# Practical guide for the diagnosis of helminth ova in stools

#### Woon-Mok Sohn<sup>10</sup>, Jong-Yil Chai<sup>20</sup>

<sup>1</sup>Department of Parasitology and Tropical Medicine, Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju, <sup>2</sup>Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul, Korea

# Abstract

In the age of globalization of infectious diseases, qualified personnel is needed for the diagnosis of parasitic diseases in the laboratory. This review aimed to introduce the methods for stool examination and identification of helminth eggs for the diagnosis of helminthic infections in laboratory and field surveys. The formalin-ether sedimentation technique (FEST) and the Kato-Katz egg counting technique (KKECT) are mainly described as representative stool examinations. The FEST is somewhat complicated and troublesome, but it is useful for differentiating small trematode eggs from opisthorchiid and heterophyid flukes. KKECT is useful in field surveys of large populations in areas endemic for soil-transmitted helminthiases. Helminth eggs are divided into four groups based on the presence or absence of the operculum and embryo. The eggs of *Clonorchis sinensis* and heterophyid flukes including Metagonimus spp. are relatively small and contain an operculum and an embryo (miracidium). Meanwhile, eggs of diphyllobothriid tapeworms, echinostomatid flukes, Paragonimus westermani, Fasciola hepatica, and Fasciolopsis buski are relatively larger, operculated, and contain germ cells and yolks instead of the embryo. The eggs of cyclophyllidian tapeworms, Taenia spp. and Hymenolepis spp., and blood flukes, Schistosoma spp., are embryonated but do not have an operculum. Nematode eggs have no operculum and embryo, but those of hookworms and pinworms sometimes have developed larvae inside. This review provides valuable insights into the methods of stool examination and helminth egg identification for the diagnosis of helminthic infections in the laboratory and field surveys.

**Keywords:** Stool examination, Formalin-ether sedimentation technique, Kato-Katz egg counting technique, Helminth eggs, Identification key

### **Background and purpose**

Helminths are commonly referred to as worms, worm parasites, or intestinal worms. Many worm parasite are members of this animal group. Taxonomically, there are five phyla related to human parasites in the helminth group: Nemathelminthes, Platyhelminthes, Nematomorpha, Acanthocephala, and Anellnida. However, members of 3 classes, Nematoda, Trematoda, and Cestoidea in the phyla Nemathelminthes and Platyhelminthes, are considered medically important groups. Members of Nematoda mainly have a life cycle including both sexes, male and female worms, and produce sexually mature eggs from adult females after mating; however, Trematoda and Cestoidea are hermaphroditic, and their eggs are commonly discharged



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Corresponding author Woon-Mok Sohn E-mail: wmsohn@gnu.ac.kr

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Microbiol, ISSN 2288-0585,eISSN 2288-6850) is an open-access journal and all published content is freely available under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND) (https://creativecommons.org/ licenses/by-nc-nd/4.0/) from fully mature adults [1–3]. Parasites, especially helminths, have the instinct for species preservation and continuously discharge their eggs from their habitats into the environment to maintain their life cycle. Therefore, helminth eggs detected from various samples are a crucial clue for some circumstances, such as helminthic infections in humans and animals, contamination in the soil of playgrounds, and endemic evidence in ancient times in archeoparasitology [4–6].

In the Republic of Korea (Korea), soil-transmitted helminthiases (STH), including ascariasis, trichuriasis, and hookworm infections, were widely prevalent until the 1970s and were the main public health threat to be solved at that time. However, their prevalence has decreased remarkably because of systematic nationwide control programs [7]. Helminthic diseases are no longer a health problem. However, the prevalence of foodborne helminthiases (FBH), including clonorchiasis and metagonimiasis, is still endemic in the Korean riverside areas. The eradication project for FBH has been continuously performed by the Division of Vectors and Parasitic Diseases of the Korea Disease Control and Prevention Agency (DCPA) [8–12]. In this eradication project, the exact identification of small trematode eggs (STE) is necessary to differentiate between liver flukes (*Clonorchis sinensis*) and intestinal heterophyid flukes including *Metagonimus* spp. in fecal specimens. The exact identification of STE is also necessary in the individual stool examination performed in the diagnosis Laboratory in the Korea Association of Health Promotion (KAHP), and the Division of Vectors and Parasitic Diseases of the DCPA [12–14]. Therefore, in the context of helminthic infections in Korea, the formalin-ether sedimentation technique (FEST) should mainly be adopted for stool examinations by well-trained laboratory personnel.

In contrast, non-governmental organizations (NGO), including the KAHP, have been used to control parasites, especially helminths, in endemic countries of Southeast Asia and Africa. Although the target parasites to be controlled in the projects differed by survey subject and region, most of the surveys were performed using the Kato-Katz egg counting technique (KKECT). This method has been very useful in helminth control projects, especially soil-transmitted helminths such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, in endemic countries of Southeast Asia and Africa [15–19].

Laboratory diagnosis of helminthic diseases is performed in two steps, detection and identification, during stool examination by trained laboratory personnel. Detection methods and skills are important for the accurate diagnosis of helminthic infections in stool examinations. The ability to recognize parasites, especially helminth eggs, should be trained and is ultimately needed to differentiate them from non-parasite artifacts for the exact identification of helminth eggs through microscopy [20,21]. In contrast, Palmieri et al. [22] emphasized the importance of parasitology education, especially the training to identify and diagnose parasitic infections, as an emerging need. These authors also mentioned the increasing need for well-trained and qualified healthcare professionals in the laboratory to diagnose parasitic diseases due to the globalization of infectious diseases. Therefore, in this review, we focused on two topics: methods for stool examination and the identification of helminth eggs.

#### Methods for stool examination

Stool examinations are usually performed to diagnose parasitic infections (helminths and protozoa). Examination of the infection status of helminths is largely divided into two methods, qualitative and quantitative. A qualitative method examines prevalence (infection rate) and is divided into two methods: smear (direct smear and cellophane thick smear) and concentration methods (sedimentation and flotation techniques). The cellophane thick smear (CTS), also known as Kato's method, has been popularly adopted in field surveys of large populations [15–19]. The concentration method, especially the FEST, is applied to small-scale surveys or individual examinations because their procedures are complicated and time-consuming. Quantitative methods check the intensity of infection (worm burden) and are only used to estimate the number of worms infecting a certain person or population. As representative quantitative techniques, Stoll's dilution egg counting and KKECT are recommended; however, the former method is complicated and troublesome. Therefore, in this review, we focus on describing the procedures for the FEST and KKECT.

### **Qualitative stool examination**

#### Smear methods

Direct smears are commonly used to detect the trophozoites of *Entaemeba histolytica* and *Giardia lamblia* from the diarrheic stools of acute-phase patients. This method is not useful for examining helminthic infections because the egg detection rate is very low, except for diphyllobothiid tapeworms.

The cellophane thick smear (Kato's method) is performed using a cellophane (No. 600) strip pre-soaked in Kato's solution instead of a cover slip. Kato's solution is a mixture of glycerin (500 mL), distilled water (DW, 500 mL), and 3% malachite green (recently, methylene blue has been highly recommended) solution (5 mL). This method is discussed in detail in the quantitative technique.

#### **Concentration methods**

The Faust's zinc sulfate flotation technique is commonly used to detect cysts and/or oocysts of intestinal protozoa, such as *E. histolytica*, *G. lamblia*, and *Cryptosporidium parvum*, in the stool of patients in the chronic phase. It is not suitable for the diagnosis of helminthic infections because the specific gravity of helminth eggs is relatively high.

FEST is somewhat complicated and troublesome; however, it is better than the Kato's method for the exact identification of helminth eggs, particularly the STE of opisthorchild and heterophyid flukes.

FEST procedure (Supplementary Fig. 1 in the online-only Data Supplement)

1. Sample a thumb head-size amount of feces into a beaker with DW.

2. Stir with a wooden applicator to form a stool suspension.

3. Filter the stool suspension through two layers of wet gauze.

4. Centrifuge the conical tip tube with the filtered stool suspension at  $500 \times g$  for 3 min.

5. Discard the supernatant in a tube.

6. Add 10% formalin (10 mL) and ether (3 mL) to the tube.

7. Shake the tube vigorously for 15 s after placing a rubber stopper or cap on it.

8. Centrifuge the tube at  $500 \times g$  for 3 min.

9. Gently remove the plug of debris and discard the supernatant.

10. Drop the sediment onto the slide glass.

11. Cover the glass slip (cover slip) and examine it under a light microscope.

In stool examinations using FEST in FBH-endemic riverside areas of Korea (2011–2023), the prevalence of clonorchiasis was the highest (4.8%), followed by heterophyid fluke infection (1.5%), trichuriasis (0.2%), and *Gymnophalloides seoi* infection (0.06%) (Table 1) [12,13]. Results of stool examinations with FEST in health checkups in the KAHP revealed a similar pattern, with a higher prevalence of FBH, such as clonorchiasis (1.5%) and heterophyid fluke infections (0.34%), including metagonimiasis, compared to STH, that is, ascariasis and trichuriasis (0.13%) (Table 2) [14]. These findings suggest that FEST is the gold standard for the diagnosis of helminthic infections, including clonorchiasis and metagonimiasis, in Korea.

Table 1. Results of stool examinations with the formalin-ether sedimentation technique in FBH endemic riverside areas of Korea [12,13]

Survey year	No. of resident examined	No. (%) of resident infected by the helminth sp.*					
	_	Cs	Het	Tt	Gs	Etc.	
2011	27,395	3,049 (11.1)	753 (2.7)	107 (0.4)	4 (0.01)	4 (0.01)	
2012	23,233	1,867 (8.0)	329 (1.4)	49 (0.2)	29 (0.12)	4 (0.02)	
2013	38,789	2,950 (7.6)	622 (1.6)	69 (0.2)	42 (0.11)	9 (0.02)	
2014	41,139	2,029 (4.9)	555 (1.3)	86 (0.2)	6 (0.01)	6 (0.01)	
2015	42,024	1,778 (4.2)	334 (0.8)	69 (0.2)	6 (0.01)	7 (0.02)	
2016	34,943	1,419 (4.1)	358 (1.0)	44 (0.1)	18 (0.05)	1 (0.003)	
2017	38,648	1,524 (3.9)	319 (0.8)	52 (0.1)	28 (0.07)	3 (0.01)	
2018	32,792	1,351 (4.1)	677 (2.1)	63 (0.2)	22 (0.07)	6 (0.02)	
2019	30,415	944 (3.1)	505 (1.7)	58 (0.2)	21 (0.07)	-	
2020	25,642	974 (3.8)	440 (1.7)	54 (0.2)	33 (0.13)	1 (0.004)	
2021	24,349	697 (3.3)	340 (1.6)	64 (0.3)	12 (0.05)	4 (0.01)	
2022	28,902	950 (3.3)	506 (1.8)	43 (0.2)	26(0.1)	2 (0.01)	
2023	25,214	463 (1.8)	391 (1.6)	33 (0.1)	11 (0.04)	4 (0.02)	
Total	413,485	19,995 (4.8)	6,129 (1.5)	791 (0.2)	258 (0.06)	51 (0.01)	

\*Abbreviations: Cs: Clonorchis sinensis; Het: Heterophyid flukes including Metagonimus spp.; Tt: Trichuris trichiura; Gs: Gymnophalloides seoi.

**Table 2.** Results of stool examinations with the formalin-ether sedimentation technique in health check-ups in Korea Association of Health Promotion [14]

Year examined	No. of resident examined	Cumulative No. (%) of positive	No. (%) resident of infected by the helminth sp.		elminth sp.
		_	Cs*	Meta <sup>†</sup>	Nematodes <sup>‡</sup>
2012	70,684	1,418 (2.0)	1,108 (1.6)	156 (0.2)	18 (0.03)
2013	140,169	2,857 (2.0)	2,397 (1.7)	505 (0.4)	239 (0.17)
2014	144,028	3,091 (2.2)	2,472 (1.7)	519 (0.4)	234 (0.16)
2015	143,949	2,460 (1.7)	1,920 (1.3)	493 (0.3)	163 (0.11)
2016	130,305	2,405 (1.9)	1,687 (1.3)	476 (0.4)	176 (0.14)
Total	629,135	12,231 (1.9)	9,584 (1.5)	2,149 (0.34)	830 (0.13)

 $Cs^*$ : Clonorchis sinensis; Meta<sup>†</sup>: Heterophyid flukes including Metagonimus spp.; Nematodes<sup>†</sup>: Ascaris lumbricoides (42) + Trichuris trichiura (765) + Enterophysic vermicularis (23).

### Quantitative stool examination

#### Stoll's dilution egg counting technique

1. Fill a 0.1 N NaOH or KOH solution into the 56 mL scale of a Stoll's flask (Supplementary Fig. 2 in the online-only Data Supplement).

2. Add the fecal sample in the Stoll's flask to a volume of 60 mL (1 g of feces is dissolved in 15 mL of fecal solution).

3. Sample the fecal solution (0.15 or 0.075 mL) with a Stoll pipette after shaking the flask, and drop it onto the slide glass when the feces are completely dissolved in the solution.

4. Cover the glass slip, examine the fecal specimen under a light microscope, and count the number of eggs.

5. EPG (No. eggs per gram of feces), n (correction factor for the condition of the stool)  $\times$  No. of eggs  $\times$  100 (200 for the 0.075 mL sample)

6. EPD (No. eggs per day): EPG × whole-day stool (200 g in adults)

7. EPDPF (EPDPW), No. of eggs per day per female (worm)

Ascaris lumbricoides: 200,000, Trichuris trichiura: 5,000, hookworms: 10,000, Clonorchis sinensis: 3,000. 8. No. of worms: EPD/EPDPF (EPDPW)

#### KKECT

Materials (Supplementary Fig. 3 in the online-only Data Supplement)

1. Plastic template: 50 mg (hole: 9 mm diameter × 1 mm thick).

41.7 mg (hole: 6 mm in diameter  $\times$  1.5 mm thick),

20 mg (hole: 6.5 mm in diameter  $\times$  0.5 mm thick).

2. Plastic spatula.

3. 60-105 nylon mesh.

4. Slide glass.

5, Newspaper (Fig. 1A).

6. Cellophane: 40–50  $\mu$ m thick (25 × 30 mm), flat-bottom jar with lid, and forceps (Fig. 1B).

#### Procedure

1. Place a small amount of fecal sample on a newspaper and press the nylon mesh on top so that some of the feces are sieved through the mesh and accumulate on top (Fig. 1C).

2. Scrape the flat-sided spatula across the upper surface of the mesh to collect the sieved feces (Fig. 1D).

3. Place the template at the center of a glass slide and add feces from the spatula to completely fill the hole (Fig. 1E).

4. Remove the template carefully so that the mound of feces remained on the slide.

5. Cover the fecal mound with the presoaked cellophane strip (Fig. 1F).

Invert the slide and firmly press the fecal sample against cellophane strips to evenly spread the feces (Fig. 1G).

7. Maintain CTS specimens at room temperature for more than 1 h for clearance (Supplementary Fig. 3 in the online-only Data Supplement).

8. Examine the fecal specimen under a light microscope and count the number of eggs.

9. EPG: No. of eggs  $\times$  20 (50 mg template), No. eggs  $\times$  24 (41.7 mg template).

10. EPD = EPG  $\times$  whole-day stool amount (200 g in adults)

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11. No. of the infected worms: EPD/EPDPF (EPDPW)
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Control and elimination programs for helminthiases have been performed using the Kato-Katz thick smear technique by NGOs including KAHP, the Korea International Cooperation Agency, and the Korea Foundation for International Healthcare in endemic countries (Myamar, Cambodia, and Lao PDR) in Southeast Asia [15–18]. The individual prevalence of helminthiasis differed between the survey regions and subjects. However, in these programs, STHs, such as ascariasis (17.7%), trichuriasis (14.0%), and hookworm infections (14.9%), were the main target diseases, and FBHs, that is opisthorchiid and heterophyid fluke infections (12.1%), were additional health problems (Table 3). Eom et al. [17] conducted a Korea-Laos collaborative project for the control of foodborne trematode infections in Lao PDR (2007–2011). They examined 6,178 fecal samples using a Kato-Katz thick smear and Stoll's egg counting technique to determine the infection status of helminths. The prevalence of STE was the highest (55.6%), followed by hookworm infection (27.8%), trichuriasis (6.5%), and ascariasis (3.7%).

In contrast, McCarthy et al. [23] reviewed the diagnostic technologies relevant to the control of helminth infections, either available or under development. They also discussed the critical gaps to be identified and the opportunities to improve diagnostic technologies for control and elimination programs as a research agenda for helminthic diseases in humans. Some research groups have adopted new devices and kits to efficiently detect helminth eggs. Boonyong et al. [24] compared the detection performance of a fully automatic digital feces analyzer (Orienter Model FA280) with that of formalin-ethyl acetate concentration technique. Lefaure et al. [25] evaluated the detection efficacy of the Parasep® device, which was adapted to the Faust's flotation

<b>Table 3.</b> Results of stool examinations with the Kato-katz thick smear technique in Southeast Asian of	countries
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Survey locality	No. of people examined	No. (%) of resident infected by the helminth sp.					
		$\mathrm{Al}^\dagger$	Tt <sup>‡</sup>	Hw <sup>§</sup>	STE <sup>∥</sup>	Etc.	
[15]	2,227	383 (17.2)	432 (19.4)	5 (0.2)	2 (0.1)	33 (1.5)	
[16]	32,201	1,478 (4.6)	1,315 (4.1)	3,101 (9.6)	1,838 (5.7)	1,325 (4.1)	
[17]	6,178	227 (3.7)	404 (6.5)	1,719 (27.8)	3,435 (55.6)	625 (10.1)	
[18]	29,846	10,416 (34.9)	7,700 (25.8)	5,701 (19.1)	3,253 (10.9)	239 (0.8)	
Total	70,452	12,504 (17.7)	9,851 (14.0)	10,526 (14.9)	8,528 (12.1)	2,222 (3.2)	

<sup>\*</sup>By projects of Korea International Cooperation Agency (KOICA), Korea Association of Health Promotion (KAHP) and Korea Foundation for International Healthcare (KOFIH) in Myamar, Cambodia and Lao PDR; <sup>†</sup>*Ascaris lumbricoides*; <sup>‡</sup>*Trichuris trichiura*; <sup>§</sup>hookworm; <sup>¶</sup>small trematode eggs including opisthorchiid and heterophyid flukes.

[15] Chai et al. (2020): Schoolchildren in Yangon Region, Myanmar; [16] Yong et al. (2014): Inhabitants of Cambodia (2006-2011); [17] Eom et al. (2014): Inhabitants of Lao PDR (2007-2011); [18] Rim et al. (2004): Primary schoolchildren of Lao PDR (2000-2002).



Fig. 1. Materials and procedures for the Kato-katz egg counting technique. (This figure was re-arranged based on Figs 1-8 [28])

technique, with conventional techniques. Sarirah et al. [26] evaluated the detection efficacy of the Mini-FLOTAC and compared it with that of KKECT, a quantitative technique recommended by the World Health Organization (WHO). Khanna et al. [27] also evaluated the detection rate of Mini Parasep® solvent-free method in the rapid diagnosis of intestinal helminthiases through comparing it with that of formalin-ethyl acetate sedimentation technique (FEAST, has been recently recommended instead of FEST by WHO) [28]. Recently, the Korea DCPA developed the ParaEgg kit and adopted it for stool examinations in the FBHendemic riverside areas of Korea (personal communication). The detection efficiency of new devices or kits is promising compared to that of conventional techniques in stool examinations; however, some limitations, such as sensitivity, specificity, practical difficulty, cost, and infrastructure demand, need to be solved preemptively for their widespread use.

The cellotape anal swab is used for the diagnosis of pinworm, *Enterobius vermicularis*, and sometimes beef tapeworm, *Taenia saginata*, infections.

### Identification of helminth eggs

The identification of helminth eggs is somewhat difficult, even for trained laboratory personnel. However, the helminth eggs are similar to the avian eggs. They commonly have hard chitinous shells, germ cells, yolks, and sometimes embryos (Supplementary Fig. 4 in the online-only Data Supplement), but they have different compositions and unique morphologies according to the helminth species. Each species of helminth eggs is differentiated according to their unique morphology and size. In this review, we discuss the morphologies and differential keys for the identification of helminth eggs from human fecal samples.

# Morphology of helminth eggs

*Group I. Operculated and embryonated smaller (trematode) eggs* (Supplementary Table 1 in the online-only Data Supplement)

The eggs of the opisthorchiid fluke (*C. sinensis*) and heterophyid flukes including *Metagonimus* spp. are relatively small and include an operculum and an embryo (miracidium).

*Clonorchis sinensis*: Ovoid and sesame seed-shaped with a miracidium inside,  $27-35 \times 15-20 \mu m$  in size, have a dominantly developed (conspicuous) operculum and shoulder rim, a shell surface with muskmelon-like wrinkles, and maximum width in the posterior 1/3 level (Fig. 2A, 2 B).

Heterophyids: Ovoid in shape with a miracidium inside, commonly having an inconspicuous operculum and shoulder rim, smooth shell surface without muskmelon wrinkle pattern, and maximum width in the median (Fig. 2C–2F). More than 11 species, including *Metagonimus* spp., *Heterophyes nocens*, *Heterophyopsis continua*, *Stellantchasmus falcatus*, *Pygidiopsis summa*, *Centrocestus armatus*, *Stictodora fuscata*, *Stictodora lari*, and *Acanthotrema felis*, have been reported in Korea. They are divided into 3 groups according to their lengths:  $< 25 \mu$ m, 26–30 µm, and  $> 31 \mu$ m.

In the CTS specimens, the eggs of opisthorchiid flukes are indistinguishable from those of heterophyid flukes including *Metagonimus* spp. (Supplementary Fig. 5 in the online-only Data Supplement). However, they can be easily differentiated based on the dominantly developed operculum and muskmelon surface appearance in FEST fecal specimens. Unlike opisthorchiid flukes, the eggs of heterophyid flukes had a smooth shell surface.

*Stictodora fuscata*: Ovoid with a miracidium inside, larger  $(34-38 \times 20-23 \mu m)$  than other heterophyid fluke eggs, with thick shells, which are sometimes dark brown in color (Fig. 2F).

*Plagiorchis muris*: Elliptical with a miracidium inside,  $32-38 \times 20-24 \mu m$  in size, thick shell, conspicuous operculum, and sometimes an abopercular knob (Fig. 2G).

*Eurytrema pancreaticum*: Oval with a miracidium inside,  $48-53 \times 25-33 \mu m$  in size, thick shell, inconspicuous operculum, dark brown in color (Fig. 2H).

*Gymnophalloides seoi*: Very small with a miracidium inside,  $20-25 \times 11-15 \mu m$  in size, very thin shell, and large operculum (Fig. 21).



**Fig. 2.** The embryonated small-sized eggs with an operculum (Group I). A. *Clonorchis sinensis*; B. SEM-view of *C. sinensis* egg; *C. Metagonimus yokogawai*; D. *Heterophyes nocens*; E. *Pygidiopsis summa*; F. *Stictodora fuscata*; G. *Plagiorchis muris*; H. *Eurytrema pancreaticum*; I. *Gymnophalloides seoi*. Scale bar = 10 µm.

#### *Group II. Operculated and unembryonated larger eggs* (Supplementary Table 2 in the online-only Data Supplement)

The eggs of *Diphyllobothrium* spp., echinostomatid flukes, *Neodiplostomum seoulense*, *Paragonimus westermani*, *Fasciola hepatica*, and *Fasciolopsis buski* are relatively larger in size and have an operculum, but do not have an embryo. They consist of germ cells and yolk inside.

Echinostomatids: The echinostomes infecting humans belong to 4 species, *Echinostoma cinetorchis* (99–116 × 66–76  $\mu$ m), *Isthmiophora hortensis* (127–139 × 71–81  $\mu$ m), *Echinochasmus japonicus* (76–87 × 52–63  $\mu$ m), and *Acanthoparyphium tyosenense* (78–93 × 63–73  $\mu$ m), have been reported in Korea. Echinostomatid eggs differentiate from other fluke eggs in this group by the presence of abopercular wrinkles,

and are roughly divided into 2 groups based on their average length:  $< 100 \mu m$  and  $> 101 \mu m$ . They are commonly oval in shape, with a thin shell, a small operculum, and abopercular wrinkles (Fig. 3A–3D).

*Paragonimus westermani*: Elliptical and asymmetrical in shape,  $80-100 \times 45-65 \mu m$  in size, have a wide operculum, shoulder rim, and abopercular thickening (Fig. 3E).

*Neodiplostomum seoulense*: Elliptical and asymmetrical in shape,  $86-99 \times 55-63 \mu m$  in size, have thin shell, and an inapparent operculum (Fig. 3F).

*Fasciola hepatica*: Very big,  $145 \times 80 \ \mu m$  in average size, with thin shell, and inconspicuous smaller operculum (Fig. 3G).

*Fasciolopsis buski*: Very big,  $147 \times 75 \,\mu\text{m}$  in average size, with thin shell, and inconspicuous smaller operculum.

*Diphyllobothrium* spp.: Ovoid and symmetrical in shape,  $58-76 \times 40-51 \mu m$  in size, with a thick shell, and smaller operculum, and sometimes with an abopercular knob (Fig. 3H).



**Fig. 3.** The unembryonated large-sized eggs with an operculum (Group II). A. Echinostomatid egg in CTS specimen; B. *Isthmiophora hortensis*; C. *Echinostoma cinetorchis*; D. *Echinochasmus japonicus*; E. *Paragonimus westermani*; F. *Neodiplostomum seoulense*; G. *Fasciola hepatica*; H. *Dibothriocephalus nihonkaiensis*. Scale bar = 50  $\mu$ m. CTS, cellophane thick smear.

#### Group III. Nonoperculated and embryonated eggs

The eggs of cyclophyllidian tapeworms, such as *Taenia* spp. and *Hymenolepis* spp., and blood flukes, *Schistosoma* spp., are embryonated, but they do not have an operculum.

*Taenia* spp.: Round, 30–45  $\mu$ m in diameter size, with thick and striated embryophore, and have a hexacanth embryo. Three species, *Taenia asiatica*, *T. saginata*, and *T. solium*, are not microscopically differentiable at the species level (Fig. 4A).

Hymenolepis nana: Round, 30–47 µm in diameter size, with transparent shell, and have a hexacanth embryo with polar filaments (Fig. 4B, 4C).

*Hymenolepis diminuta*: Round, 70–80 µm in diameter size, with thick shell, and have a hexacanth embryo with polar thickenings (Fig. 4D).

Schistosoma japonicum: Ovoid, 70–100  $\times$  50–65  $\mu$ m in size, with thin shell, a small lateral spine and a miracidium inside.

Schistosoma mansoni: Long, elliptical,  $114-175 \times 45-68 \mu m$  in size, with thin shell, a stout lateral spine, and a miracidium inside.

Schistosoma haematobium: Long, elliptical,  $112-170 \times 40-70 \mu$ m in size, with thin shell, a terminal spine, and a miracidium inside.



Fig. 4. The embryonated (cestode) eggs without an operculum (Group III). A. *Taenia* sp. in CTS specimen; B. *Hymenolepis nana*; C. *H. nana* in CTS specimen; D. *Hymenolepis diminuta*. Scale bar =  $15 \mu m$ . CTS, cellophane thick smear.

#### Group IV. Nonoperculated and unembryonated (nematode) eggs

The nematode eggs have no operculum and embryo, but those of hookworms and pinworms are sometimes observed in embryonated stages.

Ascaris lumbricoides (F): Subspherical or round,  $50-75 \times 35-50 \mu m$  in size, with 3 layered shells: outer mammilated protein coat, middle chitinous, and inner lipoid layers. In fresh stool, they retain a single blastomere (Fig. 5A, 5 B). They sometimes develop in CTS specimens as 2-, 4-, 8-, 16-, 32-cells, and morular stages in a warm environment and rarely have a decorticated (no protein coat) appearance. Fertilized eggs of *A. lumbricoides* are clearly differentiated from toxocarid (*Toxocara* spp.) eggs from cats and dogs by their morphological shape, being nearly round with a thinner shell, but nearly identical to those of *Ascaris* 

suum from pigs.

Ascaris lumbricoides (U): Long, elliptical,  $80-90 \times 40-45 \mu m$  in size, thin shell with poorly developed protein coat and granules inside (Fig. 5C, 5D). A variety of shapes are frequently observed in the CTS specimens (Supplementary Fig. 6 in the online-only Data Supplement). Characteristic broken shell fragments with a protein coat and internal granules are helpful in identifying unfertilized *A. lumbricoides* eggs.

*Trichuris trichiura*: Barrel shape,  $50-55 \times 22-23 \mu m$  in size, with thick shell and polar mucoid plugs on both sides and a single blastomere in fresh stool specimens (Fig. 5E, 5F).

Recently, two types (standard- and large-sized) of whipworm (*T. trichiura*) eggs from schoolchildren in Yangon, Myanmar were molecularly confirmed to be the same species. The size of standard-eggs was 52–58  $\times$  21–28 µm and that of large- eggs was 65–76  $\times$  30–35 µm in the study by Ryoo et al. [29]. The large eggs



**Fig. 5.** The nematode eggs (Group IV). A. *Ascaris lumbricoides* (F); B. *A. lumbricoides* (F) in CTS specimen; C. *A. lumbricoides* (U); D. *A. lumbricoides* (U) in CTS specimen; E. *Trichuris trichiura*; F. *T. trichiura* in CTS specimen; G. *Enterobius vermicularis* in CTS specimen; H. *E. vermicularis* in a specimen of cellotape anal swab; I. Hookworm; J. Hookworm (morular stage) in CTS specimen; K. Air-bubble like hookworm egg of which a mature larva already escaped; L. *Trichostorongylus orientalis*. Scale bar = 25 µm. CTS, cellophane thick smear.

were slightly smaller than the known size of *Trichuris vulpis* (dog whipworm). Some parasitologists strongly suspect *T. vulpis* eggs in CTS specimens from prevalent countries, such as Lao PDR, Cambodia, and Myanmar.

*Enterobius vermicularis*: Persimmon seed shape,  $50-60 \times 20-30 \mu m$  in size, with double-layered shell and larva. Pinworm eggs are detected in the cellotape anal swab specimens but are sometimes found in the fecal specimens of CTS (Fig. 5G, 5H).

Hookworms: Ovoid,  $55-75 \times 35-45 \mu m$  in size, have hyaline thin shells with blunting of both ends, and 4–8 cell stage in the fresh fecal specimen (Fig. 5I). They rapidly develop and are embryonated in warm environments, and sometimes show an air-bubble-like appearance (Fig. 5J, 5 K). In this case, they are difficult to recognize. To solve this problem, the microscope should be finely adjusted. The definite and continuous demarcation of egg shells may be helpful in the diagnosis of hookworm eggs. As human-infecting hookworms, 3 species, i.e., *Ancylostoma duodenale*, *A. cylanicum*, and *Necator americanus*, have been reported.

*Trichostrongylus orientalis*: Long, elliptical,  $75-95 \times 45-50 \mu m$  in size, with a thin hyaline shell and a morular stage inside (Fig. 5L). These nematode eggs are easily differentiated from hookworm eggs by their large size, one pointed end (both blunted ends in hookworms), and the internal morular stage.

The morphological characteristics of helminth eggs are summarized in Tables 4 and 5.

<b>Table 4.</b> Morphological summary on the helm	ninth ova with an operculum in Korea
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1 0	5	1
Helminth species	Size (µm)	Morphological characteristics
Clonorchis sinensis	27-35 × 15-20	Conspicuous operculum & shoulder rim, muskmelon pattern wrinkles on surface
Metagonimus yokogawai	28-35 × 17-18	Inconspicuous operculum & shoulder rim, no wrinkles, maximum width in median
Metagonimus miyatai	28-33 × 17-18	Same with those of M. yokogawai
Metagonimus takahashii	32-36 × 18-20	Same with those of M. yokogawai
Heterophyes nocens	24-29 × 15-17	Inconspicuous operculum & shoulder rim, ovoid with a little pointed both end
Heterophyopsis continua	24-28 × 16-19	Inconspicuous operculum & shoulder rim, broadly oval, no wrinkles
Stellantchasmus falcatus	25-29 × 12-14	Inconspicuous operculum & shoulder rim, elongated & oval, no wrinkles
Stictodora fuscata	34-38 × 20-23	Inconspicuous operculum & shoulder rim, oval with thick shell, no wrinkles
Pygidiopsis summa	20-23 × 11-13	Apparent operculum, small & pyriform, no wrinkles
Gymnophalloides seoi	20-25 × 11-15	Big & wide operculum, no knob, no wrinkles, elliptical with very thin shell
Plagiorchis muris	32-38 × 20-24	conspicuous operculum, elliptical with thick shell, yellow
Eurytrema pancreaticum	48-53 × 25-33	Inconspicuous operculum, elliptical with thick shell, dark brown
Isthmiophora hortensis	125-138 × 70-80	Small operculum, ovoid to elliptical with thin shell & abopercular wrinkles
Echinostoma cinetorchis	99-116 × 65-76	Nearly same with those of <i>I. hortensis</i>
Echinochasmus japonicus	75-87 × 52-63	Tiny operculum, broad oval with thin shell & abopercular wrinkles
Paragonimus westermani	80-100 × 45-65	Wide operculum, asymmetrical with shoulder rim & abopercular thickening
Neodiplostomum seoulense	86-99 × 55-63	Inapparent operculum, asymmetrical, elliptical with thin shell
Fasciola hepatica	143-147 × 78-82	Small operculum, elliptical with thin shell
Diphyllobothriids*	58-76 × 40-51	Small operculum, ovoid with thick shell & sometimes abopercular knob

\*including Dibothriocephalus nihonkaiensis (syn. Diphyllobothrium nihonkaiense) prevalent species in Korea.

1 0 9		1
Helminth species	Size (µm)	Morphological characteristics
Taenia spp.*	30-45 in diameter	Round with thick shell, striated embryophore & hexacanth embryo
Hymenolepis nana	30-47 in diameter	Round with transparent shell, hexacanth embryo with polar filaments
Hymenolepis diminuta	70-80 in diameter	Round with thick shell, striated hexacanth embryo with polar thickening
Ascaris lumbricoides $(F)^{\dagger}$	50-75 × 35-50	Subspherical or round with mammilated protein coat & single blastomere
Ascaris lumbricoides (U) <sup>‡</sup>	80-90 × 40-45	Elongated ellipsoidal with thin shell, thin protein coat & granules inside
Trichuris trichiura	50-55 × 22-23	Barrel shape with thick shell, polar mucoid plugs & single blastomere
Hookworms <sup>§</sup>	55-75 × 35-45	Ovoid with hyaline thin shell & 4-8 cell stages
Trichostrongylus orientalis	75-95 × 35-45	Long elliptical with hyaline thin shell, & morular stages
Enterobius vermicularis	50-60 × 20-30	Persimmon stone shape with double layered shell & larva
	1 * 0 84 1	1 1 1 1 1 1 1 1

Table 5. Morphological summary on the helminth ova without an operculum in Korea

\*T. asiatica, T. saginata and T. solium; †fertilized; <sup>‡</sup>unfertilized eggs; <sup>§</sup>Ancylostoma duodenale and Necator americanus.

# Differential key to helminth eggs from fecal samples of humans

Helminth eggs with an operculum	
Helminth eggs without an operculum	
1. Operculated eggs with a developed embryo (miracidium)	2
Operculated eggs with a germ cell and yolks	7
2. Egg shell rough with muskmelon appearance	Clonorchis sinensis
Egg shell smooth, no muskmelon appearance	
3. Very small (<25 $\mu m$ in length), big operculum, thin, and transparent shell	Gymnophalloides seoi
Small with thick shell	
4. Very small (<23 $\mu m$ in length), with a prominent operculum	Pygidiopsis summa
Small (>25 µm in length)	5
5. Small (25–35 µm in length)	Heterophyids*
Small (> 35 $\mu$ m in length) with thick shell	
6. Small (35–38 µm in length) with thick shell	Stictodora fuscata
Medium (45–50 $\mu m$ in length) with thick dark shellsEu	ırytrema pancreaticum
*Metagonimus spp., Heterophyes nocens, Heterophyiopsis continua, Stell	antchasmus falcatus,
Centrocestus armatus, Stictodora lari, and Acanthotrema felis.	
7. Unembryonated eggs with abopercular wrinkles	Echinostomatids <sup>#</sup>
Unembryonated eggs without abopercular wrinkles	
8. Very big (> 140 $\mu$ m in length) in size, thin shell, small or inconspicuous operculum	n
	and Fasciolopsis buski
Relatively big (< 100 µm in length) in size	9
9. Symmetrical in shape, $< 80 \mu\text{m}$ in length, with thick shell and small operculum	
	Diphyllobothrium spp.
Asymmetrical in shape, $80-100 \mu\text{m}$ in length, wide operculum and abopercular th	nickening
	Paragonimus spp.
#Echinostoma spp., Isthmiophora hortensis, and Echinochasmus japonicus.	

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10. Non-operculated eggs with an developed embryo	
Non-operculated eggs with an undeveloped embryo	
11. Round or elliptical in shape, with embryopore (egg shell) and a hexacanth	embryo 12
Very large or large, thin shell; lateral or terminal spine in the shell surface a	nd miracidium13
12. Round, with thick and striated embryopore, a hexacanth embryo	
Round or elliptical, transparent shell, a hexacanth embryo with polar filame	ents at both opposite sites
	Hymenolepis nana
Round or elliptical, thick-shelled, a hexacanth embryo with polar thickenin	gs at both opposite sites
13. Ovoid in shape, with a small lateral spine and miracidium	Schistosoma japonicum
Long, elliptical in shape, with a stout lateral spine and miracidium	Schistosoma mansoni
Long and elliptical, with terminal spine and miracidium	Schistosoma haematobium
14. Egg shell with rough surface (mammilated protein coat)	
Egg shell with smooth surface	
15. Round or elliptical, thick shell with cospicuous mammilated coat	Ascaris lumbricoides (F)
Elliptical or elongated, with thin shells and granules inside	Ascaris lumbricoides (U)
16. Hyaline thin shell	
Thick shell	
17. Ovoid, medium (<75 $\mu$ m in length) in size, with 4–8 cell stages inside	Hookworm <sup>@</sup>
Long elliptical, large (> 75 $\mu$ m in length), with a morular stage inside	Trichostrongylus orientalis
18. Barrel shape, with polar mucoid plugs	Trichuris trichiura
Persimmon seed (stone) shaped with a larva	Enterobius vermicularis

<sup>(a)</sup>Ancylostoma duodenale, A. cylanicum, and Necator americanus.

# **Concluding remarks**

The prevalence of soil-transmitted helminthiases, including ascariasis, trichuriasis, and hookworm infections, has markedly decreased owing to systematic nationwide control programs in Korea. The prevalence of foodborne helminthiases, including clonorchiasis and metagonimiasis, is continuously maintained in riverside areas. The infection status of helminths urged us to adopt FEST to differentiate STE, whether *C. sinensis* eggs or not, in stool specimens. Although some modified concentration techniques with new devices or kits have been introduced and adopted in stool examinations, conventional basic FEST is described in this review to assist laboratory personnel. Recently, Soares et al. [30] reviewed detection techniques for parasites in human stool and discussed with comparative results the advantages, limitations, and perspectives of each detection method for professionals and specialists in this field. However, this review focuses on the basic detection techniques, FEST and KKECT, as representative (qualitative and quantitative) stool examinations.

It is ultimately necessary to differentiate helminth eggs from non-parasitic artifacts for the exact diagnosis of helminthiases by microscopic stool examinations. In this review, helminth eggs were largely divided into 4 groups (Groups I–IV) based on the presence or absence of the operculum and embryo to facilitate microscopic identification by laboratory personnel. According to the basic framework for the identification of helminth eggs, members of Groups I and II have an operculum, and those of Groups I and III have a larva inside. Members in Groups III and IV have no operculum, and in Groups II and IV have germ cells and yolks instead of larvae inside (Table 6). Recently, digital identification and quantification of helminth eggs has been adopted as a new approach (automatic detection), which uses various image processing algorithms [31–35]. These studies suggest that the identification of helminth eggs using artificial intelligence-based technique can be popularly adopted in stool examinations in the near future. However, in this review, we describe only the morphologies and differential keys for the exact identification of helminth eggs from human fecal samples. In conclusion, this review provides valuable insights into the methods of stool examination and helminth egg identification for the diagnosis of helminthic infections through laboratory and field surveys.

Table 6.	The basic	frame fo	r the	identification	of	helminth	eggs
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Identification key Group		Size of helminth eggs	
	<40 μm	41~80 μm	>81 µm
Group I			
Embryo (+)	Opisthorchiid, Heterophyid & Gymnophaliid flukes	Dicrocoeliid fluke	
Operculum (+)			
Group II			
Embryo (-)		Diphyllobothriid & Spirometrid tapeworms	Echinostomatid, Paragonimus Neodiplostomum, Fasciola spp. Fasciolopsis
Group III			-
Embryo (+)		Cyclophyllidean tapeworm*	Schistosoma spp.
Operculum (-)			
Group IV			
Embryo (-)		Nematodes <sup>†</sup>	Nametodes <sup>‡</sup>

\**Taenia* spp. and *Hymenolepis* spp.; <sup>†</sup>*Ascaris lumbricoides* (F), *Trichuris trichiura* and hookworms; <sup>‡</sup>*Ascaris lumbricoides* (U) and *Trichostrongylus orientalis*.

### Supplementary materials

The online-only Data Supplement is available with this article at https://doi.org/10.5145/ACM.2024.27.2.3.

### **Ethics statement**

This article has no ethical problems.

# **Conflict of interest**

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