Rev. Biol. Trop., 11(1): 47-56, 1963

Intestinal parasites in a rural community of Costa Rica*

by

M. M. Brooke** Neva Gleason**

F. Montero-Gei***

(Received for publication February 19, 1963)

In the spring of 1957, one of the authors (MMB) participated in an examination and treatment project for intestinal nematodes at Finca Volcán-Angel, as part of the Louisiana State University (LSU) fellowship program. Lt. Col. Hershel Griffin was the other member of the team and was responsible for the medication. Our cordial host and pilot was Mr. Maxwell Cone, owner of Volcan-Angel Finca. This cattle ranch is located about 90 miles southeast of San José, Costa Rica, near the little town of Volcán de Buenos Aires.

Prior to our arrival, arrangements had been made for the children and adults on the ranch and in the nearby village to submit fresh stool specimens. In most instances, small match boxes were used for this purpose. In a field laboratory saline wet mounts were examined for nematode eggs. These examinations had to be performed rapidly so that the individuals found to be positive could be treated immediately with an appropriate anthelmintic.

In order to obtain better information on the prevalence of intestinal parasites, each specimen, quantity permitting, was preserved in two vials: one

---

* Presented at the "Segundo Congreso Latinoamericano y Primero Nacional de Microbiología", San José, Costa Rica, December 10-17, 1961.

** Communicable Disease Center, Public Health Service, U. S. Department of Health, Education and Welfare, Atlanta, Georgia.

*** Department of Public Health Laboratories, Ministerio de Salubridad Pública, San José, Costa Rica.

containing 10 per cent formalin, and the other polyvinyl alcohol (PVA)-fixative, for more thorough examination at a later time. The subsequent examinations were performed at the Central Public Health Laboratory in San José, Costa Rica, or at the parasitology laboratories of the Communicable Disease Center of the U. S. Public Health Service, in Atlanta, Georgia.

METHODS AND RESULTS

Stool specimens were submitted by 134 individuals. In 26 instances, samples were sufficient only for direct examinations to be performed in the field laboratory. The remaining 108 stools were preserved for later examination in either one or two solutions, depending upon the quantity and condition of the specimen. Fifteen were preserved in formalin only, 46 in PVA-fixative only (3), and 62 in both formalin and PVA-fivative. The 77 specimens preserved in formalin were concentrated by the formalin-ether (FE) sedimentation technique of RITCHIE (15) in the Central Public Health Laboratory in San José. The sediments were examined in unstained and iodine stained wet mounts either in that laboratory by one of us (MMB) or at CDC after shipment to Atlanta. The 108 specimens preserved in PVA-fixative were stained by a trichrome method of WHEATLEY (21) at CDC, and were examined under the oil immersion objective.

Nine species of protozoa and five nematodes were identified in the 134 specimens examined by one or more techniques. A number of additional amebae, flagellates, and larvae were encountered which could not be identified. The prevalence of the various parasites observed in the 134 individuals is given in Table 1. With the exception of *Balantidium coli,* the prevalence rates were high for all of the protozoa. A total of 62 individuals or 46.3 per cent were found infected with amebae with nuclear and chromatoidal characteristics generally ascribed to *Entamoeba histolytica.* In view of the recent publications in the scientific literature, which appear to justify the recognition of the so-called "small race *E. histolytica*" as a separate species, *Entamoeba hartmanni* (5, 6, 7, 9, 10, 11, 18, 19), we have designated those measuring less than 10 microns in diameter as *E. hartmanni* (34 per cent), and those 10 microns or over as *E. histolytica* (28 per cent). There were many mixed infections of both species.

Of the four nematodes encountered, hookworm and *Trichuris trichiura* were the most prevalent, 66 and 69 per cent respectively. No infections of *Enterobius vermicularis* were encountered, but satisfactory examinations (perianal specimens) were not included in the study. Besides the nematodes, *Hymenolepis nana* was the only other helminth identified.

Approximately the same number of males and females were examined. With the exception of *Giardia lamblia* (higher in males with $P<0.05$), there were no significant differences in prevalence rates between the two sexes (Table 1). Insufficient numbers do not permit a sex-age analysis

TABLE 1

Parasites found in 134 individuals by a combination of one or more techniques (direct wet mounts, permanently stained PVA films and/or formalin-ether concentrations).

FINDINGS	IN 69 MALES		IN 65 FEMALES		TOTALS IN 134 PERSONS	
	N°	%	N°	%	N°	%
PROTOZOA:						
Entamoeba histolytica	22	32	15	23	37	28
Entamoeba hartmanni	21	30	25	39	46	34
Entamoeba coli	33	48	28	43	61	46
Endolimax nana	35	51	30	46	65	49
Iodamoeba butschlii	24	35	18	28	42	31
Dientamoeba fragilis	11	16	10	15	21	16
Giardia lamblia	30	44	15	23	45	34
Chilomastix mesnili	30	44	23	35	53	40
Trichomonas hominis	23	33	23	35	46	34
Balantidium coli	2	3	1	2	3	2
Unidentified ameba	0	0	0	0	16	12
Unidentified flagellates	0	0	0	0	27	20
Amebic Prevalence Rate (1)	48	70	42	65	90	67
HELMINTHS:						
Hookworm	47	68	41	63	88	66
Ascaris lumbricoides	29	42	25	39	54	40
Trichuris trichura	48	70	44	68	92	69
Strongyloides stercoralis	15	22	19	29	34	25
Hymenolepis nana	3	4	1	2	4	3
Unidentified larvae	0	0	0	0	2	2

(1) Persons infected with one or more of four species *(E. histolytica, E. harmanni, E. coli, E. nana)*

Table 2 presents the prevalence rates for the various parasites according to the following age groups of the 134 persons examined: 0 thru 4, 5 thru 9, 10 thru 14, and over 15 years old. Only one in the last group was less than 20 years old. With the exception of *G. lamblia* and *H. nana,* the peaks of prevalence occurred in the 2nd, 3rd, or 4th age groups.

Three parasites showed a significant ($P < 0.05$) decline in prevalence after reaching peaks in early age groups: *G. lamblia* after the 0 to 4 year age group, *Ascaris lumbricoides* and *T. trichiura* after the 5 to 9 age group. *Dientamoeba fragilis* likewise showed a marked decline after the 5 to 9 age group, but it is not statistically significant. The amebic prevalence rate (APR) showed a significant increase in prevalence through the increasing age groups.

TABLE 2

Prevalence of parasites in various age groups of 134 individuals

FINDINGS	AGE GROUPS							
	0 - 4 YEARS (38 PERSONS)		5 - 9 YEARS (39 PERSONS)		10 - 14 YEARS (38 PERSONS)		OVER 15 YEARS (19 PERSONS)	
	Nº	%	Nº	%	Nº	%	Nº	%
PROTOZOA:								
Entamoeba histolytica	6	16	13	33	11	29	7	37
Entamoeba hartmanni	9	24	14	36	14	37	9	47
Entamoeba coli	13	34	20	51	18	47	10	53
Endolimax nana	14	37	19	49	21	55	11	58
Iodamoeba butschlii	10	26	14	36	13	34	5	26
Dientamoeba fragilis	4	11	9	23	7	18	1	5
Giardia lamblia	18	47	14	36	11	29	2	10
Chilomastix mesnili	14	37	17	44	17	45	5	26
Trichomonas hominis	13	34	15	39	10	26	8	42
Amebic Prevalence Rate	19	50	28	72	27	71	16	84
HELMINTHS:								
Hookworm	17	45	27	69	32	84	12	63
Ascaris lumbricoides	17	45	23	59	10	26	4	21
Trichuris trichura	26	68	35	90	24	63	7	37
Strongyloides stercoralis	7	18	11	28	11	29	5	26
Hymenolepis nana	3	8	0	0	1	3	0	0

Sixty-two specimens were preserved in formalin and PVA fixative, and subjected to both of two laboratory techniques: concentration and permanently stained examination. The direct examinations conducted in the field on the 62 specimens revealed less than half of the infections that were detected by the laboratory techniques, but added three infections of *Strongyloides stercoralis* missed in the laboratory. Table 3 presents the individual and combined results of the laboratory techniques. With the exception of *S. stercoralis,* the prevalence rates (of identified organisms) achieved through the more sensitive procedures, are higher than the combined rates obtained on the 134 specimens, 72 of which could not be examined by these two techniques (Table 1). For example, the prevalence rate for *E. histolytica* was increased from 28 to 36 per cent by the more complete examination of the smaller number of specimens.

Included in the three tables are the amebic prevalence rates (APR) for possible comparison with other surveys. In determining this rate, those persons are counted who are infected with one or more of the four most common species of amebae (*E. histolytica, E. hartmanni, E. coli* and *Endolimax nana*). The

TABLE 3

Parasites found in 62 individuals examined by both formalin-ether concentration and permanently stained PVA films.

FINDINGS	PROCEDURES				TOTALS BY	
	Formalin ether concentration		Permanently stained PVA films		Combination of both procedures	
	N°	%	N°	%	N°	%
PROTOZOA:						
Entamoeba histolytica	14	23	19	31	22	36
Entamoeba hartmanni	9	15	27	44	28	45
Entamoeba coli	31	50	33	53	37	60
Endolimax nana	21	34	40	65	40	65
Iodamoeba butschlii	16	26	26	42	26	42
Dientamoeba fragilis	0	0	14	23	14	23
Giardia lamblia	12	19	24	39	25	40
Chilomastix mesnili	2	3	28	45	30	48
Trichomonas hominis	0	0	25	40	25	40
Balantidium coli	2	3	1	2	2	3
No parasites found	0	0	2	3	0	0
Amebic Prevalence Rate	39	63	49	79	51	82
HELMINTHS:						
Hookworm	52	84	19	31	52	84
Ascaris lumbricoides	27	44	18	29	27	44
Trichuris trichura	45	73	27	44	49	79
Strongyloides stercoralis	11	18	5	8	14	23
Hymenolepis nana	3	5	0	0	3	5

APR has been proposed for the analysis of surveys for epidemiological purposes particularly in areas of low prevalence (4).

DISCUSSION

In conducting a study on the prevalence of intestinal parasites in a population, it is important to include techniques which will recover and identify the various types and stages of organisms present. This is particularly true of the intestinal protozoa. RUIZ and LIZANO (17) emphasized the importance of using a concentration procedure, and permanently stained fecal smears in their study at the Hospital San Juan de Dios in San José, Costa Rica. The present survey followed this principle and added appropriate preservatives (formalin and PVA-fixative), which would maintain the organisms in a satisfactory condition until

the techniques could be performed in a central laboratory. On examining the 62 specimens by both concentration and permanente stains, 36 per cent were found positive for *E. histolytica,* and 45 per cent for *E. hartmanni.* This apparently is the highest rate for *E. histolytica* reported in Costa Rica to date. The next highest rate (16 per cent) was reported by RUIZ and LIZANO (17) in their study of children between 3 and 13 years of age. This is the first survey conducted in Costa Rica that has made the distinction between *E. histolytica* and *E. hartmanni,* or "small race" *E. histolytica.* Although the pathogenicity of *E. hartmanni,* or "small race" *E. histolytica,* has not been fully ascertained, it is generally agreed to be less pathogenic than the large race *E. histolytica.*

RUIZ and ALFARO (16) were the first to report an infection of *D. fragilis* in Costa Rica. They emphasized the importance of the permanently stained fecal smear for final identification. In the present study, the examination of stained slides prepared from 62 specimens preserved in PVA-fixative revealed 23 per cent positive for *D. fragilis* (Table 3). We believe this may be among the highest rates reported anywhere to date. As evidenced in other field investigations by CDC (4, 20), stool specimens, satisfactorily preserved and examined by permanently stained smears, may prove *D. fragilis* to be a common parasite in a community. The primary purpose of PVA-fixative is to preserve the fragile trophozoites, and thus permit their identification in permanently stained films. Since *D. fragilis* is known to exist only as a trophozoite, the revealed prevalence of this organism is greatly affected by the preservation of the stool specimens.

As previously noted by many other investigators, a clear-cut age resistance is shown in the present study with *G. lamblia* (Table 2). A marked decline also occurs with *D. fragilis.* This is the first time, to our knowledge, that the development of age resistance is suggested in relationship to *D. fragilis.* The highest age prevalence for *D. fragilis* was in the 5 to 9 age group followed by a distinct decline; whereas, the other species of amebae, with the exception of *I. butschlii,* reached their peaks in the adult age group. The high prevalence of *Trichomonas hominis* in all age groups indicates that conditions exist throughout life for direct fecal spread from person to person thus permitting the transmission of organisms that exist only as trophozoites. This would tend to emphasize the validity of age resistance developing against *D. fragilis* as well as *G. lamblia.*

Previous surveys in Costa Rica, reporting on the prevalence of intestinal protozoa, involved a majority of children, principally from the province of San José (12, 14, 17). The present survey, which on the whole revealed higher prevalence rates, is the first strictly rural study to be reported. The opportunity for infection and reinfection must be very great in Volcán community. Prior to the present survey, there had been a number of similar occasions when the inhabitants of the area had the opportunity to receive anthelmintic therapy, even so, 84 per cent were infected with hookworm, and 44 per cent with *A. lumbricoides* (Table 3).

In comparison with the other surveys reported from Costa Rica, the

high prevalence of parasites (particularly the protozoa) revealed by the present study, can be attributed possibly to three factors: (a) all of the persons examined were residents of a rural area; (b) the inclusion of more adults, and (c) the examination of stained fecal smears after preservation in PVA-fixative. In regard to the third factor, the PVA-fixative technique significantly increased ($P < 0.02$) the recovery of all of the protozoa (except *B. coli* which were too few) over that revealed by the FE concentration procedures (Table 3). For example, *E. histolytica* was increased by 57 per cent and *E. hartmanni* by 200 per cent. As demonstrated before by GOLDMAN and BROOKE (8), this effectiveness of the PVA-fixative technique was due primarily to the finding of trophozoites missed by the concentration procedure. The PVA-fixative technique is less effective in revealing protozoan cysts, and is of no value in the examination of specimens for helminths. Since these two procedures supplement one another by recovering different stages of the intestinal parasites, they constitute a good laboratory regimen for parasitological examination (1). Although the PVA-fixative technique was initially developed to assist public health laboratories in the collection of specimens for the diagnosis of amebiasis, it has since been similarly utilized in the private clinic and hospital (2, 13).

SUMMARY

Single stool specimens were collected from 134 residents of Volcán de Buenos Aires, Costa Rica. After brief microscopic examinations in a field laboratory, 108 of the specimens that were sufficient in quantity were preserved in PVA fixative, and/or 10 per cent formalin. Later, the portions in PVA fixative were stained by the trichrome method, and those in formalin were concentrated by the formalin-ether sedimentation technique. The examinations revealed 5 species of helminths and 10 species of protozoa.

The effectiveness of the combination of permanently stained film and concentration technique for parasitological examinations was demonstrated by the results obtained on 62 specimens subjected to both procedures. All of the 62 were positive for intestinal parasites, 36 per cent infected with *E. histolytica* and 45 per cent with *E. hartmanni* (small race *E. histolytica*). Likewise, high prevalences of the common intestinal nematodes were obtained.

The examination of permanently stained films from specimens preserved in PVA fixative increased the percentage of recovery of *E. histolytica* from 28 per cent (by concentration) to 36 per cent. The ability of PVA fixative to preserve trophozoites resulted in the recovery of 14 (23 per cent) infections with *D. fragilis* in the 62 individulas.

The highest prevalence rates of most of the protozoa were in the adult age group, and the highest for the helminths in the children. *D. fragilis* reached a peak in the 5 to 9 year age group and declined markedly thereafter. To our knowledge, this is the first reported suggestion of the development of age resistance to *D. fragilis*.

RESUMEN

Se colectaron 134 muestras de heces de un número igual de personas residentes en Volcán de Buenos Aires, Costa Rica. Después de un breve examen microscópico de las mismas en un laboratorio de campo, 108 de los especímenes fueron conservados en fijador PVA y/o en formalina al 10 por ciento. Luego, las muestras en PVA fueron teñidas por un método tricrómico y las mantenidas en formalina fueron concentradas por la técnica de sedimentación de eterformol. El examen reveló 5 especies de helmintos y 10 especies de protozoarios.

La efectividad de la combinación de ambas técnicas (tinción y concentración) quedó demostrada con los resultados obtenidos con 62 muestras sometidas a ambos procedimientos.

Todas las 62 fueron positivas para parásitos intestinales y 36 por ciento resultaron infectadas con *E. histolytica* y 45 por ciento con *E. hartmanni* (raza pequeña de *E. histolytica*). Asimismo, se reveló una alta incidencia de los nemátodos intestinales comunes.

El examen de preparaciones teñidas de especímenes conservados en PVA, aumentó el porcentaje de *E. histolytica* de 28 por ciento (por concentración) a 36 por ciento.

La capacidad del fijador PVA para preservar trofozoitos permitió el hallazgo de 14 (23 por ciento) infecciones con *D. fragilis* en los 62 individuos.

El mayor índice de incidencia para la mayoría de los protozoarios apareció en el grupo de los adultos, y, para los helmintos, en los niños. *D. fragilis* fue más frecuente en el grupo de 5 a 9 años presentando, a partir de esa edad, un marcado declinio.

Creemos que esta es la primera vez que se sugiere un desarrollo de resistencia a *D. fragilis* en relación con la edad.

REFERENCES

1. BROOKE, M. M.
   1958. *Amebiasis: - methods in laboratory diagnosis.* Communicable Disease Center, Dept. of Public Health, Education & Welfare, Public Health Service, Washington, iii + 67 pp.

2. BROOKE, M. M.
   1960. PVA-fixative technique in the laboratory confirmation of amoebiasis. *Triangle,* 4(8): 326-335.

3. BROOKE, M. M., & M. GOLDMAN
   1949. Polyvinyl alcohol-fixative as a preservative and adhesive for protozoa in dysenteric stools and other liquid materials. *J. Lab. Clin. Med.,* 34(11): 1554-1560.

4. BROOKE, M. M., D. M. MELVIN, R. SAPPENFIELD, F. PAYNE, F. R. N. CARTER, A. C. OFFUTT, & W. W. FRYE
   1955. Studies of a water-borne outbreak of amebiasis, South Bend. Indiana. III. Investigation of family contacts. *Am. J. Hyg.,* 62(3): 214-226.

5. BURROWS, R. B.
1957. *Entamoeba hartmanni. Am. J. Hyg.,* 65: 172-188.

6. BURROWS, R. B.
1959. Morphological differentiation of *Entamoeba hartmanni* and *E. polecki* from *E. histolytica. Am. J. Trop. Med. Hyg.,* 8: 583-589.

7. FREEDMAN, L., & R. ELSDON-DEW
1958. Size variation in *Entamoeba histolytica. Nature,* 181: 433-434.

8. GOLDMAN, M., & M. M. BROOKE
1953. Protozoans in stools unpreserved and preserved in PVA-fixative. *Public Health Rep.,* 68(7): 703-706.

9. GOLDMAN, M., R. K. CARVER & N. N. GLESON
1960. Antigenic analysis of *Entamoeba histolytica* by means of fluorescent antibody. II. *E. histolytica* and *E. hartmanni. Exp. Parasitol.,* 10(3): 366-388.

10. HOARE, C. A
1950. *Handbook of medical protozoology.* Williams and Wilkins Co., Baltimore, Md. xv + 334 pp.

11. HOARE, C. A.
1952. The commensal phase of *Entamoeba histolytica. Exp. Parasitol.,* 1(4): 411-427.

12. JIMÉNEZ-QUIRÓS, O., R. R. BRENES, & P. L. VIETO
1958. Parasitosis intestinal en el universitario costarricense. I. Helmintiasis. *Rev. Biol Trop.,* 6(1): 113-122.

13. JUNIPER, K., JR.
1962. Acute amebic colitis. *Am. J. Med.,* 33(3): 377-386.

14. LIZANO, CECILIA, & J. DE ABATE
1953. Incidencia de parásitos intestinales en los niños de la Sección de Pediatría del Hospital San Juan de Dios. *Rev. Biol. Trop.,* 1(2): 223-233.

15. RITCHIE, L. G.
1948. An ether sedimentation technique for routine stool examinations. *Bull. U. S. Army Med. Dept.,* 8: 326.

16. RUIZ, A., & M. ALFARO
1958. *Dientamoeba fragilis* en Costa Rica. *Rev. Biol. Trop.,* 6(2): 201-203.

17. RUIZ, A., & CECILIA LIZANO
1954. Parásitos intestinales en niños. Estudio comparativo de los métodos diagnósticos usados. *Rev. Biol. Trop.,* 2(1): 29-36.

18. SHAFFER, J. G., W. H. SHLAES, F. STEIGMANN, P. CONNER, A. STAHL & H. SCHNEIDER
1958. Small race of *Entamoeba histolytica. Gastroenterology,* 34(6): 981-995.

19. VAN STEENIS, P. B.

1957. The problem of minuta forms in amoebic dysentery and amoebiasis. *Documenta Med. Georgr. Trop.,* 9: 325-330.

20. WEINER, D., M. M. BROOKE & A. WITKOW

1959. Investigation of parasitic infections in the central area of Philadelphia. *Am. J. Trop. Med. Hyg.,* 8(6): 625-629.

21. WHEATLEY, W. B.

1951. A rapid staining procedure of intestinal amoebae and flagellates. *Am. J. Clin. Path.,* 21: 990-991.