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ORIGINAL PAPER

Evaluation of the MGL method to detect *Paragonimus* eggs and its improvement

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Abstract Dog feces containing 500 *Paragonimus westermani* eggs per gram were examined by the Medical General Laboratory (MGL), the simple sedimentation (SS), and the Army Medical School III (AMS III) methods. The number of eggs per gram of feces (EPG) obtained by the MGL method was 17.2 and was significantly lower than those obtained by the SS method (324.0) and the AMS III method (505.6). When isolated *P. westermani* eggs were processed by the MGL method and four layers (ether, ether-fecal, formalin layers, and sediment) of the final centrifugation product were separately examined, almost 100 % of eggs were found at the ether-fecal layer. Similarly, when fecal samples containing *P. westermani*, *Paragonimus skrjabini miyazakii*, *Paragonimus ohirai*, or *Paragonimus harinasutai* eggs were processed by the MGL method, more than 95 % of the eggs were found in the supernatant layers. The formalin-ethyl acetate (FEA) method showed a similar tendency as the MGL method and over 90 % of eggs remained in the supernatant layers. Contrary to *Paragonimus* eggs, 63 and 96 % of *Clonorchis* and *Metagonimus* eggs were found in the sediment in the MGL method, respectively. When surfactant (Tween 80) was added to fecal solution, most of *Paragonimus* eggs spun down in the sediment in the MGL and FEA methods, suggesting that *Paragonimus* eggs have hydrophobic components on their surface. It is suggested that surfactant addition to the fecal solution should be considered when the MGL method is used for detection of *Paragonimus* eggs.

Keywords Diagnosis · Eggs · Fecal examination · MGL method · *Paragonimus*

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Introduction

Paragonimiasis is a typical food-borne parasitic disease caused by lung flukes belonging to the genus *Paragonimus*. Among over 50 nominal species described to date, seven are known to infect humans (Blair et al. 1999, 2007). It has been estimated that 20 million people are infected worldwide and more than 90 % of infections in Asia are with *Paragonimus westermani* (Toscano et al. 1995). Other species are commonly found in carnivorous/omnivorous animals.

Detection of eggs excreted in sputum and feces is the definitive method for proving an ongoing infection in humans and other hosts (Blair et al. 1999; Fried and Abruzzi 2010). For the diagnosis of human paragonimiasis, examination of sputum is preferred because there are fewer impurities. However, sputum examination is practically not applicable for animals because of the difficulty of sample collection. For fecal egg examination, sedimentation techniques, such

as Medical General Laboratory (MGL) method (formalin-ether method) (Ritchie 1948) and Army Medical School III (AMS III) method (Hunter et al. 1948), have generally been employed (Singh et al. 2012). The MGL method is simpler, and the morphology of parasite eggs is preserved well due to the action of formalin, whereas the AMS III method is more complicated but fecal residual matter can be more effectively removed (Hunter et al. 1948).

Previous studies suggested a relatively lower sensitivity for the MGL method. Using the MGL method, eggs could only be detected in 4.2–21.7 % of patients that showed a positive reaction in skin-patch tests (Cho et al. 1983; Joo et al. 1985). Eggs could not be detected in 26 patients diagnosed with paragonimiasis based on the presence of specific antibodies and clinical history (Nkouawa et al. 2009). In contrast, using the AMS III method, eggs were detected in 40.0–43.9 % of patients with positive reactions in the skin-patch test (Okada 1959; Katamine et al. 1960, 1972), and 65.1 % of patients clinically diagnosed with pulmonary paragonimiasis (Komiya and Yokogawa 1953). However, there has been no comparative study of the sensitivity of the MGL and AMS III methods for *Paragonimus* egg detection.

Alternatively, the simple sedimentation (SS) method has been used to detect various trematode eggs, such as *Fasciola*, *Dicrocoelium*, and *Schistosoma* (Conceição et al. 2002; Abebe et al. 2010; Habtamu and Mariam 2011). This method does not require chemicals, and still, the results are reliable. However, this method is extremely time- and space-consuming. The formalin-ethyl acetate sedimentation (FEA) method (Young et al. 1979) has been developed as an alternative to the MGL method to avoid using flammable and highly toxic diethyl ether. The method has been applied to detect various trematode eggs with the sensitivity comparable to that of the MGL method (Truant et al. 1981; Goodman et al. 2007; Songserm et al. 2012; Itoh et al. 2013; Patel et al. 2013).

In this study, we evaluated the applicability of the MGL method for detection of *Paragonimus* eggs. The results show that the conventional MGL method has poor sensitivity to detect *Paragonimus* eggs. However, simple addition of the detergent to fecal solution markedly improved the sensitivity of MGL method to detect *Paragonimus* eggs.

Materials and methods

Feces containing *Paragonimus* spp. eggs

Information of *Paragonimus*-infected dogs used in this study was summarized in Table 1. Feces containing *P. skrjabini miyazakii* eggs were obtained from an experimentally infected dog that was inoculated orally with 20 metacercariae found in brackish water crabs at Iwakuni City, Japan. Feces containing *P. ohirai* eggs were obtained from an experimentally infected dog that was inoculated intraperitoneally with 20 metacercariae found in brackish water crabs at Miyazaki City, Japan. These dogs were housed individually, fed with commercial food and received water ad libitum. The animal experiment for the dogs was approved and conducted in accordance with the regulations for Animal Experiments of University of Miyazaki (approval number 2006-043-6).

Feces containing *P. westermani* eggs were obtained from a seropositive boar-hunting dog raised in Miyazaki City, Japan. Five eggs isolated from the feces of this dog were used individually for molecular confirmation of *P. westermani* using PCR/sequence analysis of the ITS2 region (Doanh et al. 2007, 2011).

Feces containing *P. harinasutai* eggs were obtained from an experimentally infected dog in the experimental animal center, Fukuoka University, in 2008 with the ethical approval of the regulations for Animal Experiments of Fukuoka University (approval number 0711197). This dog was inoculated orally with 200 metacercariae collected from Quang Binh Province, Vietnam. The dog was housed alone, fed with commercial food and received water ad libitum.

Each fecal sample was mixed well so that eggs were evenly distributed. Samples were aliquoted into plastic tubes and used immediately or kept at 4 °C until use.

In addition to those, feces that contained a determined number of *P. westermani* eggs were artificially produced by mixing the eggs isolated from the infected dog feces with uninfected dog feces and used in some experiments. Isolation of *P. westermani* egg from infected dog feces was carried out by the following method. Feces of a dog infected with *P. westermani* was firstly mixed with enough amount of water and then passed through a 150-μm pore size wire mesh. The fecal solution was filtered through a nylon mesh with a pore size of 80 μm (Nytal, Heiden, Switzerland). Then, the filtrate was filtered again through a nylon mesh with a pore size of 30 μm (Nytal, Heiden, Switzerland). Finally, the materials trapped on the 30-μm mesh were washed off into water in a small container.

Eggs of *Metagonimus yokogawai* and *Clonorchis sinensis*

Eggs of *Metagonimus yokogawai* and *Clonorchis sinensis* that were collected from infected rats and a dog, respectively, and fixed in 10 % formalin after concentration by the AMS III method were kindly provided from Drs. Takeshi Nakamura and Daigo Tsubokawa in the Department of Parasitology, Kitasato University School of Medicine.

SS method

The SS method was conducted on feces with some modification of the method described by Conceição et al. (2002). One gram of feces was mixed with 500 ml of water and passed through a 150-μm pore size wire mesh, and the filtrate was

Table 1 Information of dogs infected with *Paagonimus* spp. used in this study

Parasite species	Mode of infection	Site where metacercariae were obtained	No. of metacercariae inoculated
P. s. miyazakii	Experimental	Yamaguchi, Japan	20
P. ohirai	Experimental	Miyazaki, Japan	20
P. westermani	Natural[a]	NA	NA
P. harinasutai	Experimental	Quang Binh, Vietnam	200

NA not applicable

[a] A dog was raised at Miyazaki, Japan

allowed to settle for 30 min. The supernatant was discarded, and water was added up to 500 ml. This process was repeated three times, and the final sediment was resuspended in 5 ml of water. The eggs in 100 μl of the final suspension were counted under a microscope. The counting step was repeated five times and the mean number of eggs was calculated. The number of eggs per gram of feces (EPG) was calculated by multiplying the mean by 50.

MGL method

The MGL method (Ritchie 1948) was used on feces with a slight modification. Briefly, 0.5 g of feces was mixed with 7 ml of formalin, passed through the wire mesh (pore size 150 μm), and 3 ml of diethyl ether was added and the mixture was vigorously shaken for 30 s. After centrifugation at 1000 rpm (190×*g*) (LC-220, Tomy, Tokyo) for 10 min at room temperature, the upper three layers were discarded, and the sediment was resuspended with a small amount of water. The number of eggs in the entire sediment suspension was counted under a microscope, and EPG was calculated by multiplying the count by 2.

FEA method

The same procedure as the MGL method was used except that diethyl ether was substituted with ethyl acetate (Young et al. 1979). The number of eggs in the entire final suspension was counted under a microscope, and EPG was calculated by multiplying the count by 2.

AMS III method

The AMS III method (Hunter et al. 1948) was used with a slight modification. Briefly, 0.5 g of feces was mixed with 7 ml of formalin, passed through a wire mesh (pore size 150 μm) and centrifuged at 1500 rpm (440×*g*) for 2 min at room temperature. After discarding the supernatant, 7 ml of AMS III solution was added, and the sediment was resuspended. Then two drops of Tween 80 and 3 ml of diethyl ether were added, and the fecal suspension was vigorously shaken for 30 s. After centrifugation at 1500 rpm (440×*g*) for 2 min at room temperature, the upper three layers were discarded, and the sediment was resuspended in a small amount of water. The number of eggs in the entire sediment suspension was counted under a microscope, and EPG was calculated by multiplying the count by 2.

Examination of eggs in the supernatant layers of MGL and FEA methods

After the final centrifugation, four layers were produced in the MGL and FEA methods (ether, ether-fecal, formalin layers, and sediment) as shown in Fig. 1, and the presence of eggs in each supernatant layer or in whole supernatant layers (ether, ether-fecal, and formalin layers) was examined.

In the examination for each layer, the supernatant layers were separately collected from the top layer by sucking up with a Pasteur pipette, and then, the number of eggs in each layer and sediment was counted under a microscope.

In the examination for whole supernatant layers, the three supernatant layers were decanted together into a tube. Then, the eggs were filtered as described above with pore size 80 and 30-μm nylon mesh. The numbers of eggs in the filtered materials and also in sediments were counted under a microscope.

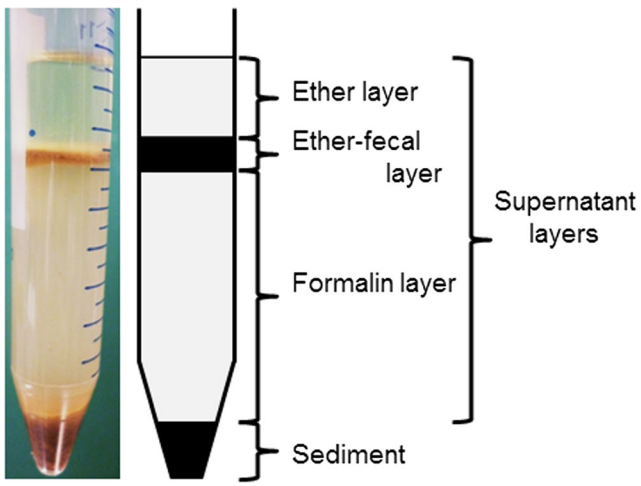

Fig. 1 Layers produced by the MGL method after final centrifugation

Measurements of dry weight of sediments

To compare the amount of sediments produced by different sedimentation techniques, sediments obtained by each method were put at a 70 °C incubator until the weight become stable. For each method, 0.5 g of 20 dog feces were processed individually and the mean of dry weight of sediments were calculated for each method.

Examination of the eggs of *Metagonimus yokogawai* and *Clonorchis sinensis*

Eggs of *M. yokogawai* and *C. sinensis* in 10 % formalin fixed fecal solution were isolated by filtering first through a nylon mesh with a pore size of 40 μm and trapping by nylon mesh with a pore size of 10 μm (Nytal, Heiden, Switzerland). Then, the eggs of each parasite were mixed with uninfected dog feces so as to attain 1000 eggs in 1 g.

Then, the MGL method was performed on the feces and the number of eggs found in the sediment and supernatant layers was counted. Finally, the percentage of eggs detected in the sediment in the total number of eggs detected was calculated.

Statistical analysis

First, the normality of the distribution of each data was evaluated by Kolmogorov-Smirnov test, and the equal variance was evaluated by *F* test. Then, significance in difference or relationship of data was evaluated by following procedures.

For comparison of the MGL, SS and AMS III methods applied on *P. westermani* eggs-mixed feces, analysis of variance (ANOVA), and Scheffe's paired comparison were performed.

For comparison of the MGL and SS methods applied on paired samples from experimentally infected dogs, Wilcoxon signed-rank test was performed. Correlation of their EPGs was evaluated by Spearman's rank correlation coefficient.

For comparison of the proportions of eggs detected in the supernatant layers in the total number of eggs detected by the MGL method among the feces containing the different species of *Paragonimus* eggs, Kruskal-Wallis test was performed after the angular transformation of data.

For comparison of the proportions of eggs detected in the sediment in the total number of eggs detected between the original and modified MGL methods and between the original and modified FEA methods, Student's *t* test was performed after the angular transformation of data.

For comparison of the dry weight of sediments obtained from different sedimentation techniques, Friedman test with Scheffe's paired comparison was performed.

Those analyses were conducted using Excel statistics 2010 for Microsoft Excel 2010 program for Windows (Social Survey Research Information Co., Ltd., Tokyo) and Program R (version 3.1.1) (R Development Core Team 2014). A difference with $P<0.05$ was considered significant.

Results

Detection of *Paragonimus westermani* eggs by the MGL, SS, and AMS III methods from dog feces mixed with eggs

Mean EPGs (standard deviation (SD)) that were obtained by the MGL, SS, and AMS III methods performed on five replicates of dog feces containing 500 *P. westermani* eggs per gram were 17.2 (8.4), 324.0 (37.8), and 505.6 (38.1), respectively. Significant difference was obtained among the three methods ($P<0.001$ by ANOVA) and in all pairs of different methods ($P<0.001$ by Scheffe's paired comparison).

Detection of *Paragonimus* spp. eggs by the MGL and SS methods from feces of experimentally infected dogs

The MGL and SS methods were performed in triplicate on 63 fecal samples from a *P. s. miyazakii*-infected dog and 135 samples from a *P. ohirai*-infected dog. Table 2 shows the number of egg-positive feces and the median EPG values obtained from the two dogs by the two methods. The median EPGs obtained by the MGL method were significantly lower than those obtained by the SS method in both species in Wilcoxon signed-rank test. While the SS method could detect *Paragonimus* eggs in all fecal samples, the MGL method failed to detect eggs in four fecal samples from the *P. s. miyazakii*-infected dog. The EPGs obtained by the SS method of those four samples were 50, 108, 108, and 133. Weak correlations were obtained by Spearman's rank correlation coefficients between EPGs obtained by the MGL and SS methods (0.465 for *P. s. miyazakii*-infected and 0.614 for *P. ohirai*-infected dog feces). Distribution of ratios of EPGs obtained by the MGL and SS methods is given in Table 3. Compared to the SS method, the MGL method provided lower EPGs in all samples except for one, and ten times lower values in 67.2 % of feces.

Presence of *Paragonimus* eggs in the supernatant layers of the final centrifugation product by the MGL method

The number of eggs in each of four layers of the final centrifugation product was counted after the MGL method was performed on isolated *P. westermani* eggs. The median numbers (minimum–maximum) of five replicates in the ether layer, ether-fecal layer, formalin layer, and sediment were 0 (0–

Table 2 Number of egg-positive feces and the median EPGs obtained by the MGL and SS methods in feces of dogs experimentally infected with *Paragonimus skrjabini miyazakii* and *Paragonimus ohirai*

	P. s. miyazakii (*n*=63)		*P. ohirai* (*n*=135)	
	MGL	SS	MGL	SS
No. of egg-positive feces (%)	59 (93.7)	63 (100)	135 (100)	135 (100)
Median EPG[a] (10th/90th percentile)	8 (1/50)	108 (50/398.4)	16 (3.4/119.2)	292 (67/1051.6)

n the number of fecal samples examined

[a] The median EPGs obtained by the MGL method were significantly lower than those obtained by the SS method in both species ($P<0.001$)

0), 96 (83–106), 1 (0–5), and 1 (0–1), respectively, resulting 98 % of eggs in total were found in the ether-fecal layer.

Then, we examined fecal samples collected from *P. westermani*-, *P. s. miyazakii*-, *P. ohirai*-, and *P. harinasutai*-infected dogs by the MGL method, and counted the number of eggs present in the supernatant layers and sediment. Table 4 shows the median numbers of eggs of five replicates for each species and the proportions of eggs detected in the supernatant layers. In all species, more than 95 % of eggs were found in the supernatant layers. There was no significant difference among the groups in the proportion of eggs detected in the sediment layers ($P=0.0581$ by Kruska-Wallis test).

Effect of surfactant addition in the MGL method

The MGL method was performed in triplicate on feces containing 500 *P. westermani* eggs per gram with modification by the addition of two drops of Tween 80 to the formalin-stool suspension, and the numbers of eggs detected in the supernatant layers and sediment were counted. Then, the proportion of eggs detected in the sediment was compared with that of the original method. The proportion (SD) of eggs found in the sediment became 98.7 % (1.6) (the mean number of eggs detected in the supernatant layers was 3.0, while that in the sediment was 251.4), which was significantly different from the proportion (5.2 % (3.2)) (the mean number of eggs detected in the supernatant layers was 167.8, while that in the sediment was 8.6) obtained by the original method ($P<0.01$ by Student's *t* test). Figure 2 shows the eggs of *P. westermani* in the final sediment of the original MGL method (a1, a2) and the modified MGL method by the addition of Tween 80 (b1, b2). The final sediment of each method was mixed with 5 ml of water, poured into a Petri dish of 10-cm diameter, and shaken and settled down so that eggs were evenly distributed. More number of eggs was observed in the final sediment of the modified MGL method, whereas the morphology of eggs was unchanged.

Table 3 Distribution of ratios of EPGs obtained by the MGL and SS methods

EPG ratio (MGL/SS)	*P. s. miyazakii* (*n*=63) No. of feces (%)	*P. ohirai* (*n*=135) No. of feces (%)	Total (*n*=198) No. of feces (%)
>1.0	1 (1.6)	None	1 (0.5)
0.5–1.0	3 (4.8)	7 (5.2)	10 (5.1)
0.1–0.5	19 (30.2)	35 (25.9)	54 (27.3)
<0.1	36 (57.1)	93 (68.9)	129 (65.2)
Negative in MGL	4 (6.3)	None	4 (2.0)

n the number of fecal samples examined

Detection of *Paragonimus* eggs by the FEA method

As observed in the MGL method, most of the eggs were detected the supernatant layers in the final centrifugation product of the FEA method that was applied in triplicate to 0.5 g of feces containing 250 *P. westermani* eggs. The proportion (SD) of eggs found in the sediment was 9.1 % (5.4) (the mean number of eggs detected in the supernatant layers was 23.2, while that in the sediment was 231.0), and the proportion significantly increased to 99.0 % (0.7) (the mean number of eggs detected in the supernatant layers was 2.0, while that in the sediment was 184.6) when two drops of Tween 80 was added to the formalin-stool suspension in the FEA method ($P>0.01$ by Student's *t* test).

Dry weight of sediments obtained by different sedimentation techniques

Mean dry weight (SD) of sediments produced by the MGL, modified MGL with addition of Tween 80, FEA, modified FEA with addition of Tween 80, SS, and AMS III methods that were performed on 0.5 g of feces were 0.062 (0.015), 0.081 (0.016), 0.071 (0.012), 0.101 (0.021), 0.052 (0.013), and 0.047 (0.013) g, respectively. The AMS III method showed the least sediment that was significantly different from those of modified MGL, FEA and modified FEA methods ($P<0.05$ by Scheffe's paired comparison). Addition of Tween 80 resulted in increase of sediment in the MGL and FEA methods.

Table 4 Median number of eggs of *Paragonimus* spp. detected in the supernatant layers and sediment after the final centrifugation of the MGL method and proportion of eggs in supernatant layers

	Median (minimum/maximum)			
	P. westermani	*P. s. miyazakii*	*P. ohirai*	*P. harinasutai*
No. of eggs in supernatant layers	154 (132/193)	72 (56/84)	319 (246/390)	263 (229/461)
No. of eggs in sediment	4 (1/8)	2 (1/4)	12 (12/30)	3 (1/13)
Proportion of eggs in supernatant layers	95.6 (92.0/96.4)	97.5 (95.0/99.5)	96.6 (95.3/98.8)	98.7 (97.3/99.6)

Detection of *Metagonimus yokogawai* and *Clonorchis sinensis* eggs by the MGL method

The MGL method was performed on dog feces containing 1000 *M. yokogawai* or *C. sinensis* eggs per gram, and the numbers of eggs in the supernatant layers and sediment were counted. The mean proportions (SD) of eggs found in the sediment were 62.8 % (4.1) and 95.6 % (5.1), for *M. yokogawai* and *C. sinensis*, respectively.

Discussion

The MGL method has been widely used to detect *Paragonimus* eggs in medical and veterinary fields (Soh et al. 1961; Min 1981; Lee et al. 1994). However, the present results clearly show that the ability of the MGL method to detect *Paragonimus* eggs was significantly lower than that of the SS and AMS III methods. Because only a part of the sediment is examined in most cases, the finding in this study implies that diagnosis for paragonimiasis using the MGL method may not represent the real status, and that previous epidemiological studies using the MGL method potentially underestimated the prevalence of *Paragonimus* infection in humans and animals. Indeed, recovery of eggs from serologically positive patients was comparatively lower with the MGL method compared to the AMS III method (Okada 1959; Katamine et al. 1960, 1972; Cho et al. 1983; Joo et al. 1985). It has been reported that the number of eggs produced per day by an adult worm in the host lung is low, especially in the early patent period (Kihara et al. 1980), and that the number of eggs detected in feces varies considerably depending on the sampling days (Yokogawa 1955; Tomimura et al. 1958; Fan et al. 1998). Considering these factors as well, we suggest that the conventional MGL method is not suitable for detection of *Paragonimus* eggs, and that more reliable methods such as the AMS III and SS methods should be selected for the purpose. Actually, the AMS III method produced the

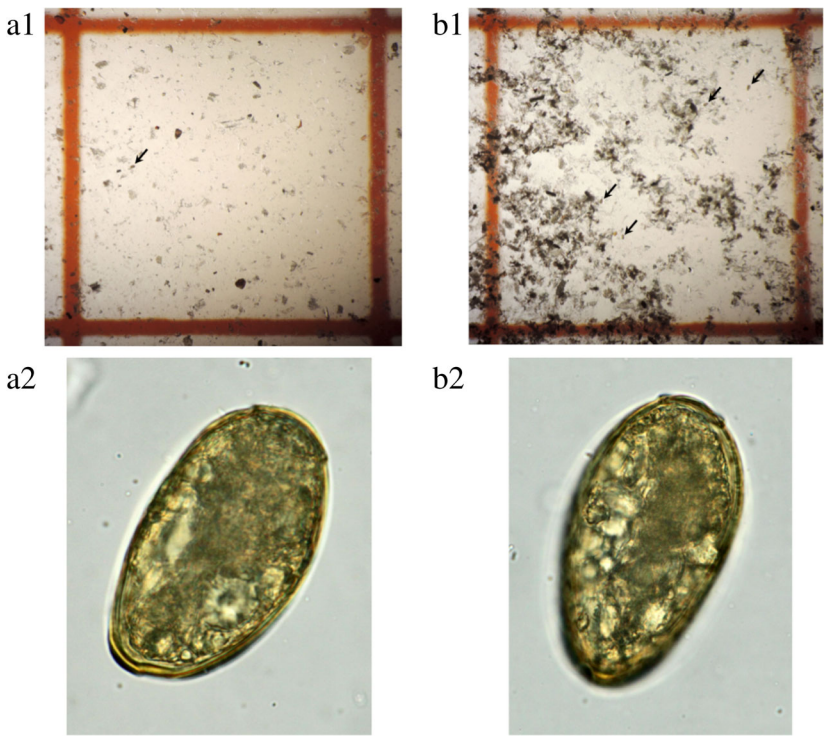

Fig. 2 Eggs of *Paragonimus westermani* in the final sediment of the original MGL method (*a1*, *a2*) and the modified MGL method by the addition of Tween 80 (*b1*, *b2*). *a1* and *b1*: Eggs (*shown by arrows*) found in 5-mm square (*orange line*) in a Petri dish. a2 and b2: Magnification of an egg found in the sediment. Note: More fecal debris was recognized in the modified MGL method

lowest amount (dry weight) of sediment among the methods we evaluated, and thus potentially reduces the time required for egg examination in whole sediment.

This study revealed that the ether-fecal layer that was produced after the final centrifugation in the MGL method contained the majority of eggs in the specimen, and that only a small portion of eggs could be detected in the sediment. This is considered to be the main reason for poor detection of *Paragonimus* eggs by the MGL method. This was commonly observed in the four species of *Paragonimus*, suggesting that this feature is common in the genus. In contrast, most of eggs of other trematodes such as *M. yokogawai* and *C. sinensis* that are commonly found in the dogs were detected in the sediment by the MGL method, indicating that the feature observed in *Paragonimus* eggs are specific to its genus.

Since the addition of the surfactant Tween 80 to formalin-stool suspension drastically improved the recovery of *Paragonimus* eggs in the sediment of the MGL method, it could be assumed that the *Paragonimus* egg surface is covered with hydrophobic substances. A major component of sputum is lipid, such as dipalmitoylphosphatidylcholine (Shaheen et al. 2009) and therefore, the hydrophobic feature of eggs could facilitate their entrapment in the lung and respiratory tract by sputum. This could be the reason why *Paragonimus* eggs were frequently detected in sputum (Komiya and Yokogawa 1953). On the other hand, the greater sensitivity of the AMS III method in detecting eggs could be explained by the addition of Tween 80 to the fecal suspension.

Recently, due to the flammable and toxoic nature of diethyl ether, other chemicals such as ethyl acetate and gasoline have been evaluated as a substitute for diethyl ether in the MGL method (Young et al. 1979; Truant et al. 1981; Goodman et al. 2007; Ahmadi and Damraj 2009; Songserm et al. 2012; Itoh et al. 2013; Patel et al. 2013). The method using ethyl acetate (FEA method) has been applied to detection of *Paragonimus* eggs in human feces (Waree et al. 2001). In this study, we evaluated the efficiency of this method in detecting *Paragonimus* eggs, showing similar detectability with the MGL method. As observed in the MGL method, addition of Tween 80 could significantly enhance the recovery rate of *Paragonimus* eggs in the FEA method.

Since the specific gravity of trematode eggs is generally higher than the standard flotation solutions, sedimentation techniques such as ones evaluated in this study are broadly chosen as an egg detection method. However, trematode eggs can be detected by flotation techniques if solution with higher specific gravity is used, and several studies showed that eggs of trematodes such as *Paragonimus*, *Fasciola*, and *Schistosoma* could be detected with flotation solution having specific gravity of 1.31 to 1.35 (Kihara 1976; Duthaler et al. 2010; Glinz et al. 2010). It is not clear that the hydrophobic nature of *Paragonimus* eggs would affect on the detectability of eggs, but flotation techniques can be considered as an alternative to sedimentation techniques for detection of *Paragonimus* eggs.

In conclusion of this study, it was revealed that the original MGL method has a low ability to detect *Paragonimus* eggs because the majority of the eggs remain in the ether-fecal layer after the final centrifugation. It is suggested that surfactant addition to the fecal solution should be considered when the MGL method is used for detection of *Paragonimus* eggs. Alternatively, since more than 95 % of *Paragonimus* eggs were trapped in the ether-fecal layer, eggs remained in this layer would be a target for examination when a survey particularly on *Paragonimus* eggs is conducted.

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