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Detection and Quantitation of *Fasciola* Eggs in Cattle Feces Using the Beads Technique

NORIYUKI TAIRA*, KYO SUZUKI* AND J. C. BORAY†

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Introduction

A new technique for the estimation of *Fasciola* eggs in cattle feces using a glass bead layer sieving technique, the "Beads-technique", has been reported previously (Taira *et al.*, 1978a, 1978b). Further studies demonstrated that the egg counts obtained could be used for quantitative estimation of the fecal egg content (Taira *et al.*, 1979). These previous experiments were mostly carried out using feces from uninfected animals to which a known number of *Fasciola* eggs were added.

However, the question of the variation in egg counts obtained due to sampling error has not been studied. Such as this error cannot be neglected, as in the field cattle feces often has very low egg concentration. In order to define the practical sensitivity of the technique, present studies were carried out using statistical analysis of data obtained from adding a known number of eggs to egg-free fecal samples and from analysis of field samples.

Materials and Methods

Feces: Fresh feces taken from dairy cattle from local farms were used. Samples were taken from a fecal mass without mixing.

Preparation of feces with known *Fasciola* egg content: Feces with known egg counts were prepared by two methods. In the first method (Taira *et al.*, 1978a) one gram of egg-free cattle feces was mixed with a water suspension containing 100 eggs in each sample. In the other method, used for materials having only one or two eggs per gram, 50 grams of egg free cattle feces were mixed with 50 or 100 eggs in a measuring cylinder and filled up with tap water to 500 ml. Ten ml of the fecal suspension equal to one gram of feces was taken with stirring for egg estimation.

Fasciola egg recovery rates using the Beads-technique: Eight fecal samples containing no *Fasciola* eggs were collected from different farms. These were used to prepare fecal samples with a known number of *Fasciola* eggs per gram and each sample was tested five times. The egg recovery rates were calculated.

Frequency distribution of *Fasciola* eggs in field cattle: Most of these datas were taken from a previous paper (Taira *et al.*, 1979) and from a published survey (Sato *et al.*, 1981). A total of 772 feces were examined by single sampling method using the Beads-technique for *Fasciola* eggs, and 278 cases gave positive results. These results were used to draw up a frequency

* *National Institute of Animal Health, Tsukuba 305, Japan.*
† *N.S.W. Govt. Veterinary Research Station, N.S.W. 2167, Australia.*

Table 1 *Fasciola* egg recovery rates using the Beads-technique in fecal samples of 8 cattle from different farms and the analysis of variance

	Feces of cattle at different farms								Grand value
	A	B	C	D	E	F	G	H	
Egg recovery* rate in %	65.9	54.5	55.2	58.1	81.8	36.9	62.9	63.2	
	69.7	50.0	71.4	61.3	65.7	82.3	61.8	76.3	
	69.6	66.1	66.0	75.3	50.0	46.7	49.1	71.9	
	70.5	59.6	71.2	70.0	67.9	79.6	56.3	65.5	
	63.9	63.5	72.2	55.9	53.7	57.4	58.1	76.0	
Mean	67.1	59.7	66.0	64.1	63.8	60.6	57.6	70.6	63.6
95% confidence limits Upper	76.3	67.9	75.2	73.3	73.0	69.7	66.8	79.7	66.8
95% confidence limits Lower	58.0	49.6	56.8	55.0	54.7	51.4	48.5	61.4	60.3
Standard deviation									10.0

	S	ϕ	V	F_0
Feces	677.23	7	96.75	0.96 NS†
error	3219.90	32	100.62	

* More than 100 eggs were mixed in each sample.

$$\%=\frac{\text{Number of eggs detected in the sample}}{\text{Number of eggs mixed in 1 gram feces contanining no eggs}}\times 100$$

† No significat difference ($p>0.05$) among the feces.

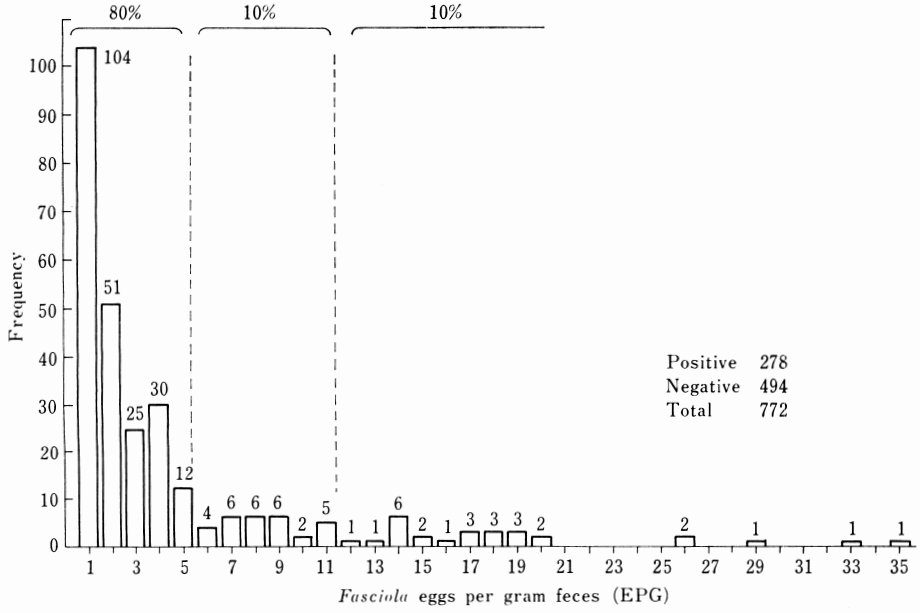

Fig. 1 Frequency distribution of *Fasciola* egg counts in one gram of cattle feces using the Beads-technique at Kanagawa and Miyagi prefectures (1977, 1978).

distribution table.

Observed and theoretical values of fecal egg content on multiple sampling: The fecal samples containing one or two eggs per gram prepared, and 5 field samples were tested 20 times respectively by the Beads-technique. Theoretical values of the frequency of the number of eggs per gram were calculated using the Poisson distribution. Statistical difference between the observed and theoretical values was calculated using χ^2 test.

Results

The recovery rate of *Fasciola* eggs from feces with a known egg content is shown in Table 1. Mean egg recovery rates for the 8 samples ranged from 57.6% to 70.6% and statistical analysis showed that there was no significant difference among the values. The overall mean of 63.6% recovery was used as a standard to correct the results.

The frequency distribution of *Fasciola* eggs found in feces from cattle in the field is shown in Figure 1. The highest egg content found was 35 EPG, but in many cases only small numbers of eggs were found in the feces. Eighty *per cent* of the samples were in the range 1–5 EPG, 10% in the range 6–11 EPG and 10% contained

Table 2 Number of *Fasciola* eggs in 20 replicate examinations of the same samples by the Beads-technique

Sampling No.		Known population*		Unknown population†-cattle name				
		EPG=1	EPG=2	As. 03	Se. 13	Se. 08	As. 32	As. 41
1	No. of *Fasciola* eggs recovered	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
No. of negative samples		12	6	10	6	2	1	0
No. of positive samples		8	14	10	14	18	19	20
Mean of eggs count $\bar{x}$		0.70	1.30	0.65	1.05	2.75	3.20	8.40
Estimated population m‡		1.10	2.04	1.02	1.65	4.32	5.03	13.21

* One or two *Fasciola* eggs were mixed into the cattle feces with no eggs.

† Cattle were naturally infected with *Fasciola*.

‡ $m=\bar{x}\cdot 100/63.6$, for the mean recovery rate was defined at 63.6% from Table 1.

Table 3 Comparison of the frequency of observed and theoretical values of *Fasciola* eggs count in 20 replicate examinations of a fecal sample using the Beads-technique

		Known population			Unknown population-cattle name					
		EPG=1		EPG=2	As. 03	Se. 13	Se. 08	As. 32	As. 41	
		Obs.*(Theo.)†		Obs. (Theo.)	Obs. (Theo.)	Obs. (Theo.)	Obs. (Theo.)	Obs. (Theo.)	Obs. (Theo.)	
0		12	(10.6)	6 (5.6)	10 (11.6)	6 (7.0)	2 (1.2)	1 (0.8)	0 (0.0)	
1		4	(6.7)	5 (7.1)	9 (6.3)	9 (7.3)	4 (3.4)	3 (2.6)	0 (0.0)	
2		2	(2.1)	6 (4.5)	1 (1.7)	3 (3.9)	3 (4.8)	2 (4.2)	0 (0.2)	
3		2	(0.5)	3 (1.9)	0 (0.3)	2 (1.3)	1 (4.4)	4 (4.5)	0 (0.4)	
4		0	(0.1)	0 (0.6)	0 (0.0)	0 (0.4)	8 (3.1)	6 (3.6)	0 (0.9)	
5		0	(0.0)	0 (0.2)	— (—)	0 (0.1)	1 (1.7)	3 (2.3)	2 (1.6)	
6		—	(—)	0 (0.0)	— (—)	0 (0.0)	1 (0.8)	1 (1.2)	1 (2.2)	
7	Frequency	—	(—)	— (—)	— (—)	— (—)	0 (0.3)	0 (0.6)	3 (2.6)	
8		—	(—)	— (—)	— (—)	— (—)	0 (0.1)	0 (0.2)	5 (2.8)	
9		—	(—)	— (—)	— (—)	— (—)	0 (0.0)	0 (0.1)	4 (2.6)	
10		—	(—)	— (—)	— (—)	— (—)	0 (—)	0 (0.0)	2 (2.2)	
11		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (1.7)	
12		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (1.2)	
13		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	2 (0.7)	
14		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (0.4)	
15		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (0.2)	
16		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (0.1)	
17		—	(—)	— (—)	— (—)	— (—)	— (—)	— (—)	0 (0.1)	
Total		20	(20.0)	20 (20.0)	20 (20.0)	20 (20.0)	20 (20.0)	20 (20.0)	20 (20.0)	
	χ^2	0.39		1.10	0.57	0.62	0.52	0.52	0.52	42.5‡
χ^2 test	ϕ	1		2	1	2	1	1	1	15‡
	p	0.5–0.7		0.5–0.7	0.3–0.5	0.7–0.8	0.3–0.5	0.3–0.5	0.3–0.5	>0.90‡

* Frequency of observed values was from Table 2.

† Frequency of theoretical values was lead from the Poisson distribution, $e^{-0.636m}0.636\,m^n/n!$ 20.

‡ Values were culculated with all the datas pooled.

Table 4 Probability in per cent with the Poisson distribution on the frequency of the number of *Fasciola* eggs detected (observed EPG) by the Beads-technique using the fecal samples mixed well with known number of eggs (theoretical EPG)

Number 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

more than 12 EPG.

Multiple estimations of *Fasciola* eggs in the samples with both known and unknown egg contents are shown in Table 2. The mean EPG counts were calculated in each case and corrected using the recovery factor determined in Table 1.

The observed values from Table 2 were compared to theoretical values obtained from the Poisson distribution (Table 3). In each case a close correspondence between observed and theoretical values was obtained. The probability in *per cent* of obtaining a particular estimate of the number of eggs in fecal samples compared with the actual number of eggs in the sample is shown in Table 4.

Discussion

Some of the techniques for the examination of cattle feces for *Fasciola* eggs are qualitative and depend on obtaining a high egg recovery rate. This is important because of the generally small egg content in the feces of naturally infected cattle. In addition, fecal egg output is not constant even in the same animal, and periodic changes with time have been reported (Dorsman, 1956b; Honer, 1965a). Quantitative techniques for estimating *Fasciola* eggs in cattle feces have included flotation with zinc sulphate solution (Honer, 1965a), sedimentation with water mixing (Coyle, 1958), sieving with multi-layer nets and the use of special egg counting slides (Dorsman, 1956a) and observing all of the sediment from one gram of feces (Dennis *et al.*, 1954).

The slanted rotation to the glass bead layer technique "Beads-technique" developed in our laboratory has been used with success for the qualitative and quantitative estimation of *Fasciola* egg in cattle feces, and has been used in the field widely Japan (Kawamura *et al.*, 1981; Kawamura *et al.*, 1982; Sato *et al.*, 1981; Yosai *et al.*, 1982).

Few studies have dealt with the problem of sampling error in the case of fecal samples with very low egg contents. Honer (1965a) used a zinc sulphate flotation technique in the diagnosis of feces from 108 cattle and found that 56.8% gave positive results for *Fasciola* eggs when a single sample was taken. The average egg content of the positive samples was 6.5 EPG, but the egg recovery rate of the technique itself was not described.

We have confirmed that our technique gives a reproducible and high recovery rate of eggs from feces of cattle with low egg contents. The average value for recovery can successfully be used as a correction factor (Tables 1, 2).

The observed frequency of the result should fit the Poisson distribution. As shown in Table 3 the observed and theoretical values fit closely, indicating that the eggs are evenly distributed throughout the fecal sample. The probability table (Table 4) can be used to estimate the likelihood of obtaining positive values for samples containing very few *Fasciola* eggs. For example if multiple samples are taken from a fecal sample processed by the Beads-technique, and that fecal sample contained one EPG, then 52.9% of the samples would contain no eggs in many times the estimation was repeated. If the fecal samples contained 2 EPG, 28% of the samples would contain no eggs. These results emphasize the importance of how to read the results and how to estimate the fecal samples collected from field cattle, as it is very likely if only one EPG count is made that a high proportion of the samples containing 1, 2 or 3 EPG will be scored as having a negative egg count.

Summary

Fasciola egg recovery rate from cattle feces using the Beads-technique was 63.6%.

Feces of 772 dairy cattle were examined with the technique, and *Fasciola* eggs were found in 278 cases. The majority of samples had low EPG. Twenty times of examinations on the same fecal materials by means of the Beads-technique were carried out on 7 fecal samples with different EPG values. The observed values were almost identical with the theoretical ones derived from the Poisson distribution. The probability of the frequency of the egg counts in field cattle using the Beads-technique is discussed.

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ビーズ法による牛糞からの肝蛭卵検出とその精度

平　詔亨　鈴木　恭
(農林水産省家畜衛生試験場)
J. C. BORAY
(オーストラリア，N. S. W. 獣医学研究所)

定量的な検査法として開発されたビーズ法を野外の牛に実際に応用した場合，どのような確率で肝蛭卵が検出されるかを調べた．まず，ビーズ法による牛糞からの肝蛭卵検出率は，既知の肝蛭卵を含む材料での検査結果から，63.6(標準偏差 10.0)% とされた．搾乳牛772例の調査では278例が陽性であった．これらの陽性牛における検出卵数の度数分布は，虫卵数 1～5 が 80%，6～11 および 12～35 がそれぞれ 10% であり，小さな値をとるものが多かった．一方，EPG が異なる7例の牛糞材料をそれぞれビーズ法で20回検査した．検出された虫卵数 0～n 個の出現頻度は，ポアソン分布から求めた理論値とほぼ一致した．また，肝蛭卵が均一に分布した糞塊であるとの条件で，牛糞1g中に肝蛭卵を1個から15個までの割合で含む場合，実際の検査ではどの位の虫卵が検出されるかをポアソン分布から値を求め一表に示し，いくつかの考察を加えた．