on the 12S NADH-dehydrogenase subunit 5 (*nad5*) gene). All of these were shown to be suitable for the detection of *E. multilocularis* in water, despite a high LOD of 10–20 ova/L which included the preceding filtration steps. After centrifugating the material from the washed filters (see section 3.1.1.), DNA was extracted from the pellet using either a Sherlock AX kit (A&A Biotechnology, Poland), or a TIANamp Micro DNA kit (Tiangen, China) with a freeze-thaw cycle to enhance eggshell rupture and minimize inhibition prior to extraction (Lass et al., 2019; Lass et al., 2020). To exclude any cross-reactions, positive samples were tested for *E. shiquicus* and *E. granulosus* using a multiplex-PCR (based on the 12S NADH dehydrogenase subunit 1 (*nad1)* gene, *nad5*, and the cytochrome C oxidase subunit I (*cox-1)* gene) able to differentiate these *Echinococcus* species developed by Liu et al. (2015).

For the simultaneous detection of, and differentiation between, *T. solium*, *T. saginata*, and *E. granulosus*, a multiplex end-point PCR was developed by González et al. with a sensitivity of less than 10 pg DNA. This system was, however, originally designed for parasitic detection and differentiation (*T. solium* and *T. saginata*) in humans. Despite the claim of applying this promising test in the future for the evaluation of environmental contamination in water, no further studies applying this test have been published (González et al., 2002).

3.1.4. Conclusion for detection in water

Depending on the source of contamination and the volume of the water body being investigated, infective eggs may be sparsely

distributed in the water matrix, thus requiring a detection tool with high sensitivity. Over the years, many different techniques for the detection, and often quantification, of taeniid eggs in water have been developed and applied. However, only studies using molecular tools determined a sensitivity or LOD of their method, with inclusion of the egg recovery and concentration step. On the other hand, although major losses of eggs can occur during processing step(s), the recovery efficiency has not been calculated in the studies based on microscopy detection. As the World Health Organization (WHO) recommends a concentration of $\leq$1 helminth egg/L in wastewater used for irrigation and a complete absence in drinking water (World Health Organization, 2006, 2017), LODs of 10–20 eggs/L are too high for determining whether the water conforms with the WHO recommendation. Also, any method standardization with regard to centrifugation time and speed, sample size, sedimentation time, storage conditions, extraction kit, etc. is lacking (Table 1, Supplementary Table B). Finally, although critical for an exact environmental-risk determination, viability assessment was only performed by Verbyla et al. (2013a, 2013b) using trypan blue staining for identifying non-viable eggs, which may overestimate viability.

3.2. Tools for the detection of Taenia and/or Echinococcus spp. in food

Monitoring and surveillance of bacteria such as *Escherichia coli, Listeria monocytogenes* and *Salmonella* spp. in food are routine practices in Europe due to EU health laws defining their presence and microbiological limits, and also standardized detection assays. However, there is no standardized methodology for the detection of taeniid eggs in food samples. This fact was also accentuated by the literature search in which 35 records were retrieved describing in total at least 24 different detection methods for taeniid eggs in food (Table 2). The food products investigated have all been fresh produce, predominantly leafy greens (e.g., lettuce) and fruits (particularly berries). An overview of the technical details is provided in Table 2 and Supplementary Table C.

3.2.1. Sample processing

No less then 23 records described a sedimentation-based technique, usually preceded by cutting the fruits and vegetables into smaller pieces or stripping of the outer leaves, and a washing step to release the eggs from fruit and vegetable surfaces and stomata. Sediments were then often subjected to one or more filters and/or centrifugation to concentrate the eggs, and the final concentrate examined by microscopy or PCR. Washing involved a whole range of different solutions according to the study, ranging from water (distilled or sterilized) to different concentrations of sodium chloride (0.85% - 0.95%) (Table 2). In addition, formalin, Tween 20 or 80, 1% sodium dodecyl sulfate with 0.1% Tween 80, sodium pyrophosphate, glycine, and Alconox® were also mentioned as solutions used to assist in detaching the eggs by washing and shaking (Table 2). One record did not mention the exact components of the detergent used (Maipanich et al., 2011), and an additional two records made use of an ultrasonic cleaner to wash vegetable parts in the detergent solution (Kozan et al., 2005; Maipanich et al., 2011). A final two records used a formalin-ether sedimentation technique, where the helminth eggs are fixed in formalin and debris is extracted by ether. After centrifugation, four layers will be formed (ether solvent at the top, a debris plug, formalin, and sediment at the bottom) from which the sediment is examined microscopically for eggs (Adenusi et al., 2015; Rostami et al., 2016).

Other than a simple sedimentation-concentration approach, a somewhat more advanced sedimentation-flotation methodology has also frequently been used for the sample processing of food. Apart from washing, sedimentation, and centrifugation/filtration, an additional flotation step was added to separate eggs from debris through differences in s.g. In these studies, either zinc sulfate, saturated sodium nitrate, saturated sodium chloride, saturated zinc chloride, or saturated sucrose were used as flotation solutions (Table 2). One paper used zinc sulfate in combination with ethyl acetate according to the modified Bailenger technique (see section 3.1.1.) (Woldetsadik et al., 2017). Notably, information on the s.g. of the flotation solutions was often missing. Furthermore, the densities of saturated salt and sucrose used were indicated as being 1.20–1.21, but densities ranging between 1.27 and 1.38 are known to be superior for recovering taeniid eggs (O'Grady and Slocombe, 1980). However, the aim of both papers using saturated sucrose was to retrieve not only taeniid eggs, but also eggs from a variety of other intestinal parasites with a lower egg density, including *Ascaris* spp., *Toxocara* spp. etc. (Maikai et al., 2012; Fallah et al., 2016).

3.2.2. Microscopic detection

After sample processing, 27 records described taeniid egg detection by microscopy (Table 2). As discussed in section 3.1.2., sedimentation- and flotation-based techniques using final microscopic examination have the advantages of being relatively easy to perform and low-cost, but do not allow differentiation between different taeniids. Nevertheless, seven records examining samples microscopically claimed that eggs could be identified to the genus or even species level (Table 2).

3.2.3. Molecular detection

As for water samples, different PCR-based techniques have been implemented to detect taeniid eggs in food and to address identification issues. Specifically, 8 records described an end-point, real-time, nested or multiplex-PCR targeting several genes of *Echinococcus* or *Taenia* spp. (Table 2). An end-point PCR targeting the 12S rRNA gene from *E. granulosus* was described by M'Rad et al. (2020). No specific DNA extraction kit was mentioned, but the DNA was extracted by alkaline lysis and proteinase K digestion, and chelating resins to trap DNA contaminants. The performance of the PCR was not determined. Secondly, a SYBR green real-time qPCR targeting the *nad1* gene from *E. multilocularis* with an LOD of 50 eggs per 250 g of berries was developed by Malkamäki et al. (2019a). A Tissue and Hair Extraction Kit (Promega, USA) was used for DNA-extraction, but no extra step to improve eggshell disruption was performed. A third PCR is the nested end-point PCR targeting the mitochondrial 12S rRNA gene from *E. multilocularis*; this was used in Poland to estimate the presence of eggs on fruits, vegetables, and mushrooms. Prior to DNA extraction with the Sherlock AX DNA extraction Kit (A&A Biotechnology) for hair and tissue, eggs that had been recovered via a zinc chloride flotation, were first subjected

to three freeze-thaw cycles to destroy the egg walls. An LOD of 100 eggs per 400 g of fruits or vegetables was calculated (Lass et al., 2015, 2017).

Finally, four different PCRs directed towards the detection of more than one single parasite genus were implemented in four different papers. The 12S rRNA, *nad1* or *nad2* gene of *E. multilocularis* was always targeted, in combination with the B1 region from *Toxoplasma gondii* or the 12S rRNA gene from *Taenia*, *Mesocestoides* spp., or *E. granulosus*. In the triplex qPCR (probe-based) from Temesgen et al. (2019) targeting *T. gondii*, *E. multilocularis*, and *Cyclospora cayetanensis*, an LOD from both egg recovery and PCR of 5 eggs per 30 g berries was calculated. DNA was extracted using DNeasy PowerSoil Kit (Qiagen, Germany), with an extra bead-beating step to break the eggshells and facilitate the release of DNA. Another multiplex end-point PCR from Guggisberg et al. (2020) that targeted *E. multilocularis*, *T. gondii,* and *Toxocara canis*, had a lower LOD of 1–2 eggs per 300 g lettuce. This again included the preceding sieving and washing step, and the PCR itself. Additionally, two eggshell disruption steps based on proteinase K digestion and freeze-thaw cycles were used to improve DNA-extraction with the Soil DNA kit (Omega Biotek, USA). In the multiplex end-point PCR used by Federer et al. (2016), the LOD, sensitivity, and specificity were not mentioned, although an extra alkaline lysis step was added prior to DNA-extraction with a QIAamp DNA mini kit (Qiagen). In a final study, a published multiplex end-point PCR was converted to a real-time PCR (probe-based) with melting curve analysis for use on berries and lettuce washes (Frey et al., 2019). Here, an LOD for both the washing and PCR was calculated and defined as 5 eggs per 35 g lettuce or 55 g berries. Finally, a 395 bp stretch from the *E. multilocularis nad1* gene, a 117 bp stretch from *E. granulosus*, and a 267 bp stretch from the small subunit of rRNA *(rrnS)* gene from *Taenia* sp. were targeted during the real-time PCR. The sensitivity for the tested taeniid species ranged from 1 pg/μL to 1 ng/μL, and DNA tested from each taeniid species individually could be distinguished by a characteristic melting peak. However, the method could not reliably identify the simultaneous presence of taeniid DNA when mixed DNAs were tested. Lastly, the authors also compared two DNA-extraction protocols. The first entailed eight freeze-thaw cycles prior to DNA-extraction with QIAamp DNA Stool mini kit (Qiagen), and the second involved the use of a FastDNA Spin Kit for Soil (MP Biomedicals, USA) that appeared to be superior to the first approach. Notably, this was the only research group from all records retrieved in the systematic review that reported evaluating the influence and performance of a chosen DNA extraction kit on the detection by PCR.

3.2.4. Conclusion for detection in food

In sum, as with the detection tools for taeniid eggs in water, many different methods with different protocols have been applied for fruits and vegetables, indicating the same lack of standardization. Even when two methods appear very similar, there are often still differences in sample size and weight, sedimentation time, centrifugation speed, and duration, etc. (Supplementary Table C). Although an LOD was calculated more often than with detection assays used for analyzing water, at least for molecular approaches, these were often still unsatisfactory for detection of eggs at low contamination levels. However, performance determination from the multiplex-PCR tools shows promising, robust, reasonably sensitive and specific tools that could be useful for further analyses in the future. For this, a thorough evaluation of the influence of the selected DNA-extraction kit and the product efficiency on different food products is essential. The structure of the food product can potentially have an impact on the level of parasitic contamination. For instance, vegetables with a dense foliage (e.g., lettuce and cabbage) or hairy fruits (e.g., raspberries) may be more likely to be contaminated than vegetables and fruits with smooth surfaces (e.g., carrots and blueberries) since they offer a larger contamination surface. On the other hand, curly leaves or hairy projections may hinder detachment of eggs during the washing step and therefore reduce recovery efficiencies. Additionally, this washing step should be performed gently, especially for fragile fruits such as berries, in order to avoid mashing of the fresh produce and thus releasing of PCR-inhibitors (Schrader et al., 2012). Finally, as for water samples, assessment of viability was only performed by Guggisberg et al. (2020) using an in vivo method in which PCR-positive samples for *E. multilocularis* were injected subcutaneously into mice. As metacestode growth was not observed, the authors suggest this is due to the low number of eggs in the sample or because the eggs were not viable. It could also reflect that only DNA was in the samples, and not *E. multilocularis* eggs.

3.3. Tools for the detection of Taenia and/or Echinococcus spp. in soil and sediments

Sensitive and specific detection tools for taeniid eggs in soil that can handle different compositions and types of soil are also important and were searched in our review. It was decided to include all records investigating “mud”, “sand”, “silt”, “sediment”, “sludge” (a complex mixture of biological and mineral compounds removed from wastewater and sewage, often used for the purpose of soil amendment) etc. as these all fall within the scope of “soil” as a matrix. As was the case for water and food, a range of different detection tools have been developed and implemented for the detection of taeniid eggs in soil. Specifically, 35 records were retrieved via the literature search describing, in total, 33 different methods subdivided into microscope-, and molecular-based techniques, and Western Blotting. An overview of the technical details is provided in Table 3 and Supplementary Table D.

3.3.1. Sample processing

As with the other sample types, prior to detection, also for soil, a protocol that started with a combination of a sieving/filtration, sedimentation, dilution/washing, centrifugation, filtration, and/or flotation step was performed. Either water, sodium chloride, Tween 20 or 80, sodium hypochlorite, PBS, ammonium bicarbonate, or trisodium phosphate were added to release eggs from debris in the dilution/washing step (Table 3). Alternatively, the formalin-ether sedimentation technique was used (see section 3.2.1.) (Huerta et al., 2008; Aghaindum et al., 2019; Sánchez-Thevenet et al., 2019). No records, with the exception of one, mentioned the efficiency of the washing solutions, nor the reason why a particular washing solution was used. Only Satchwell (1986) claimed that the use of detergents is ineffective at increasing egg recoveries and that the majority of eggs are lost at the formol-ether step from the formalin-

ether sedimentation technique. Furthermore, a variety of solutions were used for the flotation step. In particular, saturated zinc sulfate (with or without 10% formalin), magnesium sulfate, saccharose, (Sheather's) sucrose, salt, zinc chloride, and sodium nitrate were reported (Table 3). Remarkably, where the s.g. was often unspecified for flotation solutions used in water and food matrices, here it was only absent for magnesium sulfate and sodium nitrate used by Verbyla et al. and Cvetkova et al. respectively (Verbyla et al., 2013a, 2013b; Cvetkova et al., 2018). Nevertheless, a substantial number of records (nine) used a solution with an unsuitable density (< 1.27) for the recovery of taeniids eggs. Finally, although only calculated by three records, the abovementioned sample processing methods seem to suffer from a low recovery efficiency and poor LOD, at least when combined with conventional microscopy. Specifically, for *T. saginata* eggs, Scandrett and Gajadhar (2004) determined a recovery efficiency of only 2.5% after a sucrose flotation-centrifugation for egg concentration, while a recovery efficiency of 6.7–20.9% was calculated by Satchwell (1986) for the formalin-ether sedimentation and zinc sulfate flotation technique. A final performance evaluation of a sugar centrifugal flotation technique was calculated by Matsuo and Kamiya (2005) and signified an LOD of 10 *E. multilocularis* eggs/g soil.

3.3.2. Microscopic detection

Conventional microscopic detection of taeniid eggs from soil samples, was reported in 27 records, from which eleven studies again, incorrectly, claimed an identification up to genus/species level (Table 3). As a solution, IFAT, based on binding of the anti-*Echinococcus* oncosphere monoclonal antibody (4E5) to the oncospheres, which had previously been used in water samples (see section 3.1.2.), was applied to eggs from soil samples. In contrast with water samples, soil samples were first diluted and layered on to a cushion of Percoll® density gradient media. This allowed heavy particles of debris to settle rapidly through the Percoll®. The subsequent treatment with hatching solutions and addition of the murine monoclonal antibody 4D5 was identical to the IFAT protocol for eggs retrieved from water samples. Nevertheless, this method has not been used in further epidemiological studies, and, again, no information was given about the LOD, sensitivity, or specificity (Craig et al., 1988).

3.3.3. Molecular detection

For soil, also, identification issues and limited performances of conventional microscopic assays have been addressed by use of molecular diagnosis in six records. A first real-time qPCR amplifying the mitochondrial 12S rRNA gene from *E. granulosus* was developed by Shaikenov et al. (2004). It was preceded by passing the soil through a sieve, and a sodium nitrate flotation. Digestion by proteinase K and a lysis buffer was used for eggshell disruption and a QIAmp DNA mini kit (Qiagen) for DNA extraction. A specificity of 100% was confirmed, and an LOD of 2 eggs per 5 g soil sample was claimed for the PCR itself. The performance of the preceding flotation technique was not evaluated. Umhang et al. (2017), on the other hand, validated a duplex real-time qPCR (probe-based) to detect both *E. multilocularis* and *Toxocara* spp. in soil. Here, samples were first washed and subjected to a zinc chloride flotation. DNA was extracted using the NucleoSpin Tissue kit (Macherey-Nagel, Germany) with proteinase K digestion, and the *rrnL* gene of *E. multilocularis* (and *cox-1* gene for *Toxocara* spp.) was targeted. Although less sensitive for *Toxocara* spp., this method had a 100% sensitivity when 10 *E. multilocularis* eggs per 10 g of soil were present, and an LOD of 1 egg per 10 g of soil. It was also found that collecting samples of a lower weight had a better sensitivity than samples of a higher one. The method specificity was not determined. In a third record, a nested end-point PCR targeting the mitochondrial 12S rRNA gene from *E. multilocularis* was performed. Samples here were first dried, sieved, washed, centrifuged, and subjected to a saturated zinc chloride flotation step. Before DNA-extraction with a Sherlock AX Kit (A&A Biotechnology), the samples were first frozen three times at −70 °C and thawed at 30 °C to destroy the egg walls. In this PCR, a rather low LOD of 1 egg per 40 g was calculated, however, this was determined on pure suspensions containing *E. multilocularis* eggs and did not apply to preceding egg concentration procedures (Szostakowska et al., 2014). Other than a real-time or nested PCR, a third-generation quantitative PCR technology, the droplet digital PCR (ddPCR), was developed by Maganira and colleagues for the detection of *T. solium*. Soil samples were either washed or sieved, before being subjected to a zinc chloride flotation step and DNA extraction step using the QIAmp DNA mini kit for tissues (Qiagen). Samples were screened by ddPCR using primers targeting the *cox-1* gene. Despite PCR inhibitors having a marginal impact on ddPCR, a sensitivity of only 13–36% was mentioned. Such low sensitivity might be attributed to losses during preparatory steps (washing, sieving, flotation), the absence of an eggshell disruption step to improve DNA-extraction, and/or the use of a DNA-extraction kit intended for blood and tissue samples. Specificity was not determined (Maganira et al., 2019, 2020). Finally, for the simultaneous detection of, and differentiation between, *T. solium*, *T. saginata,* and *E. granulosus* in water and soil, a qualitative multiplex PCR with a sensitivity of less than 10 pg was developed by González et al. (2002). As mentioned above, this system was originally designed for parasitic detection in humans (for *T. solium* and *T. saginata*), but not applied for evaluating the environmental contamination.

3.3.4. Western blot detection

Another tool to address identification issues of conventional microscopy, is immunodetection; this not only includes IFAT (as described under microscopy; see section 3.3.2.), but also Western blot. This was implemented in one study that aimed at detecting *Echinococcus* spp. antigen in soil and sludge samples. Here, as a pretreatment, the samples were sieved and dried following mixing with an equal weight of PBS containing 0.3% Tween 20 and centrifugation. Eggs were detected in the supernatant fraction by Western blot according to Guarnera et al. (2000). Although a limited identification accuracy of the technique was mentioned as the main limitation of the study, this was not described or discussed further (Sánchez-Thevenet et al., 2019).

3.3.5. Conclusion for detection in soil and sediment

To conclude, several different detection techniques for taeniid eggs in soil samples have been developed, but their performance tends to be unsatisfactory when evaluated and, here also, there is an urgent need to improve the standardization of technical settings

Table 4
Overview of techniques for the detection of taeniid eggs in insects.

Method	Parasite species	Insect species	Qual./ Quant.	Performance	Storage conditions	Number of steps before result	Time to results	Country	Ref. and year
Formol-ether concentration method with microscopy	*Echinococcus/Taenia* spp.	Non-biting cyclorrhapan flies	Quant.	N.M.	In tubes on ice and stored at 4 °C	9	≤ 24 h	Ethiopia	Getachew et al., 2007
		Housefly			In glass bottles on ice and immediate process or stored at −4 °C	9	10-12u	Nigeria	Adenusi and Adewoga, 2013
Washing (formalin detergent) and sedimentation examined with microscopy	*Echinococcus/Taenia* spp.	Housefly	Quant.	N.M.	In capped bottle in cooler box	3	1-2 h	Lao PDR	Maipanich et al., 2011
		Housefly			In capped bottle in cooler box	3	2-3 h	Thailand	Pornruseetriratn et al., 2017
Dissection, end-point PCR targeting the *cox*-1 gene	*Taenia solium* and *Taenia saginata*	Beetles	Qual.	N.M.	N.M.	5	≤ 24 h	Peru	Vargas-Calla et al., 2018
Multiplex PCR targeting the 18S rRNA small subunit gene	T. *T. solium, T. saginata* and *Taenia asiatica*	Housefly	Qual.	LOD: one taeniid egg	In capped bottle in cooler box	6	10-12u	Thailand	Pornruseetriratn et al., 2017

PCR = Polymerase chain reaction, *cox*-1 = cytochrome C oxidase subunit I, Qual. = qualitative, Quant. = quantitative, N.A. = not applicable, N.M. = not mentioned, LOD = limit of detection.

(Supplementary Table D). Nevertheless, somewhat promising results were found in the detection of *E. multilocularis* by a real-time and nested PCR described by Umhang et al. (2017), and Szostakowska et al. (2014), with an LOD of 1 egg per 10 g and 40 g, respectively. Another main obstacle for detecting taeniid eggs in soil, are the differences in soil types that may potentially affect the recovery rates of taeniid eggs from soil and the effectiveness of DNA extraction. At least for flotation-based tools, a higher recovery was obtained from sandy soils than clay and loamy soils (Maganira et al., 2019). Finally, as for the other matrices, detection of taeniid eggs or DNA in soil does not necessarily indicate the presence of viable, infectious eggs, although none of the studies assessed this issue.

3.4. Tools for the detection of Taenia and/or Echinococcus spp. in insects

Five papers investigated carriage of taeniid eggs by insects using different techniques to evaluate environmental contamination and risk for human infection. An overview of technical details is provided in Table 4 and Supplementary Table E.

3.4.1. Sample processing

Four records investigated the presence of eggs on or in non-biting synanthropic flies after catching them with a sweep net or buckets containing bait used as fly traps. The quickest sample processing method was based on a simple washing and sedimentation technique, as described by Maipanich et al. (2011) and Pornruseetriratn et al. (2017). Briefly, flies were caught and stored in groups of 30 in formalin-detergent (FD) solution until analysis. Next, they were treated in the FD solution in an ultrasonic cleaner for 15 min at room temperature, and the sediment examined under a light microscope to identify helminth-like objects. Another relatively simple method for fly processing, which not only focused on the detection of eggs on the flies' body surface, but also their guts, is the general formalin-ether sedimentation method as explained above (see section 3.2.1.). Two research groups implemented this technique after preparing batches of ten flies. Specifically, each batch was immersed in physiological saline and vortexed to dislodge eggs from the external surfaces of the fly. The washing solution was then further processed using the formalin-ether concentration method. Simultaneously, the gut of each washed fly was dissected out, again pooled in batches of ten, macerated to liberate the contents, and finally processed with the formalin-ether concentration method (Getachew et al., 2007; Adenusi and Adewoga, 2013).

3.4.2. Detection

Both the abovementioned washing-sedimentation and formalin-ether concentration techniques successfully recovered eggs from flies in all four studies. However, both tools relied on microscopic identification and therefore have the same identification difficulties as every microscopy-dependent technique. Vargas-Calla et al. aimed at recovering *T. solium* eggs from dung and darkling beetles (i.e., *Aphodius* sp. and *Ammophorus* sp.) using an end-point PCR targeting the mitochondrial *cox-1* gene. Beetles were collected directly from the feces of domestic animals (dogs, pigs, and turkeys) and put into the same pool when caught within a radius of 5 m. Altogether 54 pools of beetles were prepared by dissection of their digestive tract and hemocoel and by addition of PBS and proteinase K to improve eggshell disruption. DNA was subsequently extracted, the *cox-1* gene was amplified, and PCR-products were sequenced for final alignment with sequences of tapeworms published in GenBank (Vargas-Calla et al., 2018). This approach confirmed the presence of *T. solium* and *T. hydatigena* in 5 pools of beetles with a 100% specificity, but the method had limitations. As beetles had been pooled in order to reduce working hours and consumables, it is impossible to determine how many beetles were carrying taeniid eggs. Furthermore, Pornruseetriratn et al. (2017) demonstrated that *cox-1* primers are unable to detect very small numbers of *Taenia* eggs on blowflies. As a solution, this research group developed a multiplex end-point PCR using *Taenia* species-specific primers based on the nucleotide sequences of the 18S rRNA gene for discrimination. Specifically, eggs obtained from flies using the washing-sedimentation step described above were first broken using a sterile coverslip on a sterile glass slide, and then heated to improve DNA-extraction. Specificity was tested by including genomic DNA from three Taenia species (*T. solium*, Ta *Taenia asiatica*, and *T. saginata*) and other helminths (i.e., *Ascaris lumbricoides*, *A. suum*, *Trichuris trichiura*, *Strongyloides stercoralis* and *Opisthorchis viverrini*). A sensitive and specific technique was reported that was successful at amplifying DNA from a single *Taenia* egg. However, the isolation from one single taeniid egg was not clearly described. Furthermore, the 18S rDNA gene is a highly conserved housekeeping gene, with numerous tandemly repeated copies present in the genome, with over 99% identity between some *Taenia* species; thus, the likelihood for cross-reactivity is high (Ale et al., 2014).

3.4.3. Conclusion for detection in insects

In conclusion, at least five papers demonstrated the presence of taeniid eggs on and in flies and beetles by using both molecular and microscopic techniques. Once again, in all records, viability determination was not addressed. In light of this, an in-depth analysis of the actual role of insects in dispersing taeniid eggs, and, transmitting disease to humans and animals, has yet to be performed, particularly as some of the methods described in the papers lack important details. Furthermore, although taeniid eggs could be isolated from the flies' guts, it is unknown whether they would have survived under those physiological conditions until the fly was consumed by a suitable host. Lawson and Gemmell reported that passage of the eggs through the arthropods' gut could cause damage to the embryophores, however when blowflies containing viable *Taenia* eggs in their gut were fed to sheep, viable *T. hydatigena* cysts could be recovered from the sheep organs (Lawson and Gemmell, 1985, 1990). The latter was also reported for experimentally *T. solium*-infected dung beetles that had been fed to pigs (Gomez-Puerta et al., 2018). Additionally, regarding the arthropods' potential capability to transfer parasites to man via food, how easily do the eggs detach from the sticky feet to the food? And, are these still viable and infective to man after exposure to the local environmental conditions? Further studies are also required on other arthropod species that come in contact with the vertebrate feces (e.g., ants and cockroaches) and thus might also contribute to spread of taeniid infections. These are all highly relevant topics that are still poorly understood and should be investigated in further research. From there

on, it could be assessed whether the presence and number of eggs in insects could also be a bio-indicator for the presence and level of environmental contamination.

3.5. Tools for the detection of Taenia and/or Echinococcus spp. on diverse objects

The systematic review retrieved two records describing the detection of taeniid eggs on objects by use of the Graham method, a technique originally invented for the recovery of *Enterobius vermicularis* eggs in the perianal region of humans (Graham, 1941) (Table 5). This entails a two-step protocol during which a clear adhesive tape of 6 × 5 cm is affixed on each site to be investigated. Thereafter, the tape is placed on a microscopic slide for examination. Pereira et al. (2016) selected different sites in a Brazilian school (toilets, restroom faucets, doorknobs, cafeteria tables, chairs, and benches) and recovered taeniid eggs on the cafeteria's table, along with *Entamoeba coli* cysts and hookworm eggs. Similarly, Diaz Camacho et al. (1991) examined unspecified utensils and objects handled by *T. solium* tapeworm carriers, although all tested negative. A final unique record aimed at assessing the contamination of air with helminth eggs by the method of Kroeger et al. (1992). This technique is very analogous to the Graham method, as it also involved the use of transparent adhesive strips (but now covering a surface of 5 × 15 cm, and with adhesives on both sides) followed by microscopic examination. These were attached on walls and pillars of houses, schools, and restaurants as a representative of the air contamination (Noda et al., 2009). However, without marking of the tape, it is difficult to know on which side the eggs had attached (and thus whether they come from the air or the object).

All three records incorrectly described the Graham method as being capable of identification to *Taenia* genus or even *T. solium* species level. Additionally, no viability, sensitivity, LOD or recovery rate was determined.

3.6. Gaps and future goals

The establishment of reliable and practical environmental detection assays for *Echinococcus* and *Taenia* spp. is recognized as being an unfilled need. The aim of this review was to present those detection methods for taeniid eggs in the environment that have been implemented in multiple studies, to compare them, and to provide recommendations for future studies. As outlined above and in accompanying supplemental files, there is a significant literature on (prototype) analytical tests for environmental detection of taeniid eggs that could be categorized as either conventional (light microscopy), molecular, or immunodetection (IFAT or Western blot). However, method performances were often not assessed, and any form of standardization within and between different matrices is entirely lacking. This makes it very difficult to compare among different studies. In the following sections we discuss this aspect further, starting with the sampling strategy.

3.6.1. Sampling strategy

Sample number varied from 3 to 432 for water, 5 to 960 for food, 2 to 608 for soil, 30 to 7190 for insects, and 35 to 159 for objects. Likewise, the sample size ranged from 250 mL to 50 L for water, 10 g to 50 kg for food, and 5 g to 36 kg for soil. With the exception of one record, the influence of sample size on sensitivity was not determined. Only Umhang et al. (2017) demonstrated that analyzing 10 g of soil was more sensitive than analyzing 20 g, when a zinc chloride flotation/sieving technique in combination with PCR was used for detection. For other matrices and detection techniques, the influence of sampling strategy was not evaluated, or often apparently not considered. For instance, is it better to collect ten times a volume of 100 mL or a single volume of 1 L? Logically, taking more samples of a huge sample volume will increase the chance of detecting the possibly very low number of parasite eggs dispersed throughout the entire environment. On the other hand, the collection of more samples from a higher volume may be difficult and time-consuming for logistical reasons and require the construction of specific in-house material. Additionally, it is important to consider the distribution of the targets (i.e., taeniid eggs) within a certain matrix, as these are often not randomly dispersed. For example, it was shown that *Cryptosporidium* oocyst densities in water may be described adequately by the Poisson distribution (Haas and Rose, 1996), whereas other micro-organisms (e.g., coliforms) often show greater variability and hence are characterized by a negative binomial distribution in water (Pipes et al., 1977). Examining the way in which taeniid eggs are distributed in different environmental matrices

Table 5
Overview of techniques for the detection of taeniid eggs on diverse objects.

Method	Parasite species	Qual./ Quant.	Performance	Storage conditions	Sample number (surface)	Percentage positive/ quantity	Country	Ref. and year
Graham method: tape affixing with microscopy	*Echinococcus/ Taenia* spp.	Qual.	N.M.	N.M.	66 (6 × 5 cm)	27.3%/N.A.	Brazil	Pereira et al., 2016[a]
				Analysis the same day	35 (N.M.)	0%/N.A.	Mexico	Diaz Camacho et al., 1991[a]
Kroeger method: tape fixed on walls for two weeks with final microscopy	*Echinococcus/ Taenia* spp.	Qual.	N.M.	N.M.	159 (5 × 15 cm)	1.26%/2 eggs in total	Vietnam	Noda et al., 2009[a]

Qual. = qualitative, Quant. = quantitative, N.A. = not applicable, N.M. = not mentioned.
a = wrongfully claimed identification at the genus/species level.

is thus important, especially with regard to reproducibility and making extrapolations to the rest of the environment.

This is closely related to another factor that affects sampling strategy: sampling location. In the case of water, for example, samples have been collected from wastewater treatment plants (influent, effluent), recreational rivers, natural ponds, sewage water, etc. but the exact location within the water such as depth, or distance from the shore, is often not specified. Here it is also important to consider the temperature and wind (season), especially for larger waterbodies, as these factors influence the circulation (i.e., mixing) of the different water layers within a waterbody. For instance, in summer, the surface water warms, making it denser than the bottom layer. As a result, the water layers turn over and mixing of debris, and possible eggs, takes place. A reverse process takes place in the fall (Macias et al., 2019).

For vegetables and fruits, samples usually originated from local and retail markets, private gardens, plantations, or forests. Specifically for berries, one paper mentioned that berries were samples at a height of 1 m, without, however, specifying the reason for this (Malkamäki et al., 2019b). Other studies suggest that the edges of gardens, plantations, and forests are suitable as sample locations when investigating *Echinococcus* eggs on food products, since foxes were often resident there (Lass et al., 2015, 2017). Moreover, for private gardens, the chances of detecting fox feces (and *Echinococcus* eggs) may increase when the garden is unfenced and surrounded by forest or grassland, or in close proximity to fruit trees or farms (Bastien et al., 2018; Poulle et al., 2017). These parameters will vary by location, but complete description of the sampling rationale and protocol is essential, yet often overlooked. Even in cases where a pragmatic, but not ideal, sampling strategy is adopted, this should be described.

Finally, topsoil, mud, (sewage) sludge, silt, dusts, sandy soil, wet soil, (toilet) sediment, clay, and loam, were all included as "soil" samples where, somewhat more, but again unstandardized information was provided on the sample location. Umhang et al. (2017) collected soil over the first 5 cm of the surface, over an area of 10 cm^2, and at a distance of between 50 cm and 1 m from carnivore feces. The same depth and surface area were selected by Sánchez-Thevenet et al. (2019) and Souto et al. (2016), but each sample's location was not particularly related to a fecal sample. In another record, the soil samples were obtained from the surface layer of the ground at a depth of less than 2 cm at several points within the space of a few meters (Szostakowska et al., 2014). A final record specifying the sample's location aimed at detecting *T. solium* eggs in soil at a depth of 3 cm at different households within a village (the backyard, around the toilet, inside the house, in front of the house, and around the waste disposal area) (Maganira et al., 2020). In other words, more research is needed to determine the optimal location and time of the sampling with the highest chance of detecting eggs. However, this may vary between study sites and parasite species/definitive hosts, as well as on the research question being addressed. Specifically, the depth, surface area, distance from a certain fecal sample or contaminated household, season (rainy or dry), the height of berry picking, etc. are all factors that may play a role, and that require further investigation in order to standardize the sample location of each environmental detection technique.

A last important factor of the sampling strategy is the need for certain storage conditions, especially when immediate analysis of samples is not possible. Once again, various storage conditions (regarding storage container, temperature, duration, preservative, etc.) have been applied, but often without investigation of the possible effects on the results obtained, including potential effects on egg viability (see section 3.6.5).

3.6.2. Elution

Before insects, food, or soil samples are subjected to any sedimentation and/or flotation procedure, many studies first aim at extracting the parasite eggs from debris, vegetable and fruit surfaces or pores, and insects. If included, this elution step is crucial, since all subsequent steps depend on it for the final egg recovery efficiency of the test. Various elution procedures, from shaking to the use of an ultrasonic cleaner, have been implemented in combination with a range of different detergents. Differences in recovery efficiency could thus be due to sample type, choice of elution procedure, duration and frequency of any agitation procedure, and types of detergents (composition, pH, and dispersing properties), if used. Moreover, all of these could also have an influence on the steps following elution. Unfortunately, few records, mentioned the efficiency of the washing solutions on taeniid egg release, nor the reason why a particular method was used. Only Satchwell (1986) claimed detergents are ineffective in increasing egg recoveries and that the majority of eggs are lost at the formol-ether step when applying the formalin-ether sedimentation technique. A possible explanation for this, is that this technique originates from the diagnostic tests for eggs in feces where the ether serves as an extractor of fat in the feces. As environmental matrices usually contain little to no fat, the suitability of this technique is questionable.

3.6.3. Concentration methods

The use of a concentration method will separate the taeniid eggs from debris and hence increase the likelihood of finding them, particularly in samples that must be eluted into a larger volume a lot and/or with a low number of eggs. The concentration methods can be divided into filtration, sedimentation (with or without centrifugation), and flotation. Filtration is a first type of concentration method often employed on the elution water from soil, food, and insects, or direct water samples, especially of large volumes. During filtration, the water is passed through filters, cotton cloths or sieves, often with the aid of a vacuum pump device. Here again, different apertures are used, ranging, if specified, from 20 μm to 1200 μm. Logically, the smaller the pore size, the more debris will be retained that masks the eggs, making it more difficult to examine. Mostly, eluate or water is first passed through two series of sieves with larger apertures ranging between 150 and 1200 μm, and in this case the larger material is removed in the filter and discarded, and the filtrate is collected. Thereafter, a final filter with a small pore size (e.g., 30 μm) is used to retain the eggs on the filter. Taeniid eggs are typically spherical to ellipsoid in shape and range in size from 22 to 44 μm (Alvarez Rojas et al., 2018). The smallest filter is washed with water or an elution buffer to retrieve the eggs on top of it, but if some eggs are smaller than the pore size (i.e., < 30 μm), then these may not be captured and this can result in a reduced recovery efficiency. Thus, filter pore size must be selected carefully.

A second concentration method is sedimentation that can be performed either directly after collection of the sample, either on the

filtered and or/eluted water. It uses the separation between solid and liquid media to concentrate parasite eggs in a sample. Following Stokes' law, the settling velocity of the eggs (and debris) depends on the egg size (defined as spherical), difference in densities between water and other particles, and the viscosity of the water (Stokes, 1851). Nevertheless, no calculations on taeniid egg sedimentation velocities that take into account all these variables have been performed to date. Hence, it seems possible that the sedimentation times in the studies retrieved by the review (ranging from 30 min to 24 h, see Supplementary Tables B-E) were chosen with no clear basis and/or that it was seen as a limiting factor for larger sample sizes. When using Stokes' law, and assuming a smooth taeniid egg (with a diameter of 30–35 μm and a density of 1225–1270 g cm^{-3}) in a clear water pond with a depth of 1 m, it would take at least 92 to 150 min for one egg to settle. In more turbid water however, helminth eggs will not settle as single entities but rather as part of flocs consisting of suspended particles. Adhesion to particles may then increase the settling velocity due to a higher apparent particle diameter, which has, in fact, been documented for protozoan parasites (Medema et al., 1998). In contrast, it was noticed in a study by Sengupta et al. (2011) that helminth eggs from *Trichuris* and *Oesophagostomum* entrapped in flocs settled more slowly than eggs in tap water. Here, floc density had presumably decreased by water making up part of the floc with a lower settling velocity as a result. Future studies should therefore assess how different types of water influence the flocculation and settling of taeniid and other helminth eggs.

Similarly, centrifugation, which serves as a third concentration method, was also applied at various times throughout the detection methods discussed in the review. Aiming at improving parasite recovery, it was often performed before and/or after filtration, flotation, and sedimentation. Centrifugation time and speed varied from 5 to 30 min and 113 to 1200 *g*, respectively, without providing any rationale for that specific choice. In the case of fecal samples, many commercial concentration kits for helminth eggs advise centrifugation at a lower speed for a longer time to prevent degradation of the eggs. However, taeniid eggs are very robust, and if the centrifugal force is insufficient, eggs may remain in suspension, resulting in a lower egg recovery. Therefore, Manser et al. (2016) determined whether variation in centrifugal force and time had effect on the recovery of helminth eggs (including *Taenia* spp.) from fecal samples and found that centrifuging at 1200 *g* for 3 min gave a satisfactory parasite recovery. A higher centrifugal force gave a slightly better recovery, but the deposit was more difficult to examine due to higher amounts of debris. In the future, the same should be determined for samples from different environmental matrices.

A final method of concentration is flotation that relies on differences in s.g. between a particular flotation solution and that of parasite eggs. Many solutions have been used to float eggs such as zinc sulfate, magnesium sulfate, zinc chloride, sodium nitrate, saturated sodium chloride, sucrose, saccharose, etc. with s.g. ranging from 1.18 to 1.45. Nevertheless, relatively heavy eggs, such as those of taeniids with an s.g. of 1.23–1.30, require a flotation solution of at least 1.27 s.g. (Bucur et al., 2019). However, a substantial number of records in the review used a solution with an unspecified or insufficient density, rendering them unreliable. Additionally, each solution has its own advantages and disadvantages. For instance, sugar solutions are cheap and allow slides to be kept for longer periods before reading, but its higher viscosity might impede egg recovery, especially when the coverslip is examined before all eggs in the sample have had the chance to float (Dryden et al., 2005). A more sophisticated, non-toxic medium used for density gradient separation of eggs, is Percoll®. This is a low viscosity, colloidal suspension of silica particles coated with polyvinylpyrrolidone that will form self-generated gradients ranging from 1 to 1.3 g/mL upon centrifugation. Here, each particle will sediment to an equilibrium position in the gradient where the gradient density is equal to the density of the particle. While often used in fecal examinations for parasites and most useful to obtain pure eggs for subsequent investigations, this medium is rather expensive, especially compared with cheaper salt and sugar solutions that are usually sufficient for environmental samples. Finally, apart from the flotation solution itself, it was highlighted by Ballweber et al. (2014) that other factors than s.g. also play a role in egg recovery, such as lipid richness, amount of debris, level of soil moisture, or even storage conditions. These issues should all be addressed specifically for taeniid eggs in different matrices before further application of flotation-based detection methods.

Table 6
Advantages and disadvantages of conventional microscopy based and molecular based tools for the detection of Taeniid eggs in the environment.

Method	Advantages	Disadvantages
Conventional microscopy-based tools	Relatively cheap	No identification to genus and species level
	Different parasites can be seen at once	Lower sensitivity and specificity
	Simple equipment requirements	Requires experience in identification
	Easily applicable in endemic poor communities	Altered morphology may impact egg detection and identification
		Dependent on preceding recovery and concentration procedure (e.g., choice of flotation solution, sedimentation time, centrifugation speed, etc.)
Molecular based tools	Identification to the genus and species level possible	Requires relatively sophisticated, expensive equipment making it less applicable in endemic poor communities
	Increased sensitivity and specificity	Detection of taeniid DNA gives no information on the viability of the egg
	Not negatively impacted by poor or altered egg morphology	Non-targeted parasites are not detected
		Influence of selected DNA-extraction kit, primer set and PCR-inhibitors
		Potential for nucleic acid contamination
		Dependent on preceding recovery and concentration procedure (e.g., choice of flotation solution, sedimentation time, centrifugation speed, etc.)
		Identifies DNA, not the actual transmission stage

3.6.4. Detection

Among the detection methods, conventional sedimentation(−flotation) concentration combined with standard light microscopy examination was most commonly used. This is usually preferred due to its relative cheapness and because many parasites can be seen at once, whereas PCR will only amplify the DNA strands bounded by the primer binding sites (Table 6). However, compared with molecular methods, conventional methods are often time-consuming and suboptimal for detection of parasites, due to their limited specificity and sensitivity in environmental samples. The latter is especially the case when a low number of eggs needs to be concentrated and separated from debris in the analyzed material. In those studies that use conventional methods, and in which recovery efficiency data were determined, these were usually low, not exceeding 20%; this is inadequate for obtaining an overview of the extent of contamination. Usually, however, studies did not provide such data. Furthermore, detection of eggs in a particular matrix is subjective and requires trained and experienced personnel. However, even the most highly trained technician will still be unable to identify a taeniid egg to genus level, let alone to species level, as they are morphologically highly similar. Nevertheless, a substantial number of records ascribed a genus, or even species, to taeniid eggs that had been detected by standard light microscopy. The only microscopy-based detection technique that should be able to identify to genus level, is the indirect IFAT used on water and soil samples proposed by Craig et al. (1986), as the staining is based on the binding of a labelled antibody. However, the use of this technique has not been reported in subsequent studies.

Immunodetection methods, such as IFAT and Western blot, have the potential for greater specificity (at least to genus level) but have rarely been implemented, and the focus has been on molecular tools for improving specificity in identification. Although not used for assessing contamination of objects such as furniture and walls, molecular approaches generally have greater sensitivity (LOD of 1 egg per 40 g soil and 1–2 eggs per 300 g vegetables reported; Szostakowska et al. (2014); Guggisberg et al. (2020)) and specificity, enabling differentiation to species level. Molecular tools may also identify and differentiate parasite eggs when their morphological features are poor or altered. One disadvantage of PCR-based tools is the requirement for sophisticated material, reliable electricity and temperature controls, and molecular grade reagents; this means they are less easily accessible to endemic poor communities, which may also be the communities where some of these parasites are more likely to be found. It is also important to keep in mind that detection of parasite DNA does not necessarily correlate with eggs, either viable or dead, in the sample. Furthermore, it is known that various matrices have different inhibitory substances that could inhibit the PCR and lead to false-negative results (Schrader et al., 2012). Besides that, there is always a potential influence of the selected primer set with regard to the design, its specificity, the number of targeted genes, its efficiency, the placement within the target sequence, etc. Lastly, to maximize the sensitivity of the PCR-based egg diagnosis, DNA-extraction must first be maximized by implementing an egg disruption procedure (e.g., sonication, bead beating, boiling, freeze-thawing, alkaline lysis, and proteinase K/SDS digestion) and selecting the appropriate DNA extraction kit (Temesgen et al., 2019). Some records did not include an eggshell disruption step, while others included two. Additionally, no single record compared egg lysis methods, but studies on DNA-recovery from parasite eggs often report better results with physical disruption than chemical ones (Klein et al., 2014; Ayana et al., 2019). Indeed, several studies in the review made use of freeze-thaw cycles or bead-beating to damage egg walls. However, proteinase K digestion was the most implemented. Frey et al. (2019) also discovered that a DNA extraction kit for soil (FastDNA Spin Kit) was superior to a DNA extraction kit for stool (QIAmp DNA Stool mini-Kit) when extracting taeniid DNA from food matrices. Similarly, it has been shown that for other helminths, such as soil-transmitted helminths, the choice of DNA extraction kit significantly influences the outcome of the result (Ayana et al., 2019). Nevertheless, no other studies retrieved in the review performed a similar evaluation for more and different DNA extraction kits for different environmental matrices. Last but not least, both molecular and immunodetection methods also require time-consuming preparatory steps (e.g., sieving, filtration, sedimentation, flotation, etc.) for egg recovery, and considerable numbers of eggs are likely to be lost during these procedures. Hence, even with a highly sensitive PCR, the result is likely to remain an underestimation of the actual egg burden in the environment, and the high sensitivity of the PCR is of lesser value when the preceding steps are of very low efficacy.

While reliable detection of parasites is crucial, quantifying them is also very important, especially to predict the human health risks from environmental exposure and to understand the epidemiology of host-parasite interactions. Microscopic examination can be quantitative by simple manual enumeration. However, this is very laborious with considerable variation in replicate experiments, and this hampers large-scale studies of parasite transmission (Mes et al., 2007). Molecular methods have significantly improved the sensitivity and specificity of detection, but conventional PCRs may only provide a semi-quantitative result, and this by subjectively comparing the intensity of the amplified bands on a gel. With the advent of quantitative/real-time PCR (qPCR) or ddPCR, however, both the presence and the quantity of a given target sequence can be objectively determined (Kralik and Ricchi, 2017). ddPCR has the advantage over qPCR methods, in that it has the ability for absolute quantification without the need for running standard curves constructed of serially diluted standard samples (Acosta et al., 2017). As production of these standard curves is relatively technically demanding, this may be one reason why the majority of the studies focused on detection, rather than quantification.

3.6.5. Viability and seasonal variation

Finally, it is important to keep in mind that detection of eggs or parasite DNA does not mean that parasites in the sample source are necessarily viable. Indeed, detection of DNA does not even demonstrate that eggs are present. Only two research groups addressed this issue and performed a viability assessment by use of either a trypan blue exclusion staining or an in vivo method in which *E. multilocularis* eggs were injected subcutaneously into mice after which metastode growth was evaluated (Verbyla et al., 2013a, 2013b; Guggisberg et al., 2020). The persistence of the Taeniidae lifecycles is linked to the long-lasting viability and infectiveness of their eggs in the environment. It is well known that taeniid eggs can remain infective for at least several months in the environment, depending on season, temperature, humidity, and rainfall, with humid and cool conditions favoring survival. Warm summer temperatures at the time of collection may therefore contribute to more rapid degeneration of eggs (Scandrett et al., 2009; Bucur et al.,

2019). In contrast, different records detected significantly more taeniid eggs in soil and vegetables during the dry season compared with cold ones (Eraky et al., 2014; Fallah et al., 2016; Maganira et al., 2020). A possible explanation for this might be the frequent use of contaminated water for irrigation during spring and summer. Alternatively, rainfall in the cold seasons intensifies erosion and wash-off of eggs from the surface to water. In sum, detailed knowledge on the physical resistance of taeniid eggs under different climate conditions is relevant to determine the influence on prevalence, viability, and distribution of parasitic contamination in the environment (Jansen et al., 2021).

4. Conclusions

Greater awareness of the role the environment plays in transmission of Taeniidae, has resulted in the development of a wide range of analytical assays. These currently lack any meaningful attempt at standardization, especially at the earlier stages in analysis. As a result, it is difficult to compare results from studies reporting different levels of the environmental contamination. Furthermore, conventional, microscope-based assays do not have the ability to identify taeniid eggs to genus/species level and have a relatively low sensitivity for environmental samples. Although PCR-based tools are more sensitive and can identify to the species level, they are also dependent on preliminary sampling and concentration techniques, and may be affected by the DNA extraction kit and PCR inhibitors. Finally, the infectious risk has been rarely addressed and tools that can replace animal experiments for determining egg viability are an acknowledged need.

This systematic review of the literature highlights the absence, and importance, of systematic sample collection, and the need for a standardized, validated detection tool that not only assesses the extent of environmental contamination but also the egg viability. Bringing this environmental part of the One Health triad into focus, will enable an improved epidemiological understanding of taeniid species contributing to measures for improving veterinary public health regarding these important parasites.

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Declaration of Competing Interest

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

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