STUDIES ON SCHISTOSOMIASIS MANSONI IN PUERTO RICO *

II. THE EPIDEMIOLOGY AND GEOGRAPHICAL DISTRIBUTION OF SCHISTOSOMIASIS MANSONI IN PUERTO RICO

2. A Survey of Intestinal Parasites in Endemic Schistosomiasis Areas in Puerto Rico

By ERNEST CARROLL FAUST, WILLIAM A. HOFFMAN, CHARLES A. JONES and JOSÉ L. JANER.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, La., and from the School of Tropical Medicine of the University of Puerto Rico, under the auspices of Columbia University.

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INTRODUCTION

In attempts to locate the incidence of *Schistosoma mansoni* infection in representative areas of Puerto Rico a supply of fresh fecal specimens was obtained for examination. In addition to *S. mansoni* these samples were carefully searched for

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protozoa and all helminth eggs and larvae. Since no similarly comprehensive parasitological survey has previously been made on the Island, the data are valuable as a present-day index of the several parasites in the different localities studied, and, in addition, furnish possible evidence of epidemiological and geographical correlations with schistosomiasis itself.

HISTORICAL

The earliest scientific studies on parasitic infections in Puerto Rico were carried out by the Puerto Rico Anemia Commission [1]. In the 1904 report of this body there is tabulated a list of the various parasites encountered in routine smear examinations of clinical hookworm patients from the Utuado area. The series included 4,482 cases, all of whom were positive for hookworm by microscopic examination (100 per cent). The other infections recorded (in order of their frequency) were as follows: *Ascaris,* 1,408 (31.6 per cent); *Trichocephalus,* 326 (7.3 per cent); *Strongyloides,* 36 (0.8 per cent); *Schistosoma mansoni,* 21 (0.46 per cent); *Balantidium coli,* 14 (0.3 per cent); *"Ameba coli",* 3; *Enterobius* 3; *Taenia saginata,* 3; *T. solium,* 2; *Fasciola hepatica,* 2: *"Tyroglyphus longior",* 2; *"Diplogonoporus grandis",* 1, and *"Ascaris canis",* 1. It is evident that most of the protozoa were missed because at that time their encysted stages were inadequately known and appreciated. Furthermore, the incidence of both protozoan and helminthic infections would have been enhanced by concentration methods such as were used in the present study. However, since that day no similarly large group from a localized area in Puerto Rico has been so carefully studied as was the Utuado group, and some of the rarer helminth infections have not again been reported from the Island. Subsequent work by the Anemia Commission and its successors was devoted almost exclusively to the study of hookworm disease. The literature on this subject has been definitely enriched by the investigations of Ashford and his colleagues [2, 3], Cort, Payne and Riley [4], Hill and Earle [5], Hill [6], Payne [7], Howard [8], Daengsvang [9], and others. Howard (l.c.)[8], states that hookworm disease was introduced into Puerto Rico in 1530 and became so extensive in its distribution that it affected 90 per cent of the population, most of whom had a heavy infection. Hill and Earle (l.c.)[5]

found that 25 per cent of the positive hookworm cases harbored *Ancylostoma duodenale,* although 99 per cent of all the worms which they recovered were *Necators.* The *Necators* were of African origin, while the ancylostomes probably came from Spain. Credit for the first finding of *Ancylostoma* in Puerto Rico belongs to Dr. Pedro Gutiérrez Igaravídez (1907). Hill [6] found that the hookworm burden varied from an average of 200 persons for coastal, to 500, for the hill regions; that a child of four years had already acquired a burden of 100 worms and that males were more heavily infected than females.

Almost at the same time that the Anemia Commission came into existence, González Martínez [10] discovered *Schistosoma mansoni* in Mayagüez on the west coast, and for years devoted time and effort in studying this disease (See section I, this study) [10a]. More recently Hoffman [11] has confirmed and added to González Martínez's biological data.

In 1930 Serra [12] reported the examination of 2,200 fecal samples from the south of Puerto Rico, of which 2,000 were from urban and 200 from rural populations approximately equally divided between males and females. Data on helminths only were presented. At the present time, George W. Bachman has nearly completed an intensive five-year survey of the helminth infections of workers on an estate near Dorado. The data are as yet unpublished.

Incidental reports on rare intestinal helminths endemic in Puerto Rico have appeared from time to time in the literature. In 1925 Martínez Alvarez [13] published a record of *Diphyllobothrium latum* from an adult female, a native of Mayagüez. The same year Hill and Sánchez [14] reported the first three cases of *Hymenolepis nana,* from three boys in an orphan asylum in Hatillo. In 1928 Hoffman [15] recorded the presence of *Dipylidium caninum* in a native child, and in 1931 a case of human gapeworm (*Syngamus*) [16] infection. A second case of syngamosis was reported by this same investigator the following year [16a].

As previously indicated, the Anemia Commission [1] listed from their Utuado series fourteen cases of *Balantidium* infection and three cases of *"Ameba coli".* There are apparently no survey records of protozoan infections from the days of the Anemia Commission until 1921, when Hegner [17]

published a brief statement based on an examination of 83 individuals (69 from San Juan and 14 from Quebradillas). In this small series single examinations showed the following incidence of infections: *Endamoeba histolytica,* 12.0 per cent; *E. coli,* 36.1 per cent; *Endolimax nana,* 7.2 per cent; *Iodamoeba bütschlii,* 6.0 per cent; *Giardia lamblia,* 9.6 per cent; *Trichomonas hominis,* 3.6 per cent; and *Chilomastix mesnili,* 3.6 per cent. In 1928 Costa Mandry and Marín [18] called attention to the relatively high incidence of *E. histolytica* infection in Puerto Rico and the extreme rarity of recognized cases of clinical amebic dysentery on the Island. Marín's attempt [19] to infect kittens with cysts from carrier cases met with failure. Recently Poindexter [20] published his studies based on stools from 564 individuals, in which microscopic and culture methods were utilized in an attempt to determine the more satisfactory technic for diagnosis. The results were as follows: *E. histolytica* direct smear examination, 11.7 per cent; culture, 10.1 per cent, including 0.8 per cent not found by direct smear, and *E. coli,* 38.1 per cent and 17.2 per cent, respectively, including 7.9 per cent not recovered from direct smear technic. The combined net positives by both technics showed 12.5 per cent for *E. histolytica* and 45.0 per cent for *E. coli.* Other protozoa were found incidentally, including *E. nana, Iodamoeba, Giardia, Chilomastix, Trichomonas* and *Embadomonas intestinalis.* Pooled cultures of *E. histolytica* were used to inoculate eleven kittens by the intracecal method. Ten of these (88.8 per cent) were positive on the 7th day. Of five puppies similarly inoculated, three became positive on the 7th, 8th and 13th days, respectively. The Puertorrican strain in the experimental host on a carbohydrate diet showed less pathogenicity than United States strains on a high protein diet. The literature also includes a report by Serra [21] on a fatal case of *Balantidium coli* infection in a 6-year old native female.

Taken as a whole, the combined studies on protozoan and helminth infections in Puerto Rico are valuable beginnings, but with the exception of the hookworm data, are not sufficiently comprehensive to be used as indices of these parasitoses on the Island.

THE PRESENT SURVEY

Material and technic.—Through the courtesy and coöperation of insular and local health officers and local physicians some 1,200 fresh fecal samples were obtained from representative non-hospitalized groups of the population. The areas surveyed included (1) an extensive rural group in the region lying around Trujillo Alto, and between Trujillo Alto, Sabana Llana and Río Piedras, to the east of San Juan; (2) persons in and near Caguas, an inland town central east in position, north of the east-west mountain range; (3) Guayama, town and estate populations, in the southeast portion of the Island; (4) Utuado, two inland country districts along mountain streams in the northwest portion of the Island, and (5) Mayagüez, suburban population, on the west coast. While the material was primarily obtained in order to determine the *Schistosoma mansoni* index of these areas, it provided an opportunity to survey these groups for all of the intestinal protozoan and helminthic infections of the persons whose specimens were examined.

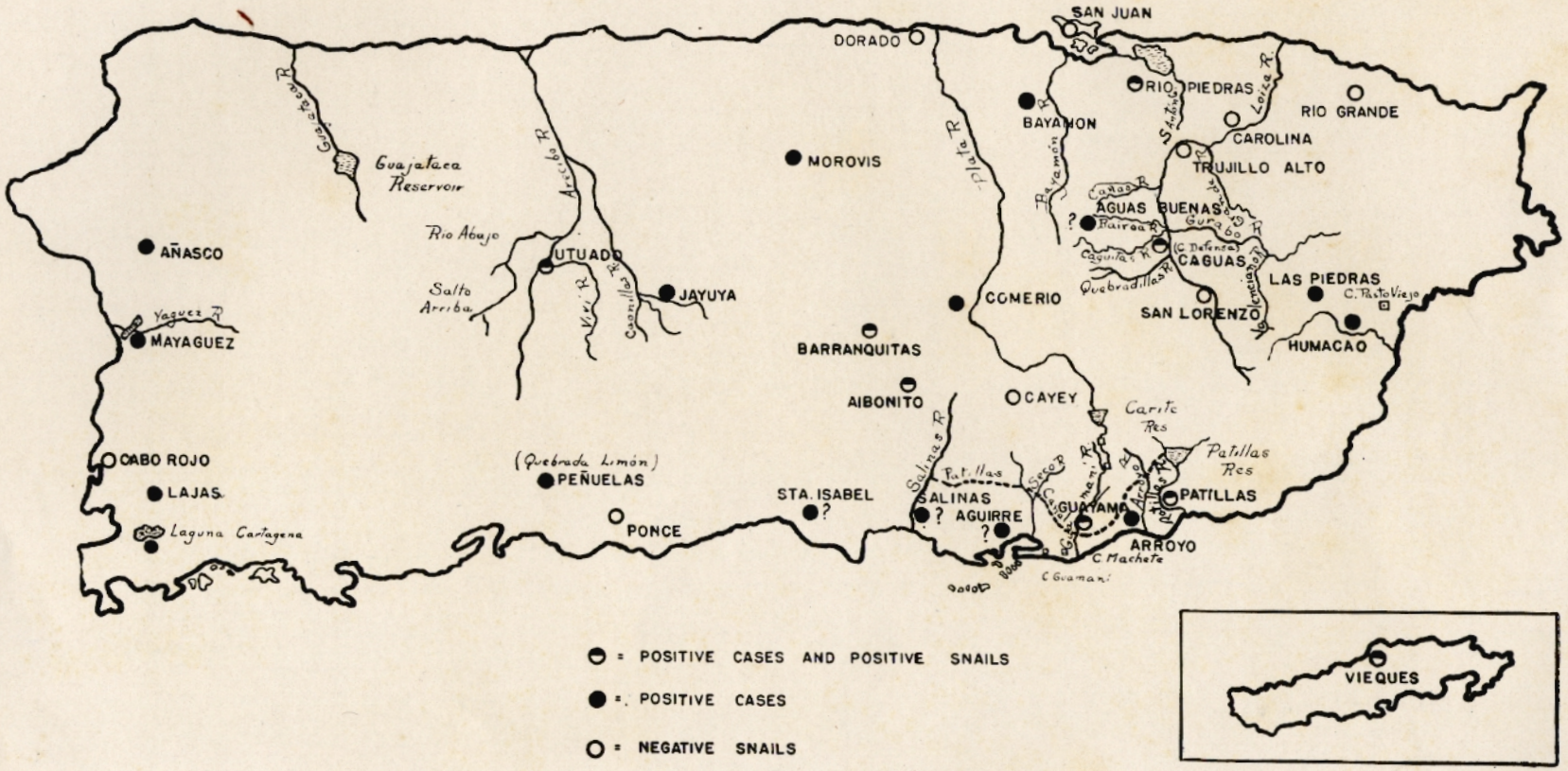
The fecal specimens were obtained fresh from the individuals, whose name, sex and age were written on the container or on accompanying lists. Groups of specimens were brought the same day by special conveyance to the Laboratory of Medical Zoölogy of the School of Tropical Medicine in San Juan. Examinations were ordinarily made within twenty-four hours after the specimen had been passed, but in about twenty-five per cent of the cases the material had to be kept in the electric refrigerator for a few days until opportunity for examination was afforded.

The examination was made on covered films of the specimen, both in physiological saline suspension and in preparations stained with Donaldson's iodine. This was always supplemented by an examination of iodine-stained films of centrifugalized concentrates of the material. The diagnoses were always made by the writers themselves, frequently after conference with one another on a questionable cyst or egg. All specimens which were too small to concentrate, those which had dried up, or had fermented, as well as those for which adequate data were not obtained, have been excluded from the survey data. It is believed, therefore, that the diagnoses are reasonably accurate and representative of the

populations surveyed, although three consecutive examinations from each person would undoubtedly have yielded a higher series of positives. Altogether 1,003 examinations from a similar number of persons are included in this report.

The Trujillo Alto–Sabana Llana–Río Piedras area.—The specimens from this area were obtained from the following sources: (1) Trujillo Alto village school, including 148 persons, ranging in age from 4 to 17 years; (2) six rural schools (Carraizo Bajo, Cuevas, Dos Bocas, Aguayo, Ida al Infierno and Quebrada Negrito), within a 10-kilometer radius of Trujillo Alto, including 283 persons, ranging in age from 6 to 16 years, and (3) a rural population in a triangular area between Trujillo Alto, Sabana Llana and Río Piedras, consisting of 102 persons from 2 to 58 years of age. Of this last group 24 individuals, all over 25 years of age, were not included in the general tables, because they were not comparable to age groups in the other units examined. There were altogether 533 specimens diagnosed, of which 509 are included in the summaries.

This area is essentially a rural one, for the most part one of hills and small valleys, with small, relatively rapid streams. It lies between the Loíza and Piedras drainage systems. Except in the village of Trujillo Alto the homes are mostly isolated. An analysis of the infections found indicates that, with the exception of *Schistosoma mansoni,* the three groups are readily comparable, and for that reason the findings have been pooled. As regards *S. mansoni,* only one case (out of 431) was found in the village and rural school in the Loíza drainage area, whereas 12 cases (out of 102) were diagnosed from the portion of the area toward Río Piedras and Sabana Llana. The incidence of this combined series by age group is shown separately for males and females (Tables I and II). The combined average percentages of infection for both males (253 individuals) and females (256 individuals) is as follows: *Endamoeba histolytica,* 17.5; *E. coli,* 36.5; *E. nana,* 21.8; *Iodamoeba,* 5.7; *Giardia,* 20.0; *Chilomastix,* 0.6; *Ascaris,* 7.6; *Necator* and *Ancylostoma,* 40.8; *Trichocephalus,* 48.5; *Strongyloides,* 6.3; *Schistosoma mansoni,* 2.2; positive, 84.1 per cent. *Enterobius* was diagnosed twice and *Fasciola hepatica* once from the Trujillo Alto village school; elsewhere these forms were not recovered. No tapeworm infection was diagnosed from this series.


SAN JUAN
DORADO
RIO PIEDRAS
RIO GRANDE
BAYAMON
CAROLINA
TRUJILLO ALTO
AGUAS BUENAS
CAGUAS
LAS PIEDRAS
SAN LORENZO
HUMACAO
MOROVIS
COMERIO
BARRANQUITAS
AIBONITO
CAYEY
JAYUYA
UTUADO
ARECIBO
AÑASCO
MAYAGUEZ
CABO ROJO
LAJAS
Laguna Cartagena
(Quebrada Limón)
PEÑUELAS
PONCE
STA. ISABEL
SALINAS
AGUIRRE
GUAYAMA
ARROYO
PATILLAS
Guajataca Reservoir
Río Abajo
Salto Arriba
Carite Res
Patillas Res
C. Machete
C. Guamani
= POSITIVE CASES AND POSITIVE SNAILS
= POSITIVE CASES
= NEGATIVE SNAILS
VIEQUES

In order to obtain a more definite estimate of the infection-trend in the pre-school and school age groups, these data were computed separately, both for males and females. They are presented in Table III.

On the whole, the series, as shown in these three tables, provides the following information: (1) A higher percentage of males is positive for each parasite, although this is not invariably the case; (2) males appear to acquire *E. histolytica* and hookworms earlier than do females; (3) in later years the female incidence rises, so that the average percentage of positives is essentially the same (males, 84.2; females, 84.0). While the tables do not evaluate the amount of infection for any particular individual (*i. e.,* the *parasite burden*), it may be said that in no single case in the series was there evidence of an overwhelming clinical infection with any particular parasite, and few persons had a combined parasite burden of clinical significance.

The Caguas area.—The 88 specimens examined from this area were obtained from two principal sources, the Bureau of Health (*Sanidad*) from the town itself and a colonia in Barrio Bairoa, just to the north of Caguas along a small stream. Males and females were almost equal in numbers (45 and 43, respectively). Since the series is small the incidence by age groups is not especially significant, but the averages are possibly valuable for comparison. The data are recorded in Table IV. The average percentage incidence for males and females is as follows: *E. histolytica,* 15.9; *E. coli,* 35.2; *E. nana,* 27.3; *Iodamoeba,* 3.4; *Giardia,* 13.6; *Ascaris,* 12.4; *Necator,* 36.1; *Trichocephalus,* 65.9; *Strongyloides,* 1.1; *Schistosoma mansoni,* 30.7; positive, 87.6 per cent. No other parasites were encountered in this series.

The Guayama area.—A total of 240 specimens, statistically usable, were obtained from persons in this area. They were secured from three principal sources: (1) the Bureau of Health (*Sanidad*); Central Machete, on the coast immediately south of Guayama, and (3) Colonia Vives, one of the holdings of Central Machete, between the Central Machete and the southern border of Guayama. All three subgroups of the series are representative of the age-groups of the populations surveyed. The male members of the series constitute about two-fifths (96 persons) and the females, the remainder (144

persons). As in the Trujillo Alto area there is little difference in the three subgroups of this series except for *Schistosoma* infection. Six cases (8.1 per cent) were diagnosed from the *Sanidad;* only one (1.0 per cent) from Colonia Vives. The results of examination of this series are presented in Table V. The combined averages for both males and females are as follows: *E. histolytica,* 12.5; *E. coli,* 30.8; *E. nana,* 8.3; *Iodamoeba,* 0.4; *Giardia,* 9.1; *Chilomastix,* 1.7; *Ascaris,* 11.7; *Necator,* 21.3; *Trichocephalus,* 32.9; *Strongyloides,* 3.8; *Schistosoma mansoni,* 17.9; positive 72.75 per cent. *Hymenolepis nana* was encountered once (Guayama *Sanidad*) and *Enterobius* once (Central Machete). On the whole, infection in the male population is considerably more common than in the female in this locality.

The Utuado area.—A total of 78 persons, residing along the banks of Río Viví and Río Abajo, near Utuado, were examined. Of this series 33 were males and 45 females. In the protozoan species of parasites found the females showed a higher incidence than did the males. The same is true for *Ascaris,* but *Necator, Trichocephalus* and *Schistosoma* are more commonly male infections. It is interesting to note that *Strongyloides* was not diagnosed in this series. The results are summarized in Table VI. The combined average percentages for males and females is as follows: *E. histolytica,* 10.3; *E. coli,* 30.8; *E. nana,* 7.7; *Iodamoeba,* 2.5; *Giardia,* 6.4; *Ascaris,* 20.6; *Necator,* 25.6; *Trichocephalus,* 28.4; *Schistosoma mansoni,* 41.0; positive 82.1 per cent. Parasites unrecorded were not observed in this series.

The Mayagüez area.—A total of 88 persons, mostly residing in the outskirts of the town near the slaughter-house, were examined. The series consisted of 30 males and 58 females. Except for *Trichocephalus,* the percentage incidence is comparatively low for Puerto Rico. The data are presented in Table VII. The combined average percentages for males and females are as follows: *E. histolytica,* 6.8; *E. coli,* 30.7; *E. nana,* 5.6; *Giardia,* 2.3; *Ascaris,* 6.8; *Necator,* 17.0; *Trichocephalus,* 45.4; *Strongyloides,* 4.5; *Schistosoma mansoni,* 10.3; positive, 75.0 per cent. Other parasites were not encountered in this series.

Comparison of the five areas surveyed.—It has been stated previously that, on the whole, the average female incidence to parasitic infections in the Puerto Rico areas investigated

is lower than that of the males, and that males show evidence of somewhat earlier exposure. As pointed out in a previous communication in this study (Hoffman and Faust [22]) the several areas considered are situated in different topographic and climatic *milieux*. The Trujillo Alto area is in the northeast portion of the Island, in a hilly region of abundant rainfall. Caguas and Utuado are inter-mountain situations on the north side of the divide, and are also supplied with abundant precipitation. Guayama is on the southeast coast, where rainfall is slight and irrigation is extensive. Mayagüez is on the west coast and rainfall is moderate. These conditioning factors of climate might be expected to play an important rôle in the amount of infection in each particular area. In addition, the relative grouping or isolation of human habitations also requires consideration. Furthermore, infections such as hookworm and *Ascaris,* have been the objects of therapeutic and preventive attack for many years in Puerto Rico. On the other hand, the amebic infections, *Giardia, Trichocephalus* and *Strongyloides,* may properly be considered as having been unaffected by public health measures, save in so far as generalized hygiene may have reduced the amount of their incidence. The data for each species of parasite are presented in condensed form in Table VIII. The infections will now be considered *seriatim.*

Endamoeba histolytica is most common in the Trujillo Alto district and is lowest in incidence in Mayagüez. In the former area this infection is significantly heavier in the males; in Mayagüez, as well as in Caguas and Utuado, it is definitely more prevalent in the females. Since the Guayama figures lie in the middle of the series it is evident either that irrigation, as well as rainfall, may contribute to the dissemination of this ameba, or that neither is an essential factor. In view of the fact that the incidence in Peiping, China (Kessel and Svensson [27]), a very dry area, is higher than it is in the Trujillo Alto district, it appears likely that other factors, such as unsanitary disposal of feces, food handling, etc., are more essential than moisture *per se.* The low incidence in Mayagüez, where better than average sanitary conditions prevail, supports this view. *E. coli* parallels *E. histolytica* throughout the series, but at a considerably higher level. This is what might be expected on the basis of other surveys. As pointed out by Faust [23] *E. coli* infection may constitute a

relatively accurate gauge for *E. histolytica* in a given locality. The incidence of *E. nana* is much less regular, while *Iodamoeba* is very erratic.

Giardia infection is primarily one of early childhood. In the Puerto Rican population it reaches its peak by the tenth year, after which it shows an irregular decline. However, isolated cases up to 60 years of age have been found in this series. Males are almost always more commonly infected than females. In the population surveyed this species more or less parallels *E. histolytica.* This might be interpreted as possibly indicating a common epidemiology. The incidence of *Chilomastix* is, indeed, low; much lower than that in the city population examined by Hegner (l.c.)[17]. *Trichomonas hominis* was never once encountered in all of the cases examined, although *Bodo caudatus* was found three times.

The incidence of *Ascaris* is consistently low, except in the rural Utuado district. Treatment of cases diagnosed by the health authorities is undoubtedly an important factor contributing to this exhibit. Likewise, the incidence of the hookworm is remarkably low, considering the estimate of 90 per cent infection from these localities when the Anemia Commission undertook its work at the beginning of the present century. Furthermore, no single case of heavy hookworm infection was encountered in the survey, indicating that the persons infected are not carrying a heavy worm burden. While egg counts or worm counts have not been made in this survey, experience in examining feces makes it possible for us to estimate conservatively that less than 10 per cent of the positives in this series harbored as many as 200 worms and that none harbored 500 worms or more. As regards *Trichocephalus* the incidence is high but the average worm burden relatively low. Nearly fifty per cent (44.6) of the persons examined were positive for this infection. Possibly a small percentage of the trichocephalids acquired had been eliminated by hookworm therapeusis, but the amount could not be very significant, since *Trichocephalus* is not appreciably affected by the usual anthelmintic procedures. The infection rates for this worm may, therefore, be regarded as nearly normal for an untreated population living under conditions prevailing in Puerto Rico. The *Strongyloides* rates in the series are low for a subtropical area where hookworm is a common infection. Any anthelmintic procedures used on the

population would not have affected this rate, and specific therapeusis (gentian violet) has not been carried out for this infection in Puerto Rico except on relatively few private and clinic patients. The incidence is, therefore, a natural one for the population. We are able to offer no explanation for the average low rate and the complete absence of this infection in the Utuado district, but this survey is supported by the experience of physicians in Puerto Rico.

As we have previously stated, *Schistosoma mansoni* is controlled by factors which limit its distribution, even in any particular district. One particular village or group of the population may have a high endemic rate, as, for example, Barrio Bairoa at Caguas, Colonia Vives at Guayama and Río Viví at Utuado, while other nearby groups may be only lightly parasitized or completely free of the disease. Thus the Guayama totals, particularly because of the Central Machete group, are unusually low for the Guayama district. Persons in the Central Machete itself are mill operatives, and their families are not in contact with the irrigation ditches, which are the principal source of the infection. The average for all groups (12.2 per cent) is probably a fair estimate of the infection in the population of the Island, using the single fecal specimen as a diagnostic unit.

In endemic areas exposure to *Schistosoma mansoni* infection begins early in childhood (2 years or less) and continues throughout life, particularly in the male populations. Infections can be most readily diagnosed in children or others who have recently acquired the infection, since in chronic cases and those subject to reinfection, fibrosis of the intestinal wall following lodgment of the eggs in its tissues prevents the egg from being regularly expelled into the lumen of the bowel and passed in the feces. Hence the diagnosed incidence for children is probably more accurate than for adults. Likewise, larger samples, comminuted, strained and sedimented (or centrifugalized), more often provide positive diagnoses than do smaller specimens, such as those utilized in this survey.

In addition to the data already considered, the evaluation of the total parasite incidence in the population is of considerable importance, particularly for purposes of comparison with other areas. This is known as the *parasite index,* and is obtained by dividing the total number of persons examined

by the total number of parasite findings in the group. It is the average of parasite species per person. These indices are included at the bottom of Table VIII. It is highest in the Caguas group (2.43), next in the Trujillo Alto area (2.09), and lowest at Mayagüez (1.29), with Utuado (1.73) and Guayama (1.50) intermediate in position. The average for all groups is 1.88. Usually the index is higher in males than in females, although the reverse is true for Caguas and Mayagüez.

Comparison with Surveys Elsewhere.—Comprehensive survey figures, in which both protozoa and helminths have been included, are available for very few localities, but data for either protozoa or helminths separately are now a matter of record for certain representative regions in various parts of the world. For the protozoa, surveys of particular value are those from England (Dobell [24,24a]); from China (Faust and Wassall [25], Faust [26], Kessel and Svensson [27], Faust [28] and Tao [29]); from Java (Brug [30]); from Sweden and Finland (Svensson [31]); from Russia (Philiptschenko [32]); from Virginia (Faust [33]); from Tennessee (Meleney [34]); from New Orleans (Faust [35]); from Los Angeles (Kessel [36]); from Pennsylvania (Arnett, Wenrich and Stabler [37] and Wenrich, Stabler and Arnett [37a]); from Panama (Faust [35]); from Colombia (Kofoid *et al.* [38]), and from Haiti (Williams, Wildman and Curtis [39], and Williams and Thomas [39a]).

In most of these surveys particular stress has been placed on the infective incidence for *Endamoeba histolytica.* Such information has been digested and for the most part summarized by Tao (l.c.)[29]. We are here particularly interested in the infections of native populations of the Western Hemisphere for comparison with our own data. Kofoid *et al.* (l.c.)[38] found 53.7 per cent infection with *E. histolytica* in Santa Marta, Colombia (average for patients, staff and servants, totalling 367 persons); 41.1 per cent for *E. coli;* 42.8 per cent for *E. nana;* 21.0 per cent for *Iodamoeba;* 1.4 per cent for *Dientamoeba fragilis;* 11.2 per cent for *Giardia;* 18.8 per cent for *Chilomastix;* 7.4 per cent for *Trichomonas hominis,* and 0.3 per cent for *Tricercomonas hominis.* Faust (l.c.)[35], in published data from Panama, reported considerable variation in positive cases of *E. histolytica* in separate urban and rural groups. In one rural community (Tuira River village) the incidence reached 90.9 per cent for *E. histolytica*