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FOODBORNE PATHOGENS (S JOHLER, SECTION EDITOR)

Assessing the Contamination of Food and the Environment With Taenia and Echinococcus Eggs and Their Zoonotic Transmission

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Abstract

Purpose of Review Cystic and alveolar echinococcosis, caused by *Echinococcus granulosus* sensu lato and *E. multilocularis*, respectively, and *Taenia solium* cysticercosis are serious but neglected zoonotic diseases, caused by extra-intestinal cestode (tapeworm) infections. Humans are dead-end hosts for *Echinococcus* spp and acquire the infections by uptake of parasite eggs, either with contaminated food or via exposure by hand-mouth contact to eggs derived from the contaminated environment, including skin or coat of definitive hosts. Data related with the production of eggs of these parasites, their survival in the environment and the methodology for detection in food and environmental samples are summarized.

Recent Findings The detection of taeniid DNA, more specifically from *E. multilocularis*, in food and soil has recently been described in some European countries. These findings have been directly connected with an increase in prevalence of human infections in countries like Poland.

Summary The isolation and molecular identification of taeniid eggs is technically challenging and little standardized. The detection of taeniid DNA per se does not imply viability of eggs, and this must be considered when interpreting molecular results for transmission risk. Finally, easy, affordable, and sensitive methods replacing animal experiments should be developed to assess the viability of taeniid eggs isolated from environmental and food/water sources.

Keywords *Echinococcus granulosus* · *Echinococcus multilocularis* · *Taenia solium* · Taeniid · Oncosphere · Viability · Detection

Introduction

Foodborne and soil-transmitted parasitic zoonoses are important but neglected human diseases [1]. Although most of them have a worldwide distribution, there is agreement that their burden is highly focal, resulting in significant morbidity and mortality among vulnerable populations [2]. A diverse range of parasites is responsible for these infections, including protozoa, nematodes, trematodes, and cestodes. The cestode group includes the most important zoonotic helminths, *Echinococcus granulosus* sensu lato (s.l.) and *E. multilocularis*, responsible for cystic and alveolar echinococcosis (CE and AE), respectively. Both diseases are characterized by extra-intestinal development of larval (metacestode) parasite stages. High endemic areas of CE exist on all continents, whereas the highest disease burden of AE is in Asia, but increasing infection rates have been reported in some areas of Europe [3, 4]. Latest estimates suggest an annual global incidence of at least 188,000 new CE cases and 18,235 new AE cases (91% occurring in China) per year [2, 5]. The global burden of the diseases were calculated as 285,407 DALYs (disability adjusted life years) for CE without adjusting for underreporting and more than 1 million DALYs when underreporting is taken into consideration [6]. For AE, a median of 666,434 DALYs was estimated [5]. CE has a mortality rate between 1 and 2%, while mortality is much higher in the case of AE, with significant regional differences: 2–5% in Western and Central Europe and North America, 10–30% in Eastern Europe, and 100% mortality elsewhere [2]. Furthermore, tapeworm infections with *Taenia saginata*, *T. asiatica*, and *T. solium* (taeniosis) in humans cause mild intestinal disorders after ingestion of the larval stages (cysticerci) in meat. In addition, eggs of *T. solium* and in rare cases also of other *Taenia* spp. such as *T. crassiceps*, *T. martis*, or *T. multiceps* [7] can also invade humans which serve as dead-end hosts, causing (neuro-)cysticercosis [8].

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T. solium cysticercosis causes high morbidity and lethality in endemic areas of America, Africa, and Asia [8]. CE and AE as well as cysticercosis develop after uptake of embryonated worm eggs from food or the environment. However, based on the available data, quantification of the ways of transmission is not possible. Taeniid eggs can be differentiated by morphology to family level only, and molecular tools are available to identify eggs to species or genotype level [9, 10•, 11]. Though such studies are important to identify transmission pathways, the methodology needs to be critically assessed.

Intestinal Reproduction and Transmission Biology of Taeniids

The taeniid life cycles involve an intermediate host, in which the larval stages develop extra-intestinally, and a definitive host harboring the intestinal tapeworms (see Table 1 for characteristics of taeniid life cycles). The development of the intestinal stages can be divided into proglottisation, i.e., the sequential formation of new reproductive units (proglottides) and their growth and maturation, yielding gravid proglottides containing hundreds (*Echinococcus* spp.) or thousands of eggs in the uteri (*Taenia* spp.). The relative small reproduction rate of *Echinococcus* spp. per worm (few mm in length, < 6 proglottides) as compared with *Taenia* spp. (several meters in length, with hundreds of proglottides) is compensated by the worm numbers per host, with up to several 100,000 Echinococcus but usually < 10 Taenia worms. *Taenia* spp. are long living parasites with a continuous proglottisation of the individual worms (Table 1). In contrast, the survival dynamics of *Echinococcus* spp. in the definitive host has to be considered at population level. Quantitative assessment revealed that the main *E. multilocularis* worm burden (95%) was eliminated during the first 27 days of patency in foxes and 43 days in dogs. Total egg numbers were comparable in both host species despite much lower worm burdens in dogs [14]. Experimental infections with *E. multilocularis* from Japanese origin in foxes and dogs confirm this relatively short major patent period [19]. A similar dynamic of egg excretion was determined for *E. granulosus* with most eggs being excreted within the first month of patency [13]. Thus, proglottisation seems of minor significance for *Echinococcus* spp., and gravid proglottides are probably predominately excreted with the entire worms. In both these experimental studies, residual worm burdens were detected in most animals 80–90 days after the inoculation [13, 14]. In general, *E. multilocularis* produces fewer eggs per worm than *E. granulosus* [20]. The average number per gravid proglottis was 178 eggs (range 158 to 210) in naturally infected foxes [21]. However, in an experimental infection, the average number of eggs excreted per worm was 114, 42, and 27 in dogs, raccoon dogs, and foxes, respectively [14]. These lower egg numbers are related to the fact that many worms are eliminated in the first month of patency before reaching gravidity and that immature eggs are dissolved in the carnivore intestine.

Taenia have much higher egg production capacities, with reports of around 55,435 (15,790–83,400) eggs/proglottis for *T. hydatigena* [22] and 88,000 for *T. ovis* [23]. On average, *T. hydatigena* produced 2.3–4.9 proglottides per day over an observation time of 41–428 days [22]. Other reports suggested that a person infected with *T. saginata* disseminates per day about half a million eggs [24] and 1–8 proglottides [15]. Egg excretion is not continuous but related to the release of proglottides or entire worms. In an experimental study, dogs

Table 1 Characteristics of the life cycle of major zoonotic Taenia and Echinococcus species

Species	Definitive host	Prepatency	Patency	Egg number per proglottis[a]	Intermediate hosts	Dead-end intermediate hosts[b]
Echinococcus granulosus sensu lato all the taxa causing CE [3, 12]	Dog, (fox), dingo, jackal and hyena [12]	34–58 days	2–3 months (residual worm burdens much longer) [13]	100–1500	Sheep, cattle, pigs, camels, goats, macropods, buffaloes [12]	Human, other mammals not in the food chain of definitive host
E. multilocularis	Fox, dog, (cat), wolf, raccoon dog, coyote, jackal [12]	26–28 days	1 month for 98% of the worm burden, residual worm burdens several months longer [14]	300	Predominantly voles (Arvicolinae) [12]	Human, domestic and wild pig, dog, monkeys
Taenia solium	Human	5–10 weeks	Less than 5 years (reviewed in [8])	50,000	Pig, wild boar	Humans with cysticercosis
T. saginata	Human	86 days [15]	Few up to 30 years [16]	50,000–100,000	Cattle	Pigs are susceptible to experimental infection [17]
T. asiatica	Human	122 days [18]	Not known	80,000	Pig, wild boar	

[a] Total egg numbers; see the "Intestinal reproduction and transmission biology of taeniids" section for more details

[b] Without epidemiological significance

infected with *T. hydatigena* excreted 64% of all proglottides spontaneously (not associated with defecation), and such proglottides harbored on average 14,820 eggs [22]. During a voluntary self-infection with *T. saginata*, "involuntary proglottides discharge throughout the infection period" was observed [15].

Ways of Taeniid Egg Transmission and Infection Risks for Humans

Humans acquire the infection by per oral uptake of infective eggs, but the exact ways of transmission are not well understood and cannot be quantified. They might vary within the endemic areas, e.g., in rural and urban environments, and might be very distinct in larger endemic areas based on socio-cultural and economic reasons. Transmission could partially be linked to a typical foodborne way after ingestion of viable eggs contaminating unwashed vegetables/fruits/berries. Hand-to-mouth is another putative way of transmission, after contact with taeniid eggs in the environment. A recently published meta-analysis reported an average hand-to-mouth frequency in children ranging from 6.7 to 28.0 contacts/h in indoor situations and 2.9 to 14.5 contacts/h outdoors [25].

Taeniid eggs are dispersed from the carnivore feces with water or adhering to objects (e.g., hoofs of sheep, shoes, and tires). Birds and flies have been speculated to be possible vectors [26]. Taeniid eggs can adhere to the coat of infected dogs or foxes, and thus, there is an obvious risk originating from direct contacts with definitive hosts. Furthermore, dogs rolling in feces can be externally contaminated without being infected. Investigations with five dogs infected with *T. hydatigena* revealed 173–210 eggs/cm^2 in the peri-anal region and 4–20 eggs/cm^2 on other body areas, including even 13 eggs/cm^2 on the nose [22]. A gravid proglottis of *E. multilocularis* has been found in the peri-anal region of a naturally infected dog [27], and examination of hair coat of 46 foxes revealed taeniid eggs in 11 animals (three cases confirmed to be *E. multilocularis*) [28]. Older data document the presence of *E. granulosus* eggs in dogs' coat. Single eggs were found in the muzzle and paws of experimentally infected dogs [29], and taeniid eggs were detected in the anal region, around the mouth and on the coat of rural dogs from Nigeria infected with *E. granulosus* (confirmed at necropsy) [30]. These data confirm the potential infection risk by close contact with dogs or foxes (e.g., for hunters).

Differences in the environmental dispersal of eggs of *E. multilocularis* and *E. granulosus* s.l. should be considered. Eggs of *E. granulosus* are often localized in the dog/livestock/human environment. In fact, feeding dogs with raw livestock viscera perpetuates transmission in a variety of endemic settings [31]. For example, a restricted local transmission on small farm level with home production of pork and vegetables has been described for *E. intermedius* (G7) in Lithuania [32]. *E. multilocularis* predominantly occurs in a wild animal cycle, with a high dispersal of eggs in the environment strongly linked to the fox defecation behavior (marking of home ranges and feeding places) [33]. Recent investigations in Central Europe have shown that the increase in fox populations affected the dispersal of eggs in rural and urban areas [27, 34, 35]. Similar trends of the urbanization of the *E. multilocularis* cycle have been observed in Canada (coyotes invading cities) and in Asia (stray dogs) [27, 36]. Even though prevalences in dogs may be low in Central Europe (e.g., 0.3–3%), they can constitute a relevant zoonotic risk [37].

A recent systematic literature review [38•] identified the following definitive host related risk factors for human AE: dog ownership or playing with dogs; vocational factors like being a farmer or handling foxes. However, the limitations of such studies are the rather small numbers of patients diagnosed after long incubation periods (up to 10 years) and the difficulty to specify certain factors that represent more a general lifestyle than the particular risk factor requested. Dog ownership and contact with dogs are key risk factors for human CE in rural endemic areas, together with the presence of free-roaming owned, community-owned and/or stray dogs in urban or peri-urban areas [31].

The extra-intestinal larval stage of *T. solium* can be acquired by humans as a truly foodborne infection, after ingestion of viable eggs present on vegetables irrigated with sewage containing human feces or on food manipulated by a person with a patent intestinal infection. In an outbreak of neurocysticercosis in an orthodox Jewish community in New York, domestic servants originating from an endemic area were identified as the most probable source of infection [39]. Risk factors associated with seropositivity in humans from old studies in Mexico included "history of passing tapeworm proglottis," "frequent consumption of pork," and "poor personal and household hygiene" [40]. Transmission of *T. saginata* independently from defecation has been evidenced by observations of very high infections in calves associated with an infected person handling the animals [41].

Tenacity of Taeniid Eggs

Detailed knowledge on the physical resistance of taeniid eggs is relevant when assessing their inactivation in food, e.g., by thermal treatment. Earlier reports showed that Echinococcus eggs remain viable at temperatures below zero for long periods of time, but only temperatures of − 70 to − 80 °C for 96 and 48 h, respectively, inactivate the eggs (reviewed in [42]). On the other hand, eggs survived temperatures of + 65 °C for 2 h but were killed after 3 h [43•]. Eggs were more resistant to elevated temperatures if suspended in water as compared to an exposure at 70% relative humidity [43•]. This is relevant since

eggs can be in water droplets on vegetables. *T. saginata* oncospheres could artificially be activated after maintaining them for 335 days at 4 °C but not after 60 days at room temperature (20 °C) (Silverman, 1956 reviewed in [44]). In the natural environment, taeniid eggs can remain viable for long periods of time. A maximal survival time of *E. multilocularis* eggs of 240 days under German autumn/winter conditions and 78 days in summer was reported [45]. Eggs of *E. granulosus* remained viable up to 41 months in the environment of the Argentinian Patagonia [46] and for only four winter months in New Zealand [47]. For Taenia eggs, fewer studies on viability in the environment have been conducted; old studies reported infection in cattle after inoculation of *T. saginata* eggs maintained for 23 weeks under winter and spring conditions in Denmark (Jepson and Roth, 1949 reviewed in [44]), and a small proportion of eggs remained infective after exposure to natural conditions in Denmark for 6.5–8.5 months depending on seasonal variation [48].

Detection of Taeniid Eggs

Diagnosis of Intestinal Taeniid Infections by Fecal Examination

The diagnostic approaches for detection of intestinal infections in fecal specimens of definitive hosts have extensively been reviewed for Echinococcus [9, 49] and for human Taenia infections [50]. Eggs of taeniid parasites can efficiently be enriched from diagnostic specimens [51] but are indistinguishable by morphology (Fig. 1). Molecular techniques allow their identification to species/genotype level which is particularly relevant to identify the zoonotic species (e.g., [10, 34, 52–55•, 56]). DNA amplification techniques are claimed to detect one single egg, which is convincing as taeniid eggs contain between 18 and 56 cells [57–59]. It was estimated that a single taeniid egg (*T. hydatigena*) contains around 7000 mitochondrial targets, and the detection limit of PCRs targeting the mitochondrial DNA was estimated at 33 copies [10•]. In our lab, a reliable method combining sieving (eggs retained in a 20-μm sieve), microscopy, multiplex PCR on DNA from taeniid-positive samples (egg-DNA PCR) and optionally confirmatory sequencing was developed [10•, 51] aiming to differentiate eggs of *E. granulosus* s.l., *E. multilocularis* and other taeniids, and the method has extensively been used for individual diagnosis (e.g., [54, 60, 61]) and in studies investigating field-collected specimens (see below). However, molecular analyses of taeniid eggs can also be performed after isolation with classical diagnostic sedimentation/flotation procedures [62, 63]. DNA amplification on DNA acquired directly from specimens, i.e., omitting the egg isolation step (copro-DNA PCR), has been shown to be successful in fresh specimens [55•, 64–66]. For example, classical PCR on fecal droppings of foxes experimentally infected with *E. multilocularis* was highly sensitive with samples from the high- and low-patent period and also detected around 20% of the samples from the prepatent period [64]. Differences in analytical sensitivity for *E. multilocularis* were described when using different DNA isolation kits and different PCR approaches [67]. Tests to detect parasite antigens by ELISA (coproantigen-ELISA) usually are genus-specific, having good sensitivities in animals with high worm burden [9, 49]. Such tests are highly useful in environmental studies involving large numbers of samples (see below).

Determination of Environmental Contamination

The investigation of fecal samples collected in the environment allows a partial estimate of the parasite contamination in the field. In the case of *E. multilocularis*, it has been proposed to express it as a "contamination index," basically because it is not possible to know if multiple samples originated from one or several individuals [9]. The identification of the animal species of the feces can be done by examination of size, shape, smell, and contents, but molecular tools allow specific host identification [56, 68]. Copro-ELISA has been applied to understand temporal aspects of urban transmission of *E. multilocularis* in Switzerland. The method was validated by confirmation of the results with egg-DNA PCR [34] and was used to assess the environmental contamination in France [69] or during fox baiting studies with praziquantel (reviewed in [37]). Egg-DNA PCRs with DNA from fecal samples from the environment followed by genetic analyses have been used in several epidemiological studies [68, 70–72]. Copro-DNA PCRs with such specimens were applied in a few studies using real-time PCRs [56, 66, 68]. As "free" DNA in these samples is increasingly degraded with time, real-time PCR targeting short stretches of DNA are mandatory; confirmation of results by sequencing the very short amplicons, however, is not straightforward. Therefore, a confirmatory PCR yielding a longer fragment was recently suggested [68].

A number of studies have been performed to detect taeniid eggs in non-fecal related, environmental samples. Taeniid eggs were microscopically detected in 9/482 flotations assays of water samples after an outbreak of *T. saginata* in Canada [73]. In the same study, the authors also described the low egg recovery from spiked water samples. Taeniid eggs have also been detected in raw wastewater using a centrifugation/flotation and biphasic separation protocol [74]. Earlier research [75, 76] done in *T. solium* endemic areas failed to detect taeniid eggs in soil samples (e.g., areas used for open-air defecation) and water samples (river). Taeniid eggs isolated from soil and water samples were identified as *E. granulosus* by using a monoclonal antibody on the oncospheres released from the eggs [52]. This approach was not further used.

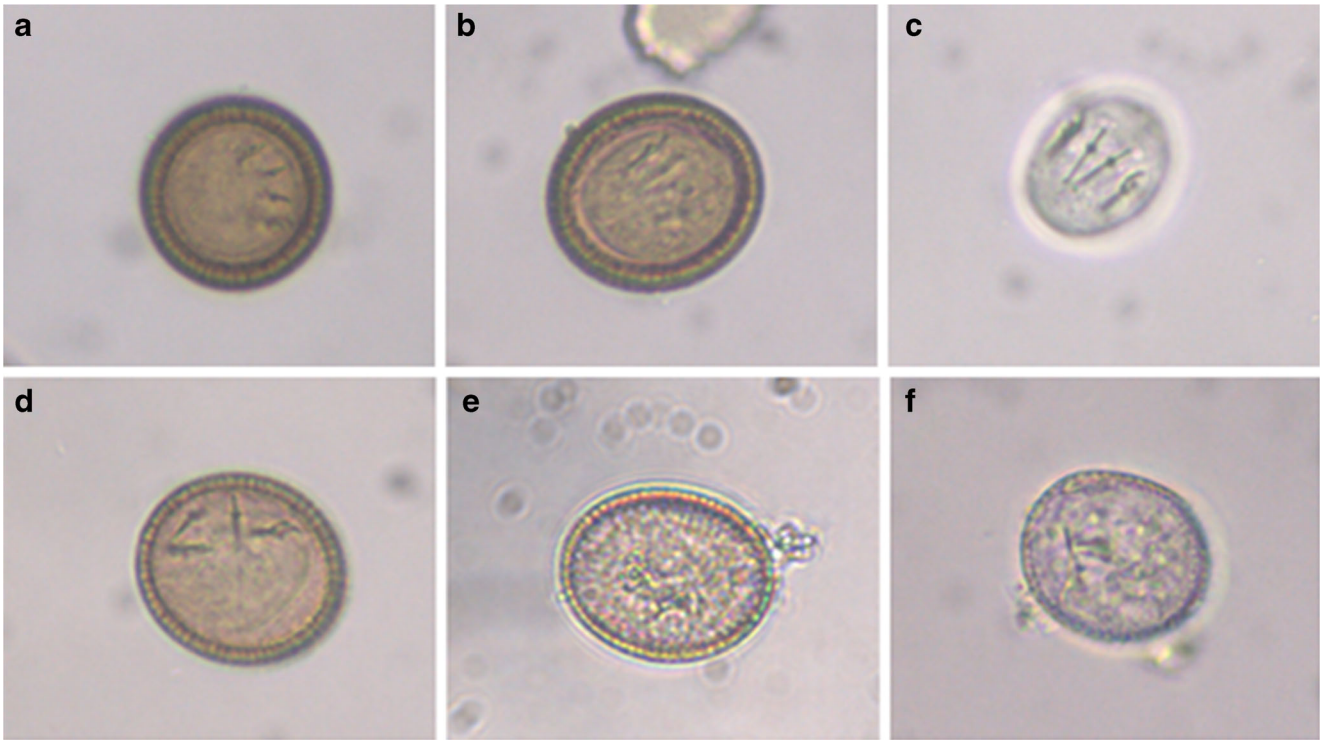

Fig. 1 In general, taeniid eggs are typically spherical to ellipsoid in shape, ranging in size from 30 to 50 μm and from 22 to 44 μm in their two diameters. All eggs in this figure were isolated from *Echinococcus multilocularis* and illustrate the typical features of a taeniid egg. **a**, **b** Mature eggs showing a thick embryophore, clearly visible oncosphere and pairs of hooks in parallel alignment; these microscopic features suggest these are viable eggs. **c** Oncosphere released from a viable egg after treatment with 2% sodium hypochlorite for 2 min. **d** Non-viable egg stored for 2 years at 4 °C in physiological saline solution showing unparalleled hooks. **e**, **f** Immature eggs showing a thin embryophore and no oncosphere; **e** was stored for 8 months in saline solution at 4 °C and **f** was freshly removed from an adult specimen of *E. multilocularis* infecting a fox

Recently, genetic tests were applied. Microscopic analyses of 120 soil samples from gardens of rural homestead in a highly *E. granulosus* endemic area in Kazakhstan revealed the presence of eggs of *Toxocara* spp., *Toxascaris leonina*, *Trichuris* spp. and taeniid eggs in several specimens. Egg-DNA PCR revealed *E. granulosus* in 5/21 taeniid egg positive samples [77]. In a study in Poland, 62 soil samples were collected, but no sampling strategy is given (random, grids) [78]. Eggs were concentrated as for microscopic examination, and taeniid eggs were identified in a subsample (8/37). Seven samples were PCR-positive for *E. multilocularis*, of which three were collected near fox dens. A major drawback of this work is the use of a classical, nested PCR which is highly prone to cross-contamination, particularly as *E. multilocularis* DNA was used as positive reaction control. More recently, the egg-DNA PCR approach was applied to investigate the presence of *E. multilocularis* (and Toxocara) in soil under and close to fox fecal samples [35]. Three soil samples collected under 25 *E. multilocularis* positive fecal samples were positive, but this was not a statistically significant difference compared to the results of the soil samples collected under/near the negative fecal samples. This illustrates that determining parasite contamination with environmental soil specimens is much less efficient than with feces. So far, there are no attempts to identify taeniids by PCR with DNA directly isolated from soil (soil-DNA PCR), and this seems to be a hopeless task due to the patchy distribution of eggs, the degradation of free DNA and the presence of PCR-inhibitory substances in soil.

Determination of Food Contamination

The scientific literature provides several reports on microscopic findings of taeniid eggs in vegetables, mainly in Asia and Africa, with contamination rates ranging between 0.9 and 18.3% (Table 2). Presently, there is no standardized methodology for the detection of taeniid eggs in food samples. We are aware of two studies using egg-DNA PCR to assess food contamination with *E. multilocularis*. In a first study [91], 103 samples (fruit, vegetable, mushrooms) were subjected to the procedure as mentioned above for soil samples [78], i.e., concentration of eggs as for microscopic examination, but no effort was made to demonstrate the presence of eggs. Cross-contamination-prone nested PCRs as used in their earlier work [78] revealed nearly one-fourth positive samples. This publication triggered some discussion in the literature, particularly questioning the finding of positive raspberries collected from the bushes at some distance

Table 2 Studies documenting microscopic detection of taeniid and other parasite stages in vegetables and fruits

Country	Number of food samples	Samples with parasites detected (%)	Weight of sample	Samples with taeniid eggs (%)	Other parasites	Reference
Iran	772	14.8%	200–300 g	0.9%	*Ascaris lumbricoides* (3.3%), *Trichuris trichiura* (2.2%), hookworms (2.9%), *Toxocara* spp. (1.6%), *Trichostrongylus* spp. (1.5%) and *Hymenolepis nana* (2.2%)	[79]
Iran	304	32.6% unwashed 1.3% traditionally washed	200 g	9.2% unwashed	*A. lumbricoides* (14.1%), *Toxocara* spp. (3.3%), *Trichostrongylus* spp. (4.3%), *Giardia* spp. (8.2%) *Entamoeba coli* (9.2%)	[80]
Iran	453 unwashed 448 washed	25.2% unwashed	250 g	4.8% unwashed	*A. lumbricoides* (8.1%), *Cryptosporidium* spp. (2.8%), *Enterobius vermicularis* (2.6%), *Strongyloides* spp. (1.1%), *Toxocara* spp. (3.9%), and *Entamoeba coli* (2.4%)	[81]
Iran	218 unwashed 436 washed	9.6% unwashed 0 in washed	200 g	1.8%	*H. nana* (0.4%), *T, trichiura* (0.9%), *A. lumbricoides* (2.2%), *Tricostrongylus* spp. (2.7%), *Dicrocoelium* spp. (33.5%)	[82]
Jordan	133	29%	250 g	6%	*Ascaris* 28 (21.1%), *Toxocara* (7.5%), *Giardia* (6.8%), *Fasciola* (4.5%), *Entamoeba histolytica* (3.8%)	[83]
Libya	126	58%	100 g	22%	*Ascaris* (68%), *Toxocara cati* (26%), *Toxocara canis* (18%), *Giardia* (10%)	[84]
Nigeria	960	8.4%	250 g	1.2%	*A. lumbricoides* (4.5%), *T. trichiura* (3.9%), hookworms (1.5%), *Strongyloides stercoralis* (0.7%), *E. vermicularis* (0.4%) and *H. nana* (0.42%)	[85]
Nigeria	1130	3.5%	200 g	0.5%	*Ascaris* (0.5%), hookworm (1.2%), *Trichuris* (0.5%), *S. stercoralis* (0.6%)	[86]
Nigeria	199	57.8%	250 g	18.3%	*Toxocara* spp. (48.3%), *Strongyloides* spp. (19.2%), *Ancylostoma* spp. (10%), *Trichuris* spp. (3.3%) and *Enterobius* spp. (0.8%)	[87]
Nigeria	120	68.3%	Unknown	4.2%	*A. lumbricoides* (16.7%), *hookworm* (18.3%), *S. stercoralis* (45.8%), *B. coli* (0.8%)	[88]
Turkey	203 unwashed 406 washed	5.9% unwashed 0 in washed	200 g	3.5%	*Toxocara* spp. (1.5%) and *A. lumbricoides* (1%)	[89]
Turkey	111	6.3%	100 g	2.7%	*Toxocara* (2.7%), *A. lumbricoides* (1.8%) and *E. vermicularis* (0.9%)	[90]

from the ground [92], though such a contamination cannot be excluded as flies were shown to transmit such eggs [26]. A second investigation on the presence of cestode eggs in feed (vegetables, fruits) [60] was triggered by frequent cases of alveolar echinococcosis in primates kept in captivity at a Zoo. Egg-DNA PCR using multiplex PCR/sequencing [10•] on filtered samples revealed non-zoonotic *Taenia* spp. of dogs, foxes, or cats in 14 of the total 95 samples (each consisting of the washing of around 40 heads of lettuce enriched with a day ration of fruits and vegetables) originating from Switzerland. Taeniid-DNA was further detected in 13 (28%) of 46 samples of vegetables originating from different parts of Europe (vegetables and fruits as

mentioned above), including *E. granulosus* s.l. (2), *T. crassiceps* (1), *T. hydatigena* (2), *T. multiceps/serialis* (2), *T. saginata* (1) and *T. taeniaeformis* (5). Although DNA of *E. multilocularis* was not identified in this study, the detection of DNA of other taeniids of foxes reveals that feed potentially pose a source for *E. multilocularis* eggs. So far, methods used to estimate the environmental or food contamination with taeniid eggs/DNA are not allowing to assess their viability, and hence, the results of such studies have to be carefully interpreted, especially considering that parasite DNA (e.g., Trichuris) [93] and also taeniid eggs [94] can be detected in archeological samples.

Determination of the Viability of Taeniid Eggs

Taeniid eggs develop to the embryonated stage within the proglottis, a further maturation of taeniid eggs in the environment has to our knowledge not convincingly been documented. Experimental studies with taeniid eggs collected from the last proglottides of gravid worms showed that only a variable proportion of these eggs are mature and viable. Experimental inoculations with taeniid eggs obtained from gravid proglottides revealed usually low (< 2.5%) development rates into larval stages in suitable hosts (for *E. granulosus infections* [95, 96], for *Taenia* spp. [97–99]).

A qualitative estimation of the viability of taeniid eggs collected from worms or isolated from feces can be achieved by microscopy by experienced scientists; criteria for viable (mature) eggs are the thick embryophore, the clearly visible oncosphere and the pairs of hooks in parallel alignment (Fig. 1). An elegant technique to assess the viability of taeniid eggs freshly collected from proglottides is their treatment with sodium hypochlorite for a few minutes to dissolve their embryophore. Immature eggs are destroyed by this procedure while viable oncospheres are protected by a resistant membrane [100•, 101]. Based on this observation, a sodium hypochlorite resistance test (SH-RT) was introduced and compared with the in vitro activation and in vitro development of oncospheres [102]. However, the results of the SH-RT did not correlate with the in vitro activation and development rate of *T. hydatigena* oncospheres after 270 days preservation in water at + 4 °C or − 28 °C. Furthermore, SH-RT was not reliable to assess the viability of heat-exposed eggs of *E. multilocularis* [43•] or *E. granulosus* [103]. Therefore, SH-RT is useful to determine the rate of mature eggs after isolation from gravid worms, and this is needed to standardize infection doses in experimental studies [101]. An in vitro method including treatment with sodium hypochlorite and assessing the permeability of oncospheres to eosin was used to estimate the viability of *E. granulosus* eggs after exposure to different temperatures [103]. This method could be used in the future for tenacity studies; however, no in vivo validation of the method was performed. Another recent study reported the survival of *T. hydatigena* eggs exposed at different conditions of relative humidity using 0.1% trypan blue to assess viability [104]; similar to other studies, no in vivo confirmation of infectivity of the treated eggs was included. So far, the most reliable viability test for taeniid eggs is the experimental infection in susceptible animals. For example, Veit et al. [45] used peroral inoculations of mice to test egg viability after exposure to several climatic conditions. The sensitivity of peroral inoculation is rather low, e.g., only 50% of mice inoculated with 100 mature eggs developed an infection. Subcutaneous inoculation was recently described as being more sensitive, as metacestode growth was observed in all animals inoculated with 20 eggs [43•]. Therefore, the differentiation of viable and non-viable eggs in the environment remains as an important issue that needs to be addressed in the future; however, so far, no laboratory method replacing experimental infections of suitable hosts has been developed.

General Recommendations

A critical view on the strategies and the methodology used for risk assessment based on the identification of eggs from zoonotic taeniids in food, water, and environment is necessary.

- Agreement on a standardized procedure for egg isolation including rigid control measures against cross-contamination.
- Professional validation (in silico, diagnostic samples) of specificity and sensitivity of assays.
- Assessment of the environmental contamination from a systematic collection of samples (host feces, soil samples) is required.
- The detection of DNA per se does not imply viability of eggs, and this must be considered when interpreting molecular results.
- Easy and affordable sensitive methods to assess the viability of taeniid eggs isolated from environmental and food/water sources should be developed to replace animal experiments.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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