Sieving Technique with the Glass Beads Layer for Detection and Quantitation of *Fasciola* Eggs in Cattle Feces

By NORIYUKI TAIRA*

First Research Division, National Institute of Animal Health
(Yatabe, Ibaraki, 305 Japan)

Fascioliasis of livestock occurring frequently in the world is regarded as an important helminthiasis, because it causes reduced production and quality deterioration of milk and meat. In Japan, 8.1% of slaughtered cattle had *Fasciola* sp. and 30% of the cattle liver was condemned by the lesion caused by the fluke in 1980.[12] Detection of the eggs in feces is the most basic method for the diagnosis of the disease. Many fecal examination techniques have been reported,[1,2,3,4] and various kinds of the modifications have been applied in the world. However, most of those are not quantitative but qualitative techniques which evaluate only negative or positive of the egg in the feces. The reason for that is attributable to the lower egg concentration in feces of cattle. The quantitative data which can express the egg density in feces is obviously better than the qualitative one.

For the detection and the quantitation of *Fasciola* eggs in cattle feces, a new sieving technique using the glass beads layer, that is Beads-technique, has been developed in our laboratory.[8,9,10,11] This technique has shown an advantage of less technical error by different workers,[5] and it has been widely applied in the field.[6,7,13] The principle and the procedures of the Beads-technique and the egg recovery rates by the techniques are presented in the first half in the present paper. The last half is the statistical consideration to the use of the Beads-technique for quantitative examination.

* Present address: Kyushu Branch Laboratory, National Institute of Animal Health (Chuzan, Kagoshima, 891-01 Japan)

The egg recovery by the Beads-technique

1) Principle of the Beads-technique

In the pile of glass beads, many fine spaces are recognized among the glass beads. Using the beads of suitable size, the spaces which are able to contain a few *Fasciola* eggs are formed (Plate 1). The fecal suspension is poured to a tube containing the beads, and stand still for a minute. Then the *Fasciola* eggs settle down faster than many other fecal components, and the eggs touch to surface of the beads layer. The eggs are taken into

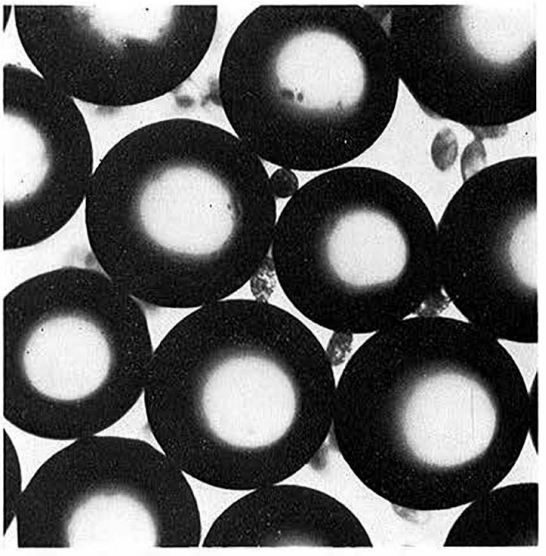

Plate 1. *Fasciola* eggs and the glass beads

spaces of the layer when they receive the power such as the vibration[8] or the slanted rotation.[9] But the layer do not catch comparatively large-sized fecal components. Many small fecal components are also remaining in the suspension fluid because the fluid is shaked before the components sink down. Thus, the selective sieving for the fluke eggs can be carried out.

2) Equipments

Equipments necessary for this technique are beads, a pair of centrifuge tubes and a rotator.

(1) Beads: Fine glass beads have been produced industrially, with the purpose of using them for the signal plate or the line mark of the traffic. The beads of the special size 590–710 μm in diameter with 2.5 in specific gravity are available for this technique (General industrial bright beads 0.59–0.71, Bright Marking Industry Co., Ltd., Osaka; Toshiba glass beads GB501M, Toshiba Electric Co., Ltd., Tokyo). The suitable beads can also be obtained from the common beads of the traffic purpose by sieveing them with 24 and 32 mesh nets.

(2) Centrifuge tubes A and B: A pair of the glass centrifuge tubes with rounded bottom of 60 m*l* capacity, 30 mm in inside diameter, and 100 mm in height are used. One (tube A) is empty and another (tube B) contains three gram of the glass beads.

(3) Apparatus of the rotator: The electric rotator is convenient for this technique, though the simple hand-made rotator is also sufficient for this purpose. The simple rotator was made by two round trays, a centrifuge tube stand and a cap screw. The tube stand was fixed on one of the trays. A small ring was fixed at the center of back side of the tray as a fulcrum. The vertical axis using a cap screw was set to the center of the other tray. The inclination of the tray was adjusted at 35 degree by the length of the screw as illustrated in Plate 2.

The special kit of the Beads-technique is already on the market (*Fasciola* egg detection rotator KT-1, Taiyo Kagaku Industry Co., Ltd., Tokyo; *Fasciola* egg detector for the Beads-technique, Fujihira Industry Co., Ltd., Tokyo).

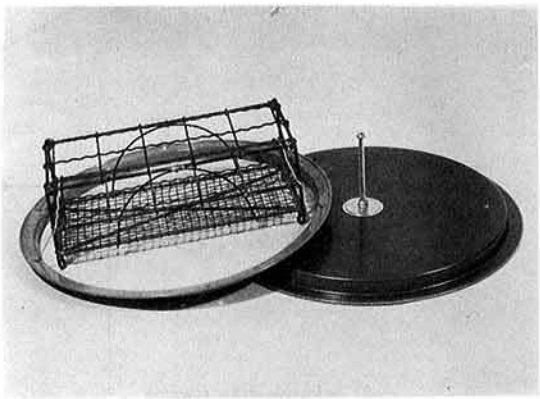

Plate 2. A simple rotator with two trays and a cap screw

Procedures

The process of the Beads-technique is composed of three steps, pre-treatment, selective sieve and sedimentation as shown in Fig. 1. The numbers in parentheses which appear in the following text corresponds to the numbers in Fig. 1.

1) Pre-treatment: (1) The tube A receives one gram of feces and about 10 m*l* of tap water. (2) The suspension is transfered to the tube B through the 60 mesh net. The tube B is filled up with water used to rinse the tube A. (3) The tube B is kept still for five min for sedimentation.

2) Selective sieve: (4) The tube B is placed into the tube stand and it is rotated five times at the velocity of about 10 sec per a rotation. (5) All of the supernatant containing debris on the beads layer are discarded by a siphon. (6) Pouring strongly 50 m*l* of tap water to the tube, the beads layer are agitated and the eggs are redistributed in the suspension. The procedures (3) to (6) must be repeated two times for selective sieving.

3) Sedimentation: (7) The beads sink in a moment, then all of the fluid is returned to the tube A. (8) The tube A is kept still for 5 min for sedimentation. (9) The super-

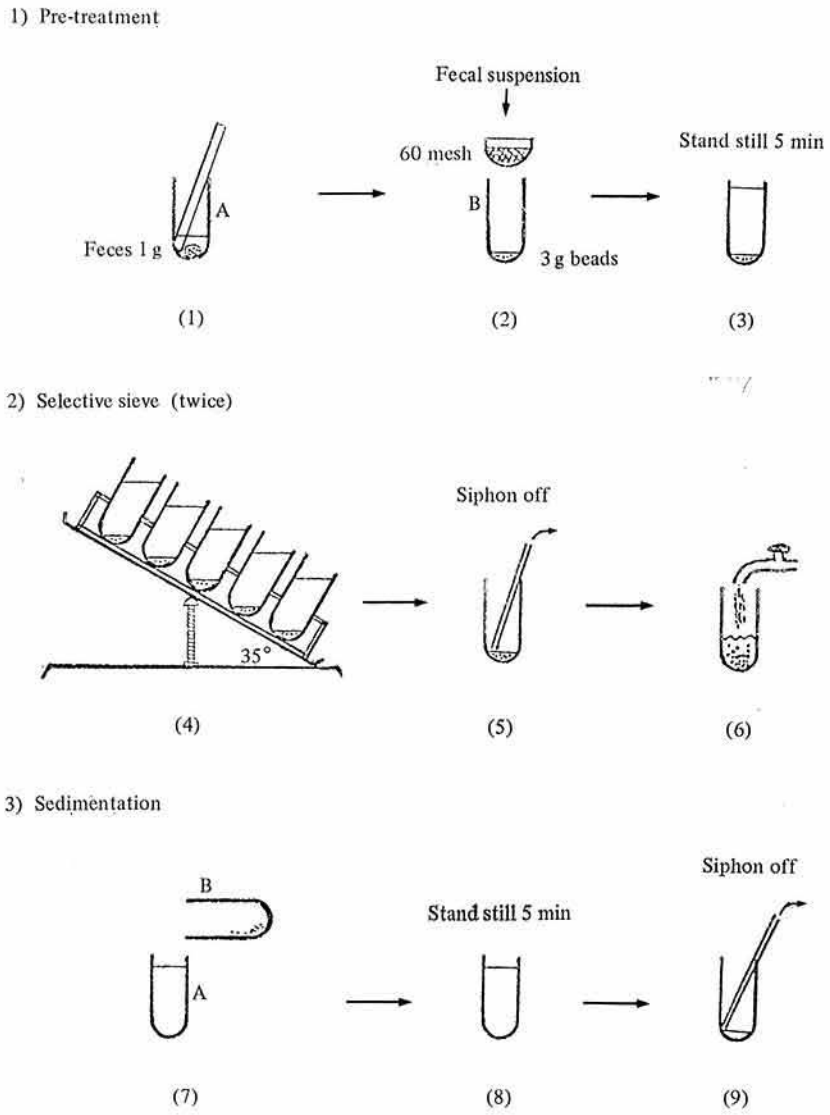

Fig. 1. Procedure of the Beads-technique

natant is removed by a siphon to get about 2 m*l* of sediments. All of sediments are placed on a slide glass by a pipett and are mixed with a drop of 1% methylen-blue solution for staining of the debris. After 20 to 30 min, the egg count is done under a microscope.

Notes and attentions on the application

1) Amount of feces: The Beads-technique can be applied for fecal samples of any kind of animals. One gram of sample is used to examine cattle feces. But, 0.5 gram is enough for the feces of sheep, goats, rabbits and rats, because they have higher percentage of dry matter contents with higher egg concentration.

2) Slide glass: All of the sediments are transferred on a slide glass. To protect the overflow of sediments, it is a good way to paint a kind of car-wax or a lip-stick along the edge of the slide glass. Special slide glass, Chamber-slide-glass (Fujihira Industry Co.,

Table 1. Number of *Fasciola* eggs detected by the Beads-technique from the samples which were prepared by mixing a serial number of eggs to one gram of egg-free feces

Sample No.	Number of *Fasciola* eggs mixed						
	1	2	4	8	16	32	64
1	0	1	1	6	8	24	48
2	0	0	3	6	13	19	49
3	1	1	4	6	8	25	51
4	1	1	2	6	14	16	47
5	1	2	3	3	12	21	45
6	1	2	0	7	15	20	41
7	0	1	2	6	11	22	37
8	1	1	1	5	11	18	43
9	0	1	4	5	14	19	42
10	0	2	2	7	12	26	38
11	1	1	4	4	10	25	44
12	0	2	4	4	14	29	53
13	1	1	4	6	11	23	46
14	0	2	3	7	11	17	55
15	1	2	1	4	11	18	45
16	1	2	3	6	11	27	47
17	1	2	3	6	14	21	43
18	0	1	2	6	11	20	44
19	1	2	3	7	9	21	54
20	0	2	3	6	13	25	41
Number of positive samples	11	19	19	20	20	20	20
Positive of sample in %	55	95	95	100	100	100	100
Total number of eggs mixed	20	40	80	160	320	640	1,280
Total number of eggs detected	11	29	52	113	233	436	913
Recovery rate of egg in %	55	73	65	71	73	68	71

No. of *Fasciola* eggs detected[1)]

1) Fixed the velocity at 10 second per a rotation and rotated 5 times

Ltd., Tokyo) is conveniences for this purpose.

3) Methylen-blue staining: Staining is an additional procedure. It is easy to find the egg in stained specimen. *Fasciola* eggs are brightly yellow in contrast with the blue color of the debris.

The egg recovery rate

Rates of detecting *Fasciola* eggs were examined by using 20 replicate fecal samples of known EPG value of 1, 2, 4, 8, 16, 32 and 64, respectively. These samples were made by mixing the eggs to one gram of egg-free cattle feces. The positive rate was 11/20 (55%) for EPG=1, 19/20 (95%) for both EPG=2 and 4, and 20/20 (100%) for EPG> 8 as expressed in Table 1. On the other hand, the egg recovery rate was determined on fecal samples mixed with comparatively many *Fasciola* eggs. The standard rate of the egg recovery in percent was shown as mean 63.6, standard deviation 10.0, 95% of upper and lower confidence limits 66.8 and 60.3.[11)]

The egg recovery rate was less affected by different velocities of rotation in practice. Higher egg recovery rates were obtained at the velocity slower than 4 sec per a rotation as shown in Table 2. Therefore the manual rotation, at the velocity of approximately 10 sec per a rotation, was good enough for the examination.

Table 2. Effect of rotation velocity on the recovery rates of *Fasciola* eggs in feces by the Beads-technique

Sample No.		Second per a rotation[1]						
		0[2]	2	4	8	10[3]	16	32
1	Recovery rates in %	35.2	37.1	79.6	50.9	53.3	64.4	60.7
2		24.5	56.0	59.5	76.0	78.7	51.2	63.6
3		26.1	54.2	75.0	42.8	78.8	52.8	74.7
4		33.3	38.5	49.1	57.5	61.3	62.7	76.1
5		33.8	45.3	67.2	54.9	48.0	68.0	73.7
6		22.1	48.9	66.2	60.3	79.0	79.5	69.2
7		35.9	27.6	47.6	78.0	60.5	61.3	79.7
8		28.4	41.3	70.0	73.8	64.5	77.1	46.5
9		17.6	26.2	67.8	76.2	67.3	74.9	77.0
10		22.2	40.0	65.4	73.2	77.0	63.4	66.0
Mean		27.9	41.5	64.7	64.4	66.8	65.5	68.7
Confidence limits		±4.6	±7.1	±7.3	±9.0	±8.1	±6.7	±7.1

1) Velocity of the rotator was fixed at 5 levels (2, 4, 8, 16 and 32)
2) Kept still without rotation
3) Estimated velocity of manual rotation

Statistical consideration to the quantitation

1) *The value of EPG and the number of eggs detected by the Beads-technique*

The important point in the present work was not only to get the higher egg recovery rates but also to obtain EPG values with a reduced time. No significant differences were observed in the egg recovery rates among the Beads-technique, Dennis' technique, Watanabe's sedimentation technique and Watch glass method.[10] Therefore, the Beads technique was also satisfactory as the qualitative method of detecting *Fasciola* egg in the feces.

The quantitative data is advantageous than the qualitative one for the analysis of the data. Number of eggs detected by the Dennis' technique has been considered as EPG, because all sediments from one gram of feces were observed under a microscope by this technique. In order to evaluate the Beads-technique as the EPG counting method in the practical fecal examination, the number of the eggs counted by the Beads-technique <x> was plotted against the values by the Dennis' technique <y> in Fig. 2.

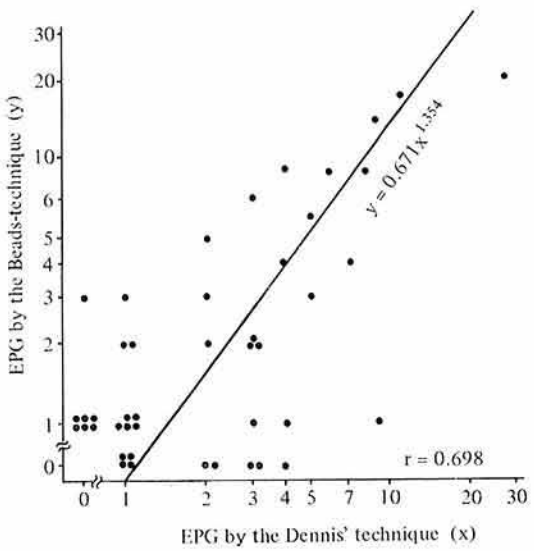

Fig. 2. Correlation between *Fasciola* EPG values counted by the Beads-technique and those by the Dennis' technique

From the result, the following equation was obtained:

$$y=0.671x^{1.354} \qquad (r=0.698)$$

Thus, the number of eggs detected by the Beads-technique can be regarded as the EPG value.[10]

2) *Correlation between the theoretical EPG and the observed EPG*

It is necessary to determine the accuracy of the Beads-technique in the examination of the field cattle, which have often lower egg concentration in the feces. Samples containing the eggs were prepared by well-mixing of fecal masses. Results of the twenty replicate examinations of each of different samples were shown in Table 3. These data, the probability of the egg count in the feces, were almost coincided with theoretical values calculated by the Poisson distribution.[11] On the samples of theoretical EPG=1 to 15, the probability (%) of the egg count in the practical fecal examination was calculated with the Poisson distribution as shown in Table 4. Examining repeatedly, the samples taken from feces containing EPG=1 (theoretical EPG), no egg expected in 52.9%, one egg in 33.7%, two eggs in 10.7% and three eggs (observed EPG) in 2.3%. For the feces containing EPG=2, no egg expected in 28.0%, one egg in 35.7%, two eggs in 22.7%, three eggs in 9.6% and four eggs in 3.1% on the practical observation. In the sample of theoretical EPG=15, all cases were expected as egg-positive and the value of the probability showed the normal-distribution centering EPG=8–10 (Table 4).

3) *Frequency distribution of Fasciola EPG in the survey data*

Almost no attention has been paid to quantitative measurement in the diagnosis of bovine fascioliasis, because it has been an experiential idea of veterinarians that EPG values are very low. To clarify the density

Table 3. Number of *Fasciola* eggs detected in 20 samples (1 gram of feces) taken from each of the same fecal masses by the Beads-technique

Sampling No.	Known population[1]		Unknown population—cattle name[2]				
	EPG=1	EPG=2	As. 03	Se. 13	Se. 08	As. 32	As. 41
1	3	2	0	1	2	3	7
2	0	1	0	0	6	4	8
3	1	3	0	2	4	3	8
4	3	1	2	0	5	1	6
5	0	0	0	3	2	2	7
6	1	3	3	2	4	0	8
7	0	2	1	1	0	4	9
8	1	2	0	1	1	1	13
9	0	0	1	1	3	5	9
10	0	0	0	0	4	3	5
11	0	0	1	1	3	2	9
12	0	2	0	0	4	6	13
13	2	2	1	1	4	4	8
14	0	3	1	2	1	4	10
15	0	1	0	0	1	5	7
16	2	2	1	1	2	1	9
17	0	1	1	3	1	4	5
18	0	0	1	0	4	3	10
19	0	0	0	1	4	4	8
20	1	1	0	1	0	5	9
Number of negative samples	12	6	10	6	2	1	0
Number of positive samples	8	14	10	14	18	19	20
Mean of eggs count x	0.70	1.30	0.65	1.05	2.75	3.20	8.40
Estimated population m[3]	1.10	2.04	1.02	1.65	4.32	5.03	13.21

Number of *Fasciola* eggs recovered

1) One or two *Fasciola* eggs were mixed into the egg-free feces
2) Cattle were naturally infected with *Fasciola*
3) $m = \bar{x} \cdot 100/63.6$

Table 4. Probability in percent by the Poisson distribution of the frequency of the number of *Fasciola* eggs detected (observed EPG) by the Beads-technique in fecal sample mixed well with the known number of eggs (theoretical EPG)

Numbre of *Fasciola* eggs detected (Observed EPG)	Probability in %	Number of *Fasciola* eggs mixed (Theoretical EPG)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0		52.9	28.0	14.8	7.9	4.2	2.2	1.2	0.6	0.3	0.2	0.1	0.0	0.0	0.0	0.0
1		33.7	35.7	28.3	20.0	13.2	8.4	5.2	3.1	1.9	1.1	0.6	0.4	0.2	0.1	0.1
2		10.7	22.7	27.0	25.4	21.0	16.0	11.6	8.0	5.4	3.5	2.2	1.4	0.9	0.5	0.3
3		2.3	9.6	17.2	21.6	22.3	20.4	17.1	13.5	10.2	7.4	5.2	3.6	2.4	1.6	1.0
4		0.4	3.1	8.2	13.7	17.7	19.5	19.1	17.2	14.6	11.8	9.1	6.9	5.0	3.6	2.5
5		0.0	0.8	3.1	7.0	11.3	14.8	17.0	17.5	16.7	15.0	12.8	10.5	8.3	6.3	4.7
6			0.2	1.0	3.0	6.0	9.4	12.6	14.9	16.0	15.9	14.9	13.3	11.4	9.4	7.5
7			0.0	0.3	1.1	2.7	5.1	8.0	10.8	13.0	14.4	14.9	14.5	13.4	12.0	10.3
8				0.1	0.3	1.1	2.5	4.5	6.9	9.3	11.5	13.0	13.8	13.9	13.3	12.2
9				0.0	0.1	0.4	1.0	2.2	3.9	5.9	8.1	10.1	11.7	12.8	13.2	13.0
10					0.0	0.1	0.4	1.0	2.0	3.4	5.2	7.1	9.0	10.6	11.7	12.4
11						0.0	0.1	0.4	0.9	1.8	3.0	4.5	6.2	7.9	9.5	10.7
12							0.0	0.1	0.4	0.8	1.6	2.6	4.0	5.5	7.0	8.5
13								0.1	0.2	0.4	0.8	1.4	2.3	3.5	4.8	6.3
14								0.0	0.1	0.2	0.4	0.7	1.3	2.1	3.1	4.3
15									0.0	0.1	0.1	0.3	0.6	1.1	1.8	2.7
16										0.0	0.1	0.1	0.3	0.6	1.0	1.6
17											0.0	0.1	0.1	0.3	0.5	0.9
18												0.0	0.1	0.1	0.3	0.5
19													0.0	0.1	0.1	0.2
20														0.0	0.1	0.1
21															0.0	0.1
22																0.0

of *Fasciola* EPG in the field cattle, the survey data in two reports[11,13] were summarized in Table 5. The egg-positive rate was 36.0% in the survey 1, 22.8% in the survey 2, and 31.31% in the total. Number of cases of lower EPG values were observed with higher frequency; the cases for EPG=1–6, 7–12, 13–19 and above 20 were 78.7%, 10.4%, 6.4% and 4.5%, respectively.

There were only a few reports which described the EPG value of fascioliasis. Only the positive rate in the herd has been discussed in bovine fascioliais surveys by means of the fecal examination. The present study which succeeded in the quantitation of the fecal egg counts using the Beads-technique will contribute to future field and fundamental studies on bovine fascioliasis.

Acknowledgments

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Table 5. Frequency distribution of EPG values counted by the Beads-technique obtained in two surveys of bovine fascioliasis

EPG	Frequency			Ratio
	Survey 1	Survey 2	Total	%
1	104	29	133	
2	51	17	68	
3	25	13	38	
4	30	4	34	78.7
5	12	2	14	
6	4	5	9	
7	6	4	10	
8	6	4	10	
9	6	3	9	10.4
10	2	2	4	
11	5	0	5	
12	1	0	1	
13	1	1	2	
14	6	0	6	
15	2	1	3	
16	1	1	2	6.4
17	3	2	5	
18	3	0	3	
19	3	0	3	
20–29	5	5	10	
30–39	2	3	5	
40–49	0	1	1	4.5
>50 (62)	0	1	1	
Positive	278	98	376	100
Negative	494	331	825	
Total	772	429	1201	
% Positive	36.0	22.8	31.3	—

Survey 1: Kanagawa & Miyagi Pref. (Taira et al., 1983a)
Survey 2: Tsuchiura, Ibaraki Pref. (Yosai et al., 1982)

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