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Ascariasis: Endemic Foci in Shiga Prefecture, Japan, and a Trial of Masstreatment with Dry Syrup of Pyrantel Pamoate

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Pyrantel pamoate (Combantrin) is now widely used as a broad spectrum of anthelmintics; the drug has been evaluated to be effective against *Ascaris*, hookworm and pinworm infections. As far as the treatment of patients with ascariasis concerned, more than 90% of cure rate may be obtained by the oral administration of pyrantel pamoate with a single dose of 20 mg/kg of body weight for adults and of 10 mg/kg for school children (Yokogawa and Kagei, 1970). Kobayashi *et al.* (1970) used a single dose of 5 mg/kg with a satisfactory cure rate comparable to that obtained by the treatment with 10 mg/kg. Furthermore, it has been suggested by Campos (1969) and, quite recently, by Seo (1977) that the anthelmintic effect of pyrantel pamoate against *Ascaris* infection may be obtained even by a low dose of 2.5 mg/kg. If this is the case, it is apparent that such a low dose may provide certain advantages in views of reducing economical problems as well as avoiding undesirable side effects by the drug.

The purpose of the present study is to evaluate the efficacy of a newly developed preparation of the drug, i.e. dry syrup, by using low doses for the masstreatment of patients with ascariasis. Despite the general impression that ascariasis has been eradicated completely from Japan, we found endemic foci of the infection still present in the west coast area of Lake Biwa, Shiga Prefecture, during the survey on metagonimiasis. Thus, results of epidemiological studies in the area are also included in this paper.

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Materials and Methods

Places of studies.

Imazu-machi lies on the west coast of Lake Biwa where the intestinal fluke *Metagonimus yokogawai* and the liver fluke *Clonorchis sinensis* are known to be endemic among inhabitants. The Imazu Health Center covers not only Imazu-machi but its neighbouring four towns and one village which with a total population of about 50,000 belong to Takashima-gun, Shiga Precture (Fig. 1).

Epidemiological studies.

Epidemiological studies on ascariasis were carried out from May to November 1977. In early studies on metagonimiasis carried out in Shin-asahi-machi, AMS III centrifugation-sedimentation technique (Hunter *et al.*, 1948) was the first choice of the stool examination. During the course of the study, we found *Ascaris lumbricoides* eggs in some specimens by the centrifugation technique which we had thought to be good enough for the detection of *Ascaris* eggs. However, our preliminary results of comparing the AMS III technique with Kato's

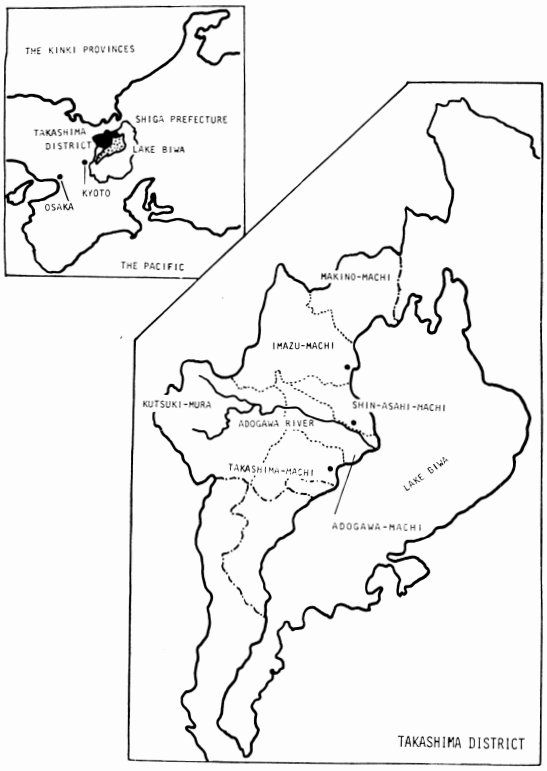

Fig. 1 Location of study sites in Shiga Prefecture, Japan.

cellophan thick smear method (Kato and Miura, 1954) modified by Martin and Beaver (1968) revealed that the former was not suitable for the *Ascaris* eggs in terms of the detection rate. Thus, the Kato's method was used for further epidemiological studies in the other areas (see Table 1). The AMS III technique was also used in areas where metagonimiasis or clonorchiasis was expected endemic.

Masstreatment with pyrantel pamoate.

A hundred and three patients with ascariasis were divided into 4 groups. A single dose of 5 mg/kg or 2.5 mg/kg (as base) of dry syrup, a newly developed form, of pyrantel pamoate, with the formula trans-1-methyl-1, 4, 5, 6-tetrahydro-2-[2-(2-thienyl) vinyl]-pyrimidine pamoic acid salt, was given orally for Group 1 or Group 2, respectively. As control, the other two groups of patients were treated with 5mg/kg of the drug by the administration of tablets (Group 3) or syrup (Group 4). The method of the administration was identical as previously described (Yokogawa *et al.*, 1970a). Briefly, the patients were gathered to a certain place in a village or town and they were randomly divided into the groups mentioned above and administered either of the preparations with a cup of water. Side effects were checked at least for 30 minutes after the administration under doctor's observation.

Table 1 Prevalence of helminthiases among inhabitants and workers of gloceries in the jurisdictional area of the Imazu Health Center, Shiga Prefecture

Group & district	Method of stool examination	No. of examined	No. (%) of positive for eggs of			
			A. lumbricoides	*T. trichiura*	*M. yokogawai*	*C. sinensis*
Inhabitants						
Shin-asahi-machi	AMS III	379	8(2.1)	37(9.8)	31(8.2)	33(8.7)
Takashima-machi	Kato's	476	9(1.9)	ND	6(1.3)	1(0.2)
Imazu-machi						
(Mukugawa)	Kato's	158*	39(24.7)	55(34.8)	ND	ND
(Hamabun)	Kato's & AMS III	214*	3(1.4)	ND	26(12.1)	17(7.9)
Kutsuki-mura	Kato's & AMS III	221*	36(16.2)	70(31.7)	2(0.9)	0
Workers of gloceries	Kato's & AMS III	384	17(4.4)	13(3.4)	17(4.4)	10(2.6)
Total		1,832	112(6.1)	175	82	61

* Including school children.

Table 2 Prevalence and incidence of ascariasis in Kutsuki-mura and Imazu-machi, Shiga Prefecture

Village	May 1977		November 1977				
	Total No. of examined	No. of positive for *Ascaris* eggs (prevalence)	Total No. of examined	No. of positve for *Ascaris* eggs (prevalence)	No. of followed up since May 1977	No. of negative in May 1977	No. of converted to positive (incidence)
Kutsuki-mura	192	33(17.2%)	221	36(16.2%)	69	45	15(33.3%)
Mukugawa (Imazu-machi)	149	30(20.1%)	158	39(24.7%)	133	102	15(14.7%)

Taking breakfast or lunch was not allowed before the treatment. Evaluation was performed by the Kato's thick smear method about four weeks later in December, 1977. In order to know whether the worm burden somehow affects the efficacy of the drug, the number of eggs per gram of feces (EPG) was counted by both of the Stoll's dilution egg-count technique (Stoll and Hausheer, 1926) and a modified Kato's method (Martin and Beaver, 1968) in some instances.

Comparison of stool examinations suitable for detecting Ascaris *eggs.*

Various concentration techniqes were compared in order to examine whether any of the techniques is more sensitive for the detection of fertilized or unfertilized *Ascaris* eggs. This seems to be reasonable since ascariasis is not rarely found to be associated with trematode infections in such places as Shiga Prefecture, as presented here, or some other developing countries. Among these techniques are examined the formalin ether centrifugation technique (MGL) (Ritchie, 1948), a modified Weller-Dammin's concentration technique (Wakejima *et al.*, 1961), Tween 80-citrate buffer sedimentation procedure (Oshima *et al.*, 1965) in addition to the AMS III technique. We followed to the original procedures as possible as we could, except that 0.5 g of feces was applied for all the concentration techniques. Sedimentation gradient and some other quality of each reagent used were strictly checked. Results were expressed as percent recovery of eggs in comparison with that of the Stoll's dilution technique. The same stool specimen containing either of fertilized or unfertilized *Ascaris* eggs was used for each series of examination with different techniques. Comparison was also made on *Trichuris trichiura* or *M. yokogawai* eggs.

Results

Epidemiological studies.

Epidemiological studies carried out in the jurisdictional area of the Imazu Health Center have revealed that ascariasis is still endemic at least in the mountain areas seveal kilometers interior of the west coast of Lake Biwa. As shown in Table 1, a high prevalence rate of ascariais was observed in Mukugawa village of Imazu-machi (24.6%) and in Kutsuki-mura (16.2%) where villagers were utilizing their excreta as fertilizer for vegetables. The infection with *T. trichiura* was also endemic in these villages. Metagonimiasis and clonorchiasis were found more endemic among people living close to the lake side if compared with those living in Kutsuki-mura (Table 1). Incidence of the infection with either of the soil-transmitted helminths was also examined in two villages. The results are summarized in Table 2, suggesting that new infections occurred within six months since May of 1977.

Concerning to a question whether there exists any difference of the prevalence of helminthiasis between males and females a statistical analysis by the chisquare test showed the fact that metagonimiasis and clonorchiasis were more prevalent among males than among females ($P<0.01$), whereas the infection with *T. trichiura*

Table 3 Difference of the prevalence rate of helminthiases between males and females

	No. of examined		No. of positive for stool examination*		P**
	m.	f.	m.	f.	
A. lumbricoides	179	200	34	37	N.S.
T. trichiura	363	395	59	104	<0.01
M. yokogawai	284	309	31	13	<0.01
C. sinensis			29	15	<0.01

* Data derived from results of the Kato's thick smear technipue for *A. lumbricoides* eggs, of the AMS III method for trematode eggs, and of both techniques for *T. trichiura* eggs.

** *P* value yielded by chi-square test. N. S.; not significant.

Table 4 The efficacy of different preparations of pyrantel pamoate against *Ascaris lumbricoides*

Group	Preparation	Dose	No. of patients treated	No. of patients followed	No. of patients cured(%)*
1	dry syrup	5 mg/kg	42	41	40(97.6)
2	dry syrup	2.5 mg/kg	23	23	23(100)
3	tablet	5 mg/kg	20	20	20(100)
4	syrup	5 mg/kg	18	17	16(94.1)

* Follow-up stool examination was carried out about four weeks after the administration of pyrantel pamoate.

was more prevalent among females ($P<0.01$) (Table 3).

Figure 2 shows the age distribution of patients with ascariasis and trichuriasis, in which a tendency of higher prevalence rate was present in groups of preschool children and aged people at least in Mukugawa village of Imazu-machi (Fig. 2A).

Masstreatment of patients with ascariasis with various preparations of pyrantel pamoate.

Results of the masstreatment of ascariasis with low doses of pyrantel pamoate are shown in Table 4. Nobody made complains of side effects after the administration of the drug. A newly developed form of the drug (dry syrup) was quite effective against *A. lumbricoides* infection just as preveously developed preparations were. Even a single administration with such a low dose as 2.5 mg/kg resulted in complete elimination of the infection (Group 2, Table 4). A case of Group 1 in which patients were treated with a dose of 5 mg/kg was, however, positive for a few number of unfertilized eggs. Another case of Group 4 was positive for fertilized eggs, although EPG determined by the Stoll's dilution technique and the modified Kato's method reduced from 19,000 to 720. As far as the EPG concerned, if it well corresponds to the intensity of the infection, any difference of the efficacy of pyrantel pamoate was not observed in patients with different intensity (Table 5).

Comparison of stool examinations suitable for detecting Ascaris *eggs.*

Since we were aware of having some discrepancies concerning the detection of *Ascaris* eggs between results of the AMS III technique and the Kato's method, we examined various concentration techniques to determine which of them is the best one in terms of the recovery rate of *Ascaris* eggs. The results are summarized in Table 6, showing that among the techniques as far

as examined, the MGL method was the best, although the EPG was less than half of that obtained by the Stoll's dilution technique. The modified Weller-Dammin's method was fairly good for fertilized eggs but not for unfertilized eggs. Contrary as expected, the recovery rate by the AMS III technique was always less than 2% of that obtained by the Stoll's technique. However, the AMS III method was the best for recovering ova of *T. trichiura* and/or of *M. yokogawai*, although the variations in each examination were relatively large.

Discussion

Endemic foci of soil-transmitted helminthiases, i.e. ascariasis and trichuriasis, were found in the west coast area of Lake Biwa, Shiga Prefecture, where villagers were utilizing their night soil as fertilizer. Parasite control program had already been introduced in these areas since 1975: Masstreatment of patients with ascariasis had been carried out by the administration of a mixture of santonin and kainic acid twice a year; ovicidal drugs had extensively been applied into latrines. Educations necessary for the improvement of sanitary conditions had also been provided repeatedly for all villagers through efforts of visiting public health nurses. Nevertheless, high incidence of the infections was demonstrated in the present study, indicating that new infections occurred actively in these areas (Table 2). According to Professor Morishita (personal communication), a mixture of santonin and kainic acid was not any more efficient in terms of the cure rate when he applied the mixture for the masstreatment performed in Kutsuki-mura in late of 1960s. Kobayashi *et al.* (1970) also have pointed out such a sort of resistance of *Ascaris* against a santonin-kainic acid mixture in a trial in which they obtained a cure rate of 37.3%. Probably this is the reason why the rate of prevalence and incidence of ascariasis is still maintained in high level in these areas.

A tendency that the number of patients with the helminthiases increases among children and aged individuals was observed, whereas the prevalence of the infections was low among youg adults working outside of the villages (Fig. 2). Interestingly, trichuriasis was more prevalent among females than among males. Application of chi-square test yielded a P value of <0.01 for the difference of the prevalence rate in females vs. that in males. On the other hand, infections with *M. yokogawai* or *C. sinensis*, i.e. infections transmitted by fresh water fishes, were more prevalent among males than among females ($P<0.01$) (Table 3). This is probably due to the fact that females have a habit of visiting each other at tea time taking home-made pickles in-

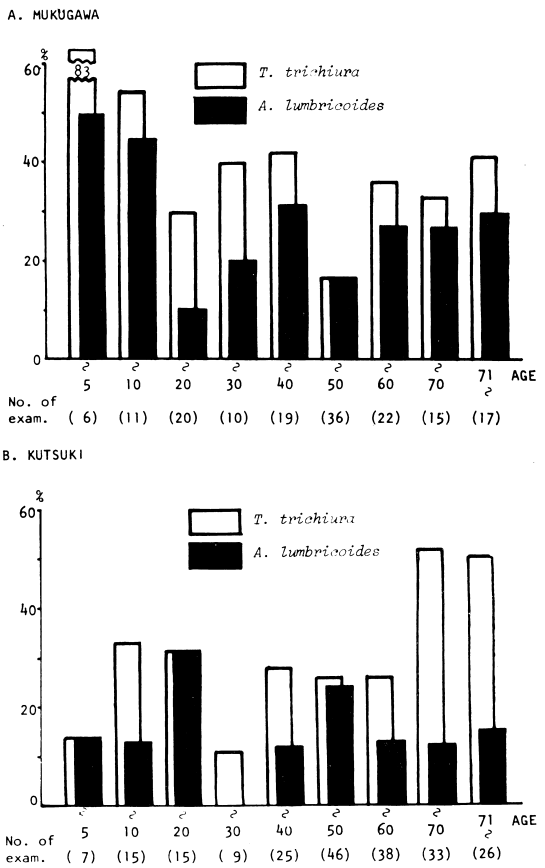

Fig. 2. Age distribution of patients with ascariasis or trichuriasis in Mukugawa village, Imazu-machi (A) and Kutsuki-mura (B). Ordinate; percentage of positives for stool examinations.

stead of cookies, whereas males have more chances of eating raw fresh water fishes. The reason why the frequency of ascariasis is just the same in both sexes is not clear. However, it is very likely that the mass-streatment with a mixture of santonin and kainic acid, which had been carried out twice a year since 1975, might affect on the prevalence rate, resulting in no accumulation of the *Ascaris* infection. On the other hand, *T. trichiura* is resistant to the drug resulting in the accumulation of the infection among females.

Pyrantel pamoate is known to have a broad spectrum of anthelmintic effect against *A. lumbricoides* (Campos, 1969; Desowitz *et al.*, 1970; Kobayashi *et al.*, 1970; Yokogawa and Kagei, 1970), hookworm (Desowitz *et al.*, 1970; Yokogawa *et al.*, 1970a; Hori, 1975; Ishizaki *et al.*, 1971; Kobayashi *et al.*, 1971), and *Enterobius vermicularis* (Bumbalo *et al.*, 1969; Yokogawa *et al.*, 1970b; Yamamoto *et al.*, 1971). Quite recently, it has been reported that the efficacy of the drug against *A. lumbricoides* may clearly be shown even by a single administration of a low dose of 2.5 mg/kg in terms of the cure rate as well as of the egg reduction rate (Seo, 1977). In the present study, a newly developed form of pyrantel pamoate, i.e. dry syrup, was proved to be effective against the *Ascaris* infection. We obtained 100% of the cure rate with a single dose of 2.5% mg/kg of the dry syrup preparaton. The intensity of the infection when expressed as EPG did not seem to affect the efficacy of the anthelmintic; patients, even though they have such a large count of EPG as 20,000 or 40,000 were cured of the infetion completely (Table 5). The egg reduction rate was 96.2% in one case showing positive for *Ascaris* eggs after the treatment with the syrup preparation (Table 1, G.O.). These results are in accordance with those obtained by Seo, indicating that the administration dose of pyrantel pamoate, regardless of the preparation form, may be reduced to as low as 2.5 mg/kg for ascariasis. Reducing dosage would provide a great advantage in economical point of views and in avoiding side effects of the drug.

It is well known that even the simple smear method may be enough for the detection of *Ascaris* eggs because of a large number of eggs deposited by a female worm per day. It is, however, apparently convenient if one can use a technique by which the detection of *Ascaris* eggs as well as trematode eggs is possible at the same time in such an endemic area as in the west

Table 5 Efficacy of pyrantel pamoate against ascariasis in patients excreting various numbers of EPG

Initials (sex)	Treated with 5 mg/kg of	EPG before treatment determined by			EPG after treatment determined by
		Stoll	MGL	Thick smear	Thick smear
C.S. (f)	D*	23,000	3,620	22,440	0
Y.O. (m)	D	4,250	1,382	13,200	0
G.I. (m)	D	380	1,822	5,480	0
U.N. (f)	D	ND	6,788	34,060	0
A.S. (f)	T	400	22	480	0
Y.S. (m)	T	600	66	620	0
S.O. (m)	T	2,600	124	560	0
Y.S. (f)	T	ND	ND	39,980	0
K.O. (f)	S	ND	156	4,320	0
G.O. (m)	S	19,000	1,772	19,040	720

* Prearation of pyrantel pamoate; D: dry syrup, T: tablet, S: syrup.

Table 6 Comparison of various stool examination techniques in terms of the recovery rate of helminth eggs

Eggs of	Stoll's dilution method	AMS III	MGL	Tween 80 citrate buffer	Weller-Dammin	Thick smear technique
A. lumbricoides fertilized	20,645.6 ±2,045.4*	224.8±184.4	10,978.0 ±2,250.4	561.0±248.2	4,945 ±2,294.6	30,150.5 ±5,411.6
(Sample No. 1)	(100)**	(1.1)	(53.2)	(2.7)	(24.0)	(146.0)
unfertilized	565.0±158.2	7.6±14.6	230.6± 92.1	27.4± 13.2	15.8± 10.0	ND***
(Sample No. 2)	(100)	(1.3)	(40.8)	(4.9)	(2.8)	
unfertilized	985 ±285.8	0.6± 0.97	331 ±335.2	199.2±196.3	0.8± 4.9	2,028.8 ± 860.7
(Sample No. 3)	(100)	(0.06)	(33.6)	(20.2)	(0.3)	(205.8)
T. trichiura (Sample No. 4)	15.0± 33.7	28.0±11.7	18.8± 20.3	16.0± 12.0	27.6± 10.5	ND
M. yokogawai (Sample No. 5)	0	8.6± 7.1	2.8± 5.4	3.2± 3.7	5.2± 3.3	ND

* Mean±SE of EPG was calculated from results of ten preparations for each technique.
** Per cent of EPG (mean) vs EPG (mean) determined by the Stoll's dilution method.
*** Not done.

coast of Lake Biwa where these infections coexist. Our preliminary results on various techniques of stool examinations showed that the AMS III technique was not always sensitive for the detection of *Ascaris* eggs, although it was one of the excellent methods for detecting trematode eggs. Thus, we further examined to find out the most sensitive and reproducible method for *Ascaris* eggs among several concentration techniques. A series of examinations were carried out by using the same specimen obtained from each person. As shown in Table 6, reproducible egg counts were obtained by the Stoll's dilution technique (Stoll and Hausheer, 1926), and the modified Kato's thick smear method (Martin and Beaver, 1968). Among centrifugation techniques so far examind, the most sensitive method was the MGL method (Ritchie, 1948) in which 30 to 50% of *Ascaris* eggs were recovered if compared with EPG determined by the Stoll's dilution. If one considers that *Ascaris* may produce as much as 200,000 eggs per female per day and that 0.5 g of feces may be applied for the MGL method, the method would be the first choice for epidemiological studies in areas where many kinds of parasitic diseases including protozoa and trematode infections are endemic in association with ascariasis. It should be noted in Table 6 that especially on *Ascaris* unfertilized eggs the lowest recovery rate was obtained by the AMS III techniques, although the technique was superior in the recovery of eggs of *T. trichiura* and *M. yokogawai*. Furthermore, only 37.5% of positives for *Ascaris* eggs determined by the Kato's method were found to be positive by the AMS III technique, indicating less sensitivity of the latter in terms of the detection rate (data not shown in Table 6). On the contrary, the Kato's method was more sensitive on *Ascaris* unfertilized eggs than any other stool examination techniques. Martin and Beaver (1968) also have pointed out that the Kato's method is sensitive and reliable for the diagnosis of infections not only with intestinal nematodes but with *Schistosoma mansoni*. Tween 80-citrate buffer centrifugation technique was better than the AMS III method but the recovery rate was 3 to 20% of the Stoll's dilution, which was far behind of the modified Kato's method. According to Kagei (1975), the Tween 80-citrate buffer sedimentation technique was as sensitive as

the Kato's method in terms of the detection rate of *Ascaris* and hookworm eggs.

Finally, as mentioned already, the eradication program against ascariasis had been in progress in Kutsuki-mura and Mukugawa village where we used a low dose of pyrantel pamoate with a great success against *Ascaris* which seemed to be somewhat resistant to a mixture of santonin and kainic acid. In the villages, however, new infections may occur, because there is a possibility that *Ascaris* eggs with the infective larva may still be present in latrines and that the excreta may be used as fertilizer soon later. Therefore, further follow-up studies will apparently be needed in the areas described here.

Summary

Endemic foci of soil-transmitted helminthiases, i.e. ascariasis and trichuriasis, were found in the mountain area several kilometers interior of the west coast of Lake Biwa, Shiga Prefecture, where villagers were utilizing their excreta as fertilizer for vegetables of their own use. Masstreatment of patients with ascariasis was carried out by a single administration of low doses of pyrantel pamoate (Combantrin). A newly developed preparation of the drug (dry syrup) was quite effective against *Ascaris lumbricoides* just as the other preparations were; almost 100% of the cure rate was obtained by a low dose of either of 2.5 mg/kg or 5 mg/kg of the dry syrup. Side effects were not observed at all in individuals of any of the groups examined. Using the same specimen containing either of fertilized or unfertilized *Ascaris* ova, a series of stool examinations were carried out to determine the most sensitive and reliable centrifugation technique for the detection of the eggs. Among the techniques so far examined, the MGL method was the best which would be the first choice for epidemiological studies in areas where ascariasis coexists with other endemic parasite infections such as trematode infections.

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滋賀県下で見出された蛔虫症流行地とピランテルパモエート新剤形による集団駆虫成績

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滋賀県今津保健所管内の高島郡6カ町村において，AMS Ⅲ 法およびセロファン厚層塗抹法により検便を実施したところ，鞭虫，横川吸虫，肝吸虫各虫卵陽性者のほかに，対象者のほとんど全員が検査を受けた今津町椋川および朽木村（西地区）において，それぞれ24.6%および16.2%と多数の蛔虫卵陽性者が見出された．そこで，　これら蛔虫卵陽性者 103 名を無作為に 4 群に分け，第1群にはピランテルパモエート （Combantrin）の新剤形 （ドライシロップ）5 mg/kg を，第2群には同 2.5 mg/kg を，第3群には本剤の錠剤 5 mg/kg を，第4群にはシロップ 5 mg/kg を，それぞれ1回経口的に投与した．その結果，4週後の各群の陰転率はそれぞれ 96.7%，100%，100%，94.1%であつた．　これらの成績から，この新剤形はすでに広域駆虫剤として十分評価されている他の剤形の本剤と同様の効力が認められること，また蛔虫症に対しては，本剤の 2.5～5 mg/kg 1回投与によつても満足すべき陰転率が得られることが明らかとなつた．

さらに，蛔虫が他種寄生虫とくに吸虫と混合感染している場合に，蛔虫卵について最も検出率の高い遠心沈殿法をあらかじめ知つておくことは有意義と考えられたので，蛔虫受精卵または不受精卵をそれぞれ含有する検体を用いて，Stoll 法を規準に各種の遠心沈殿法について虫卵回収率を比較したところ，MGL 法が最もすぐれていた．これに対し，AMS Ⅲ 法は，鞭虫卵・横川吸虫卵について回収率がすぐれていたが，　蛔虫受精卵で Stoll 法の 1.1%，不受精卵ではわずか 0.06～1.3%の回収率にすぎず，別に試みた検出率についての実験でも，厚層塗抹法の40%以下であつた．