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Egg Discharging Patterns of *Ascaris lumbricoides* in Low Worm Burden Cases

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INTRODUCTION

It is evident that all of the infected cases with *Ascaris lumbricoides* was not always recognized by egg detection from the stool specimens, because of larval or sole male adult infection. And in the many lightly infected cases, only the unfertilized ova used to be detected, particularly in a single infection.

Therefore, according to the egg detectability, the infected cases are grouped into the following three; the fertilized ova passer, the unfertilized ova passer and the false negative case. The probability of the false negative findings is almost negligible in the highly endemic areas, where the heavy worm burden is indicated. However, it is suggested that the increasing tendency of false negative findings is expected in the low endemic areas, where the worm burden is decreasing due to the regularly repeated mass treatment. In Korea, it is well known that the lowering rates and worm burden of soil-transmitted helminths are partly contributed to the recent progress of their control activities. Especially in case of *Ascaris* infections, the mass diagnostic procedure has brought on it's quality control problem.

In the present study, authors present information on sex combination of *Ascaris lumbricoides*, their egg discharging pattern, sex-related difference etc. among the infected cases of the above three groups with low worm burden.

MATERIALS AND METHODS

A total of 853 inhabitants, randomly selected from six villages in Hwasung Gun, Machun Dong in Seoul, Hoengsung Gun, Jangheung Gun, Jinyang Gun and Kangjin Gun were treated with the dosage of 10.0mg/kg pyrantel pamoate. In order to detect all worms expelled, whole stool specimens of these inhabitants were collected for two consecutive days immediately after drug administration(Cho, 1977).

A total 1,861 *Ascaris lumbricoides* were confirmed under the stereomicroscope and they were subjected for the analysis of the relationship between their appearance in the host and the egg detectability in the stool specimens.

The cellophane thick smear technique was applied for the egg detection from all specimens collected. During the whole course of examination, only a smear specimen from each case was examined by one experienced technician. The results obtained through the egg detection were classified into three categories; the false negative, the positive only with unfertilized eggs and

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the positive with fertilized eggs. The egg discharging patterns among these three groups were studied.

RESULTS

1. The relationship between ova detection and worm burden

The sex ratio and sex combination in a host were observed. As presented in Table 1, the sex ratio of *Ascaris lumbricoides* in the surveyed areas was 1 : 0.74 (Female: Male) in average. The number of male worms was very small in area F because of the dominant female brood in some heavily infected cases. However, it seems that the degree of endemicity does not apparently affect the sex ratio of worm in the population.

According to the sex combination of the worm in a host, the number of cases was calcula-

Table 1. Sex ratio of collected *Ascaris lumbricoides* by surveyed area

Area (Code)	No. of cases	No. of worm-posit. case (%)	Sex of worm			Sex ratio** (X)
			Male	Female	Unident.*	
Hwasung Gun (A)	540	211 (39.1)	246	288	38	0.85
Machun Dong (B)	136	79 (58.1)	183	245	1	0.75
Hoengsung Gun (C)	32	19 (59.4)	33	40	1	0.85
Jangheung Gun (D)	47	29 (61.7)	62	71	3	0.87
Jinyang Gun (E)	39	31 (79.5)	155	173	1	0.90
Kangjin Gun (F)	59	42 (71.2)	90	220	11	0.41
Total	853	411 (48.2)	769	1,037	55	0.74

*Unidentified because of degeneration of worms in 16 cases **Male: Female=X : 1

Table 2. Sex combination of *A. lumbricoides* in low worm burden cases

Worm burden per case	No. of cases	*Sex or sex combination	Observed frequency	Freq. by binomial distribution	Probability of identity
1	145	M1	46	61.7	$.005<P<.01$
		F1	99	83.3	
2	67	M2	12	12.1	$.75<P<.9$
		M1 + F1	31	32.8	
		F2	24	22.1	
3	45	M3	7	3.5	$.1<P<.25$
		M2 + F1	16	14.1	
		M1 + F2	16	19.0	
		F3	6	8.5	
4	29	M4	0	1.0	$.5<P<.75$
		M3 + F1	5	5.1	
		M2 + F2	8	10.4	
		M1 + F3	12	9.4	
		F4	4	3.2	
5	25	M5	0	0.4	$.1<P<.25$
		M4 + F1	3	2.4	
		M3 + F2	2	6.4	
		M2 + F3	15	8.6	
		M1 + F4	4	5.8	
		F5	1	1.6	

* Mn: Male worms, 'n' in number Fn: Female worms, 'n' in number

ted as shown in Table 2. The case number of each sex combination was subjected to the statistical analysis. The fitness test was attempted from the theoretical values of binomial distribution, calculated from the equation, $(m+f)^n$; $(0.426+0.574)^n$. Except in case of worm burden '1', it was revealed that the distribution of cases by each sex combination was identical with the theoretical one.

The results of stool examination, which were categorized to false negative cases, unfertilized ova passers and fertilized ova passers, were related with each sex combination (Table 3). The single sex combination of male worm(s) was exclusively manifested by the negative ova detection. However, the frequency of only male worm infection was theoretically and practically rare when the worm burden was over 4 in a case.

The single sex infection of female worm(s) was possible up to 5 worms. Those cases were manifested by the unfertilized ova passers in majority, but not all. As shown in Table 3, there were false negative cases in different degrees of chance. The chance to be false negative cases was higher in lower burden cases of single sex infection of female.

Not all of the cases with male and female worm infection was manifested exclusively as fertilized ova passers. Some of them was false negative case or unfertilized ova passer. Unfertilized ova passers among the mixed sex combinations were usually observed in the female dominant infections, such as M1+F2; M1+F3;

Table 3. Relationship between sex or sex combination of infected *Ascaris* worms and the result of stool examination for eggs

Worm burden per case	*Sex or sex combination	No. of cases	Results of stool examination					
			False neg. case		Unfertil. egg case		Fertil. egg case	
			No.	(%)	No.	(%)	No.	(%)
1	M1	46	46	(100.0)	0	(0.0)	0	(0.0)
	F1	99	37	(37.2)	62	(62.6)	0	(0.0)
2	M2	12	12	(100.0)	0	(0.0)	0	(0.0)
	M1 + F1	31	6	(19.4)	2	(6.5)	23	(74.2)
	F2	24	7	(29.2)	17	(70.8)	0	(0.0)
3	M3	7	7	(100.0)	0	(0.0)	0	(0.0)
	M2 + F1	16	4	(25.0)	0	(0.0)	12	(75.0)
	M1 + F2	16	1	(6.3)	1	(6.3)	14	(87.5)
	F3	6	0	(0.0)	6	(100.0)	0	(0.0)
4	M4	0	0	(0.0)	0	(0.0)	0	(0.0)
	M3 + F1	5	1	(20.0)	0	(0.0)	4	(80.0)
	M2 + F2	8	1	(12.5)	0	(0.0)	7	(87.5)
	M1 + F3	12	0	(0.0)	1	(8.3)	11	(91.7)
	F4	4	1	(25.0)	3	(75.0)	0	(0.0)
5	M5	0	0	(0.0)	0	(0.0)	0	(0.0)
	M4 + F1	3	0	(0.0)	0	(0.0)	3	(100.0)
	M3 + F2	2	0	(0.0)	0	(0.0)	2	(100.0)
	M2 + F3	15	1	(6.7)	0	(0.0)	14	(93.3)
	M1 + F4	4	0	(0.0)	1	(25.0)	3	(75.0)
	F5	1	0	(0.0)	1	(100.0)	0	(0.0)
6~10	—	44	1	(2.3)	1	(2.3)	42	(95.4)
11 & over	—	40	0	(0.0)	0	(0.0)	40	(100.0)
Total		**395	125		95		175	

* Mn: Male worm, 'n' in number Fn: Female worm, 'n' in number

** 16 cases among the 411 worm positive cases were excluded.

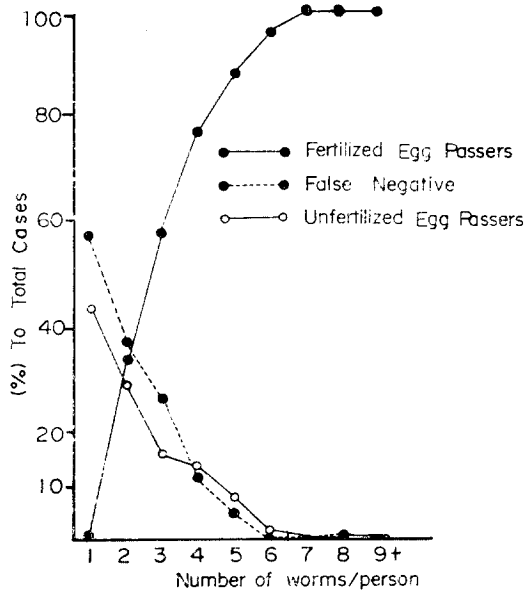

Fig. 1. The probability of stool examination results according to the worm burden per case.

and M1+F4.

The causes of false negative results in ova detection will be discussed in the next section, however, it is evident that the false negative cases other than male infection were usually observed in young female infections.

In Fig. 1, the observed probability of stool examination results by each burden was presented. This figure was derived from the results in Table 3. In each burden, the ova detection results were quantitatively plotted. This shows the exponential decrease of the false negative result and unfertilized ova passers with the increase of worm burden per case. Also the probability of fertilized ova detection was increased just like natural logarithmically from the burden of two worms per case to the asymtote, 100% in the burden of over 6 worms.

2. The cause of the false negative cases in ova detection

The causes of the false negative result in stool examination were analysed (Table 4) with the data of worm collection. The single sex infection of the male worm(s) was contributed to the false negative result only in 52% out of 125 cases. The remaining 48% were associated with female(s) infection either with or without male (s). The known and acceptable causes of sterile female infection were the young females evidently small to reproduce (in 24.8% of all cases) and the older females which were pigmented, longer than 30cm and heavier than 3.0gm (in 8.8% of all case). The easily recognizable and morphologically distinguishable characters of sterility were not shown in total 19 cases(15.2%).

There was no recognizable relationship between the causes of the false negative result of ova detection and the endemicity of the *Ascaris*.

DISCUSSION

Yokogawa and Wakejima(1932) collected *Ascaris* worms from 377 Taiwanese schoolchildren after treatment of santonin and reported the sex ratio was 1,626 males and 2,039 females(0.79

Table 4. Analysis of the false negative results in stool examination by observation of the collected worms from each case

Feature of worms	No. of false negative cases in areas of						Total	
	A	B	C	D	E	F	No.	%
Male worm(s) only	50	7	1	2	3	2	65	52.0
Young female(s) with or without male(s)	13	15	1	0	2	0	31	24.8
Old female(s) with or without male(s)	8	2	0	1	0	0	11	8.8
Females of no distinct character	8	1	2	3	0	5	19	15.2
Total	75	25	4	6	5	7	125	100

: 1). Fushimi(1959a) summarized the sex ratio *Ascaris suum* which had been reported by previous workers, which was 0.55 : 1. Morishita (1972) recorded that the sex ratio in many heavy burden cases reported by earlier workers were in the range from 0.17 : 1 to 0.98 : 1. Considering these reports, the sex ratio obtained in this study, 0.74 : 1, is quite reasonable even though one of the surveyed area showed low sex ratio.

As expected, the sex combination of infected *Ascaris* in each burden was determined by probability of the binomial distribution. And the sex combination itself is very important factor determining whether they produce eggs or what types of eggs are produced. It is because male and female worms are not paired permanently. In case of *Schistosoma* spp., the male and female are coupled and produce eggs. So the probability of pairing in different worm burden is determined by the equation, $1-0.7979n^{-0.5}$ (MacDonald, 1973).

In this study, we discriminated the sex of all the collected *Ascaris* including the young worms. So not all of the male or famale are in their reproductive stage. This is why many of the female associated infections were false negative by ova detection.

The single sex infection of the male worms was exclusively manifested by negative for ova. However, all of the single sex infection of female worms was not manifested by the unfertilized ova passer, and many of them were negative for ova. The lower the worm burden of female, the higher the frequency of false negative as shown in the present study. This phenomenon must be explained on the basis of small size of single brood infection. In other words, because only small number of *Ascaris* larvae arrived intestine time after time, the single young worm infection can be manifested by false negative result more frequently, and when the worm burden becomes higher, the chance of only young female infection is reduced.

Another possible factor involved in the false negative result in female sex infection is the suppression of egg production in case of sperm paucity. The diagnostic sensitivity of the stool examination for ova must be another possible explanation for higher rate of false negative results in the single sex infection of females. Authors are now checking the egg production amount of various sex combination of *Ascaris* infection because it is closely related with the egg detection ability.

The majority of the false negative result or unfertilized ova passers in mixed sex infection can be explained as mentioned in the above paragraphs.

The false negative result of stool examination is not caused by the male worm infection only as Japanese workers thought (Fushimi, 1959b; Morishita, 1972). It is rather complex in aetiology as shown in the present study, and probably is related with the worm burden, sex combination, the size of one infection, the interval between repeated infections and the host immunity that destroys the migrating larvae and thus reduces the number of worms arriving intestine.

In any way, the progress of *Ascaris* infection to the lower endemicity raised the problem of diagnostic sensitivity of stool examination. As Seo *et al.*(1979) suggested, the relative proportion of the lower burden cases is increased when the endemicity is lowered. So the relative frequency of the false negative result of stool examination becomes never negligible in so far as the diagnostic sensitivity of stool examination is concerned.

SUMMARY

In *Ascaris lumbricoides* infection, the faecal

examination, undertaking for ova detection, is not always diagnostic. It is just because some of the infected cases has *Ascaris* worm(s) which do not produce eggs. In the present study, the authors attempted to analyse quantitatively the egg discharging patterns in *Ascaris* infected cases with low worm burden. The following results were obtained:

1. In 1,861 *Ascaris* worms collected from 853 cases, the sex ratio was 1 : 0.74(Female : Male). Sex combinations in each burden of case were always fitted with theoretical values from the binomial distribution; $(m+f)^n=(0.426+0.574)^n$.

2. In each worm burden, their sex combination indicated different egg discharging patterns; false negative cases, unfertilized ova passers and fertilized ova passers. When the relative frequency of the above three egg discharging patterns was plotted to worm burden per case, a definite relationship was found. The cases with six or more worms have nil probability to be false negative case or unfertilized ova passer.

3. Out of 853 cases, we found 129 false negative cases. The collected worms from 125 cases were morphologically analysed. It was found that 52% of them were infected with only male worm(s) and 24% were infected with young female worm(s). And in 8.8%, old female(s) with empty uterus were infected. The cause of 15.2% was remained unexplained, even though the collected worms were scrutinized.

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＝國文抄錄＝

蛔蟲 輕感染者의 蟲卵排出 樣相

서울大學校 醫科大學 寄生蟲學敎室 및 風土病硏究所
徐 丙 嵩・趙 昇 烈・蔡 鍾 一

蛔蟲感染에 있어서 蟲卵檢査가 항상 絕對的 診斷價値를 갖는 것은 아니다. 그것은 감염자에 있어서도 감염된 蛔蟲이 蟲卵을 생산하지 않는 경우가 있기 때문이다.

이연구에서는 특히 輕感染者의 경우에 蟲卵排出 樣相을 관찰하여 受精卵 排出者, 不受精卵 排出者 및 蟲卵 不排出者의 量的 分布를 분석하고자 하였다.

對象은 京畿道 華城郡 住民 540명을 비롯하여 서울市 馬千洞, 江原道 橫城郡, 全南 長興郡 및 康津郡, 慶南 晋陽郡 주민등 모두 853명으로서, 대상자 전원을 pyrantel pamoate로 치료하고 얻은 蟲體所見과 그 以前에 얻은 蟲卵檢査 성적을 비교분석한 것이다.

그 結果를 要約하면 다음과 같다.

1. 對象者 853명中 411명(48.2%)로부터 모두 1,861마리의 蛔蟲을 수집하였다. 이의 性比(雌：雄)는 1：0.74이었다. 1人當 感染蟲體數에 있어서 雌雄蟲의 組合에 따른 例數分布는 二項分布公式에 의한 이론치 즉$(0.426+0.574)^n$와 일치하고 있었다.

2. 各 感染蟲體數에 있어 雌雄蟲의 性組合에 따라 蟲卵排出樣相은 蟲卵 不排出者, 不受精卵 排出者, 受精卵 排出者로 명백하게 구별되었고 이는 生物學的 理論과 일치하고 있었다. 다시 各 感染蟲體數에 따른 위 세가지 蟲卵排出樣相의 상대적 비율을 계산하였다. 結論을 略述하면 蛔蟲 6마리 또는 그 이상 感染되었을 때 蟲卵不排出者나 不受精卵排出者가 나타날 確率은 무시할 정도가 되었다.

3. 對象者 853명중 蟲卵不排出 感染者는 129명이었다. 이중 125명에서 수집한 蛔蟲의 형태학적 원인분석을 통하여 이중 52%는 雄蟲만의 감염, 24%는 어린 雌蛔蟲 감염, 8.8%는 자궁에 충란이 없는 老衰雌蟲의 감염임을 알 수 있었다. 나머지 15.2%에서의 蟲卵不排出 원인은 알 수 없었다.