

COMPARISON OF CIRCUMOVAL PRECIPITIN TEST (COPT) FILTER PAPER METHOD AND FORMALIN-ETHER CONCENTRATION TECHNIQUE IN SCHISTOSOMIASIS FIELD SURVEY

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INTRODUCTION

This report is one of several studies undertaken during field trips to Jaro, Leyte. Although the junior author had for priority project the study on *Paragonimus* and paragonimiasis, research on other equally important parasitic diseases like schistosomiasis and taeniasis were also included in the original research plan.

The circumoval precipitin test (COPT) is a serologic test for schistosomiasis. It was first reported by Oliver-Gonzales in 1954 wherein he demonstrated the formation of bleb-like globules or chain of precipitates around the *Schistosoma* egg incubated in sera of cases and experimentally infected animals. The use of this test in the Philippines was reported by Yogore *et al.*, (1962, 1968) followed by other reports by Garcia *et al.*, (1963, 1968). In both studies they have demonstrated that the test was adequately sensitive and specific.

In later studies to overcome resistance to withdrawal of blood from the vein and adopt or develop a procedure for collecting blood and serum suitable for rural areas as well as for field surveys, we evaluated the COPT using blood obtained from finger prick and received or collected on filter paper. Our results showed that performing the test on blood collected in this manner did not

result in loss of sensitivity of the test. Also it was found that blood dried on filter paper kept at room temperature for as long as 70 days still gave positive results (Cabrera *et al.*, 1968). These modifications made the COPT suitable for field surveys.

In the evaluation of serologic tests for schistosomiasis it was also demonstrated among chronic cases with hepato-splenomegaly that almost two-thirds would be negative by stool examinations (Garcia *et al.*, 1968). This indicated that a stool survey would underestimate the prevalence of schistosomiasis and a serologic test would give a more accurate estimate of the prevalence of the disease.

This study was undertaken to determine the suitability of the filter paper collection of blood for COPT for surveys and the difference in estimate of prevalence of schistosomiasis by stool examination using formalin-ether concentration and circumoval precipitin test (COPT).

MATERIALS AND METHODS

The study area included Barrio Pitogo and Barrio Buri of the municipality of Jaro, Eastern Leyte. The two barrios are about a kilometer apart, $2\frac{1}{2}$ kilometers from the town of Jaro. This town on the other hand is 38 kilometers from the town of Palo where the Schistosomiasis Control Project Laboratory is located, and 50 kilometers from

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Tacloban City, the capital of the province. There are no roads or any means of transportation going to these barrios except for a foot-trail. Several fresh water streams have to be crossed in going to these barrios (Fig. 1). Both Pitogo and Buri are typical of any Philippine barrio with farming as the principal means of livelihood.

The authors together with laboratory technicians made a number of field trips to the study area which is known to be endemic both for schistosomiasis and paragonimiasis. Because of lack of time and working space in the area and in order to perform a more accurate and reliable laboratory diagnosis using concentration techniques, we preserved all specimens and took them back with us to Manila.

Inhabitants from both barrios were requested to submit stool specimen wrapped in waxed paper. With an applicator stick, a

sufficient amount of the faeces was transferred into a screw-cap vial containing 10 per cent formalin. The specimen was then emulsified thoroughly to insure complete fixation and preservation of protozoan cysts and helminthic ova and/or larvae. The screw-cap was replaced, then sealed with masking tape. After the vials were properly identified and labelled, they were packed for shipment.

Two to three drops of blood from finger pricks were also obtained from the inhabitants and received on filter paper strips. These were allowed to dry, properly labelled and packed for shipment. Paired specimens of stool and blood were therefore collected from the inhabitants of Pitogo and Buri. There were occasions, however, when stool specimens were submitted but no blood samples and were therefore disregarded for this purpose.

In order to insure the collection of a large

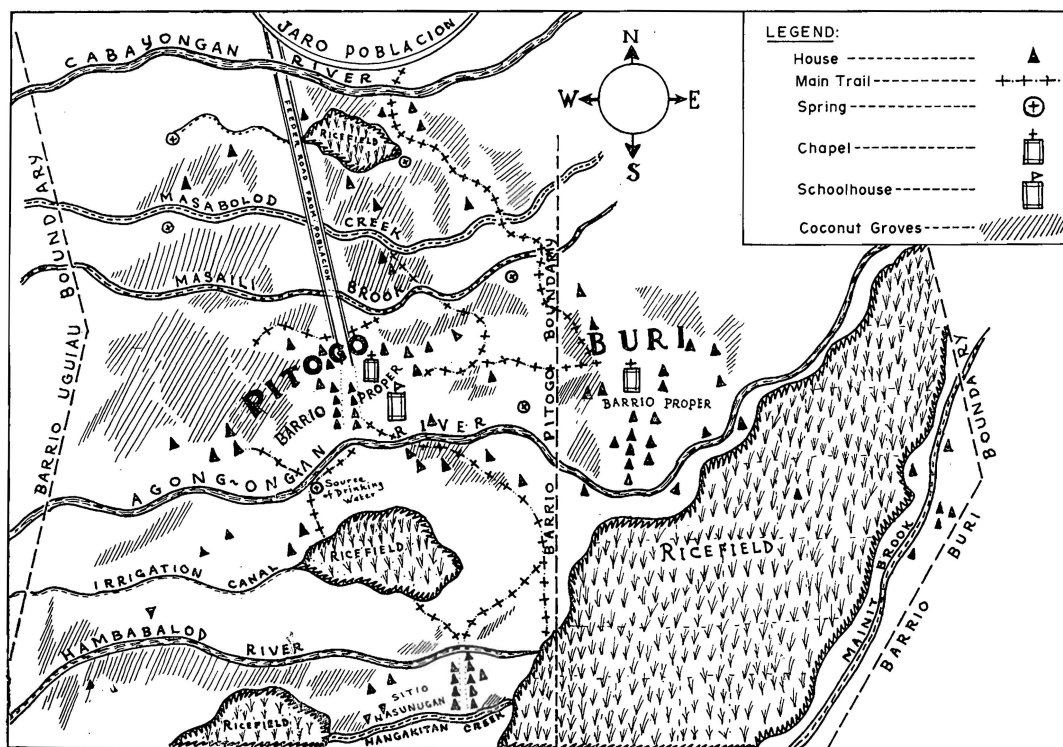


Fig. 1—Sketch map of Barrios Pitogo and Buri, Jaro, Leyte del Norte.

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sample, a house-to-house survey was done, intended particularly for those who failed to deliver specimens to us, as per our request. One team was assigned to Barrio Buri while the other team to Barrio Pitogo. We had also availed of the help of two school teachers in the collection of specimens from school children.

The preserved stool specimens were taken to the Department of Parasitology, Institute of Public Health, University of the Philippines where they were processed using formalin-ether concentration technique. The examinations included all helminthic ova and/or larvae as well as protozoan cysts. The dried blood on filter paper were processed and examined at the Department of Microbiology and Immunology, University of the East Ramon Magsaysay Memorial Medical Center. The procedure followed was based on the original article on the subject reported by Cabrera *et al.*, (1968).

RESULTS AND DISCUSSION

Table 1 shows the prevalence of schistosomiasis by barrio, kind of specimen and laboratory technique used among 312 inhabitants that submitted paired specimen of stool and blood. It appears that schistosomiasis is more prevalent in Barrio Buri than

in Barrio Pitogo although the sample obtained in Buri is small compared to Pitogo. The difference may be due to the fact that the former has more extensive rice fields than the latter which may account for more chances of exposure of the inhabitants to the *Schistosoma cercariae* (Fig. 1). What is important to note here is the number of schistosomiasis cases missed by using stool examination (formalin-ether) when compared to COPT (filter paper) method. In Barrio Pitogo 46 cases or 16.31 per cent were missed while in Barrio Buri 3 cases or 10 per cent were missed. This gives a total of 49 or 15.7 per cent schistosomiasis cases missed by stool examination.

Table 2 shows the age and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT (filter paper) examinations. It appears that schistosomiasis is more prevalent in adults than in children as shown both in stool and blood examination results. With stool (formalin-ether) examination a prevalence rate of 18.84 per cent for children and 30.45 per cent for adults were obtained. Hence 18 per cent of cases were missed among children and 14 per cent of cases among adults. Under all ages, one will note that the prevalence rate of schistosomiasis in males is higher than in females in both methods of exami-

Table 1

Prevalence of schistosomiasis by Barrio, kind of specimen and laboratory technique used, Jaro, Leyte, 1973.

Barrio	No. Examined	Stool (Formalin-ether)		Blood COPT (filter paper)	
		No.(+)	%(+)	No.(+)	%(+)
Pitogo	282	68	24.11	114	40.42
Buri	30	11	36.66	14	46.66
Total	312	79	25.32	128	41.02

Table 2

Age and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT (filter paper) examinations. Jaro, Leyte 1973.

Age and Sex	No. Examined	Stool (formalin-ether)		Blood COPT (filter paper)	
		No.(+)	%(+)	No.(+)	%(+)
Children					
Male	82	19	23.17	36	43.90
Female	56	7	12.50	15	26.78
Both sexes	138	26	18.84	51	36.95
Adult					
Male	85	30	35.29	44	51.76
Female	89	23	25.84	33	37.07
Both sexes	174	53	30.45	77	44.25
All Ages					
Male	167	49	29.34	80	47.90
Female	145	30	20.68	48	33.10
Both sexes	312	79	25.32	128	41.02

nation. It is also worth noting that blood COPT (filter paper) method is much more sensitive than stool (formalin-ether) concentration method because it detected 16 per cent more cases. This statement holds true for both children and adults as well as for both sexes.

Table 3 shows the age and sex distribution of schistosomiasis cases among children detected by both methods of examination. One will note that the infection was more prevalent among males than among females. This may be due to the fact that boys are usually asked to help the father in the farm while girls usually stay with the mother in keeping house. Males also acquire the infection earlier than females and the youngest case was a five-year-old male from Barrio Pitogo. Whereas males got infected at age 5 years, females got it at age 7 years.

There were 17 males and 8 females or a total of 25 schistosomiasis cases among children missed by stool (formalin-ether)

concentration method but which were detected by blood COPT (filter paper) examination. The sensitivity of both methods in detecting schistosomiasis cases appears the same at ages 5 and 6. However from 7 up to age 12 the blood COPT (filter paper) method was definitely more efficient in detecting cases. At age 7 in males none was missed, 1 case at age 8; 2 at age 9; 8 at age 10; none at age 11 and 6 at age 12 or a total of 17 males. At age 7 in females, 2 cases were missed; 2 at age 8; 2 at age 9; 1 at age 10; 1 at age 11; none at age 12 or a total of 8 females. One has to take into account the possibility of tissue proliferation and repair, taking place in the damaged intestinal mucosa, which accounts for the failure of eggs to be discharged into the intestinal lumen and hence a negative stool examination but a positive COPT (filter paper method), due to the presence of antibodies.

The prevalence of helminthic and protozoan infection among inhabitants of Barrio

Table 3

Age and sex distribution of schistosomiasis cases among children detected by stool (formalin-ether) and blood COPT (filter paper) examinations, Jaro, Leyte, 1973.

Age in years	No. Examined			No. positive on stool (formalin-ether)			No. positive on blood COPT (filter paper)		
	M	F	Total	M	F	Total	M	F	Total
4	0	0	0	-	-	-	-	-	-
5	7	4	11	1	0	1	1	0	1
6	5	5	10	3	0	3	3	0	3
7	4	8	12	1	1	2	1	3	4
8	7	8	15	1	1	2	2	3	5
9	10	11	21	3	2	5	5	4	9
10	25	5	30	4	1	5	12	2	14
11	8	7	15	5	2	7	5	3	8
12	16	8	24	1	0	1	7	0	7
Total	82	56	138	19	7	26	36	15	51

Pitogo and Barrio Buri is shown in Table 4. A total of 448 stool samples were collected and examined, 281 from Barrio Pitogo and 167 from Barrio Buri. It is interesting to note that the prevalence rates for *Ascaris*, *Trichuris*, hookworm, *Schistosoma* and *Paragonimus* were higher among inhabitants of Barrio Buri than those from Barrio Pitogo. On the other hand, the prevalence rates for *Taenia*, *Entamoeba histolytica*, *Entamoeba coli* and *Giardia* were higher among inhabitants from Barrio Pitogo than those from Barrio Buri. Other interesting findings in this table are the almost equal or parallel prevalence rates for *Ascaris*, *Trichuris* and hookworm, and the high rate for *Taenia* infection. A review of similar surveys done by the junior author and his group revealed that this is the first survey done with a prevalence rate for *Taenia* of more than 10 per cent of people examined. The eating habits of the people in the area with respect particularly to animal meat, no doubt, contributes a major factor in taeniasis.

Table 5 shows the age and sex distribution

of helminthic and protozoan infection in the two barrios. The prevalence rates for *Ascaris* and *Trichuris* in children were higher than those of adults, while the prevalence rates for hookworm, *Schistosoma*, *Paragonimus*, *Taenia*, *E. histolytica* and *E. coli* were higher in adults than in children. Among female children, the prevalence rates for *Ascaris*, *Trichuris*, *Paragonimus* and *Giardia* were higher than among males, but higher prevalence rates for hookworm, *Schistosoma* and *E. coli* infections were observed among males.

Among adult males the prevalence rates for all the parasitic infections were higher than in females except for *Ascaris*, *Taenia* and *E. histolytica* infections. When one consider the all age group, the males have higher prevalence rates than females for *Trichuris*, hookworm, *Schistosoma*, and *E. coli* infections while females have higher prevalence rates than males for *Ascaris*, *Paragonimus*, *Taenia*, *E. histolytica* and *Giardia* infections. The prevalence rates for all ages on helminths was highest in *Ascaris*

Table 4

Prevalence of helminthic and protozoan infections in two barrios, Jaro, Leyte, 1972.

Barrio	No. Exam.	Helminths										Protozoa							
		<i>Ascaris</i>		<i>Trichuris</i>		Hookworm		<i>Schistosoma</i>		<i>Paragonimus</i>		<i>Taenia</i>		<i>E. hist.</i>		<i>E. coli</i>		<i>G. lamblia</i>	
		No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +
Pitogo	281	180	64.05	171	60.50	169	60.14	54	19.21	3	1.06	38	13.52	2	0.71	19	6.76	2	0.71
Buri	167	116	69.46	116	69.46	113	67.66	34	20.35	2	1.19	8	4.73	1	0.65	11	6.58	0	0.00
Total	448	296	66.07	287	64.06	282	62.94	88	19.64	5	1.11	46	10.26	3	0.66	30	6.69	2	0.44

Table 5

Distribution by age and sex of helminthic and protozoan infections in two barrios, Jaro, Leyte, 1972.

Age and Sex	No. Exam.	Helminths										Protozoa							
		<i>Ascaris</i>		<i>Trichuris</i>		Hookworm		<i>Schistosoma</i>		<i>Paragonimus</i>		<i>Taenia</i>		<i>E. hist.</i>		<i>E. coli</i>		<i>G. lamblia</i>	
		No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +	No. +	% +
Children*	189	146	77.24	123	65.08	117	61.90	28	14.81	3	1.58	1	0.52	0	0.0	12	6.34	1	0.52
Male	117	85	72.64	72	61.54	74	63.24	19	16.24	1	0.85	1	0.85	-	0.0	8	6.84	0	0.0
Female	72	61	84.72	51	70.83	43	59.72	9	12.50	2	2.78	0	0.0	0	0.0	4	5.56	1	1.38
Adults**	259	150	57.92	165	63.70	165	63.70	60	23.16	5	1.93	45	17.37	3	1.16	18	6.94	1	0.38
Male	124	62	50.00	35	68.54	81	65.32	38	30.64	3	2.41	21	16.94	1	0.81	11	8.87	1	0.81
Female	135	88	65.18	80	59.26	84	62.22	22	16.30	2	1.48	24	17.78	2	1.48	7	5.18	0	0.0
All Ages	448	296	66.07	288	64.28	282	62.94	88	19.64	8	1.72	46	10.26	3	0.66	30	6.69	2	0.45
Male	241	147	61.00	157	65.14	155	64.32	57	23.65	4	1.66	22	9.12	1	0.42	19	7.88	1	0.42
Female	107	149	71.98	131	63.28	127	61.35	31	14.98	4	1.98	24	11.59	2	0.96	11	5.31	1	0.48

* = 1-12 years of age are children

** = 13 and over are adults.

(66.07 per cent); *Trichuris* (64.28 per cent); *Schistosoma* (19.64 per cent); *Taenia* (10.26 per cent) and *Paragonimus* (1.78 per cent). With regard to protozoan infection, the prevalence was highest for *E. coli* (6.69 per cent) followed by *E. histolytica* (0.66 per cent) and *Giardia* (0.44 per cent).

SUMMARY AND CONCLUSION

Schistosomiasis prevalence rate is higher among inhabitants of Barrio Buri than Barrio Pitogo as shown in both stool (formalin-ether) and blood COPT (filter paper) examination. The latter method of examination appears to be more sensitive than the former in the detection of schistosomiasis cases because about 16 per cent of cases are liable to be missed with stool (formalin-ether) concentration technique. There was not a single schistosomiasis case detected by stool (formalin-ether) concentration method that was not picked up by blood COPT (filter paper) method. Schistosomiasis is more prevalent in adults than in children and higher in males than in females in all age group.

Among children, schistosomiasis is also more prevalent in males than in females with the youngest case found in a 5-year-old male. Males appear to acquire the infection earlier than females.

A blood COPT (filter paper) method is preferred than a stool (formalin-ether) concentration technique because it requires less work and less time; it is not messy yet is very sensitive and highly specific. The method of obtaining blood from a finger prick (COPT filter paper) is more readily acceptable by subjects than the standard COPT method where blood specimen has to be obtained by veni-puncture.

Stool samples from 448 inhabitants of Barrios Pitogo and Buri of the Municipality of Jaro, Leyte were examined and analyzed. There was a more or less equal overall

prevalence rates for *Ascaris*, *Trichuris* and hookworm in the area. As expected, prevalence rates for *Ascaris* and *Trichuris* are slightly higher in children than in adults while rates for hookworm, schistosomiasis, paragonimiasis and taeniasis are higher in adults than in children. Except in few instances, males have, higher parasitic prevalence rates than females. In addition to the aforementioned intestinal helminths, the area is likewise endemic for schistosomiasis, paragonimiasis and taeniasis at relatively high prevalence rates.

In conclusion we can say that circumoval precipitin test (filter paper) method is superior than formalin-ether concentration technique in the detection of schistosomiasis cases. We therefore, recommend this method for all schistosomiasis surveys, as well as for rural physicians in remote endemic areas, for diagnosis of suspicious subclinical cases, whereby specimens may be sent through the mail to a central laboratory doing circumoval precipitin test (COPT) for schistosomiasis.

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