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A Survey of Intestinal Parasites in a Unit of U.S. Troops in Burma, with Comments on Methods for Detecting the Presence of Endamoeba histolytica in Stools

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A SURVEY OF INTESTINAL PARASITES IN A UNIT OF U. S. TROOPS IN BURMA, WITH COMMENTS ON METHODS FOR DETECTING THE PRESENCE OF ENDAMOEBA HISTOLYTICA IN STOOLS

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In September, 1944, the writer and one enlisted man were ordered to Burma from the Ninth Medical Service Detachment (Laboratory) to complete an intestinal parasite survey of the 1888th Engineering Aviation Battalion then engaged in constructing the air field two miles north of Myitkyina near the Irrawaddy River. Another team from the Ninth Medical Service Detachment(Laboratory) had surveyed a part of the unit in India several weeks earlier, and reported an incidence of *Endamoeba histolytica* of approximately 15 percent. The enlisted personnel of the organization, which was entirely colored, was drawn largely from southern states, though there was a liberal sprinkling of northern Negroes.

The examinations were made entirely by the saline and iodine smear method, as will be brought out in subsequent comments. Identifications of protozoa were made on either cysts or trophozoites, or both. A summary of the results follows:

Total stools examined	451
Total individuals examined	435
Individuals positive for *Endamoeba histolytica*	125
Endamoeba histolytica alone	76
Endamoeba histolytica and other intestinal protozoa	49
Percent positive for *Endamoeba histolytica*	28.79
Individuals positive for *Endamoeba coli*	48
Percent positive for *Endamoeba coli*	11.04
Individuals positive for *Iodamoeba butschlii*	31
Percent positive for *Iodamoeba butschlii*	7.13
Individuals positive for *Endolimax nana*	47
Percent positive for *Endolimax nana*	10.8
Individuals positive for *Dientamoeba fragilis*	1
Individuals positive for *Trichomonas hominis*	10
Individuals positive for *Enteromonas hominis*	4
Individuals positive for *Chilomastix mesnili*	10
Individuals positive for *Giardia lamblia*	2
Individuals positive for hookworm	1
Individuals positive for whipworm	1
Individuals positive for unidentified amoeba trophozoites	2
Individuals positive for intestinal protozoa	51.3
Percent positive for intestinal protozoa	51.3

The finding of 29 percent positive for *Endamoeba histolytica* in this outfit of United States troops was about three times the estimate of 9 percent sometimes set for the people of the United States

*Survey was made while the writer was on detached service as a Sanitary Corps Officer assigned to the Ninth Medical Service Detachment (Laboratory), Chabua, Assam, India.

as a whole. So high an incidence uncovered by the microscopic examination of but 1 stool from each subject, except for 16 mess attendants and cooks who submitted stools on 2 successive days, is surprising, since it is claimed by certain authorities that it is unsafe to pronounce an individual *Endamoeba histolytica*-free until negative smears are obtained on at least 6 successive days. It is not to be doubted that the true incidence in this case was much higher, although a second examination of 16 mess attendants' stools did not disclose further infections. Although this region of Burma is notorious for amoebic infection, there were 2 arguments against recent acquisition of the parasite: (1) The previously mentioned finding of a 15 percent incidence in another part of the outfit before its departure from India, and (2) statements of the medical officer (Captain Douglass) to the writer that there had come to his attention comparatively few intestinal disorders ascribable to *E. histolytica*. The incidences of *E. coli, I. butschlii, Endolimax nana, Trichomonas hominis, Enteromonas,* and *Chilomastix mesnili* were also comparatively high for single stool examination. The writer became thoroughly familiar with *Dientamoeba fragilis* while in the service, so it was quite unexpected that but one positive stool would be found even though practically all the stools were freshly passed. Worm eggs and larvae were searched for alertly, so the finding of 1 light hookworm and 1 light *Trichuris* infection indicated that the worm burdens of these troops were insignificant. This was decidedly not true of Burmese and Indian laborers whom the writer and his collaborators examined on various occasions.

The low incidence of 0.5 percent for *Giardia lamblia* deserves special comment. The reports of various surveys made in the United States and abroad vary considerably in respect to the incidence of this flagellate. Boeck and Stiles (1923) reported an incidence of 6.0 percent for troops with only home service and 5.5 percent for those who had seen foreign service. Other authors estimate that 12-15 percent of the population of the world in general harbors this parasite. It would be expected that a group which showed 51.3 percent positive for intestinal protozoa would have a higher than average incidence of *Giardia* also. The writer believes that the explanation lies in the fact that the battalion was on a regimen of 0.1 gm. atabrine per diem for suppressing malaria. It is well known that atabrine is a specific for *Giardia lamblia* in man. The writer cautiously confronted 1 of the 2 subjects who were positive for *Giardia* with the accusation that he was not taking his atabrine. He frankly admitted his delinquency in this regard.

As previously indicated ,the stool examinations were made by the well-known "smear method". A physiological saline preparation was made on one end of the slide and an iodine-stained preparation on the other. The staining agent for the latter had the following formula: iodine, 0.5 gm.; potassium iodine, 1 gm.; acetic acid (glacial), 1 cc.; distilled water, 100 cc. It should be made fresh every week.

The saline preparation is useful for (1) the detection and study of worm eggs and larvae. (2) trophozoites of flagellates, amoebae and ciliates, and (3) chromatoid bars in *Endamoeba histolytica* cysts. The iodine preparation is useful for rendering the nuclei of protozoan cysts visible for study and staining the glycogen formations. Ordinarily the chromatoid bars of *E. histolytica* are not so apparent in an iodine preparation as in a fresh smear. The staining solution diluted from 2 to 3 times with saline solution makes a better stain for the nuclei of trophozoites of *Endamoeba histolytica, Endamoeba coli,* and *Iodamoeba butschlii* than any solution containing dyes (such as Quensel's) which the writer has tested.

The success of the smear method depends to a considerable extent on the experience and visual acuity of the observer with the microscope. When the low-power of the microscope (X10 eye piece and 16 mm. objective) and a mechanical stage are used it doesn't take a competent microscopist long to scan an entire 25mm. square coverglass preparation in search of parasite stages. When the latter or simulacra are detected a switch to the high-dry or oil immersion objective is usually necessary for definite recognition of their nature.

In the I-B Theater *Endamoeba histolytica* was by far the most troublesome intestinal parasite and most of the fecal examinations made were for the purpose of establishing or excluding its presence in the bowel. *Blastocystis* in iodine is easily distinguished from *E. histolytica* cysts when the switch is made to the high power. *Iodamoeba butschlii* cysts have a sharply delimited glycogen body that is readily seen in the unstained preparation under the high-dry lens owing to its differential refraction and which, of course, stains mahogany-brown in iodine. The large karyosome of the nucleus in iodine preparations is a feature readily recognized by the experienced worker. *E. coli* cysts ordinarily present no problem on account of their large size, 8 nuclei when mature, and other peculiarities of the 2-nucleate and 4-nucleate stages, but occasionally they are puzzling. *E. coli* cysts were occasionally encountered in unstained smears with chromatoid bodies closely resembling the bars characteristic of *E. histolytica.* In a number of instances *E. coli* cysts with 4 or 2 nuclei were practically the only parasites found in the stool, even on 2 successive days. Sometimes in these cases the nuclei, glycogen formations, chromatoid bars, and cyst size so closely resembled the large race of *Endamoeba histolytica* that the writer was actually confounded until prolonged search revealed 1 or 2 8-nucleate cysts. *Endolimax nana* cysts are ordinarily characteristic and easily spotted, but the uninucleate cyst may frequently resemble the uninucleate cyst of the small race of *E. histolytica* in respect to size, nuclear structure, and characteristics of the glycogen. In fact, were other stages not also present accurate identification would not be possible. Cysts of the large and small races of *E. histolytica* were both encountered. In certain stools the nuclei of a large race stained but faintly in iodine, making necessary careful study under the oil immension lens.

The trophozoites of *Iodamoeba butschlii* generally present no problem in identification. Those of *Endolimax nana,* however, may so closely resemble those of the small race of *Endamoeba histolytica* in respect to shape and rapidity of streaming in tropical climates that it is impossible to tell them apart until they are stained in the diluted iodine solution. If the specimen is *E. histolytica* its characteristic nucleus can be observed, but *E. nana* nuclei will not show well. *Dientamoeba fragilis* usually presents a perfectly rounded appearance when the saline smear is first made, but in the tropics it soon protudes a broad pseudopod on one side with a more or less serrated margin. Thus when active it can be recognized as composed of 2 parts, viz., a ball of protoplasm and an attached apron of clearer protoplasm with a serrated border.

The trophozoites of the large race of *E. histolytica* are likely to be confused only with those of *E. coli.* Ordinarily *E. coli* is markedly more sluggish and contains more bacteria and debris, but in the heat of the tropics it may at times become very active and stream in the limax manner charactive of *E. histolytica.* Such ascribed characteristics of *E. histolytica* as the greenish hue, freedom from bacteria, invisibility of the unstained nucleus, and included erythrocytes are, in the writer's experience, unsafe to depend upon. Erythrocytes are practically never found in the cytoplasm of this amoeba from subjects not experiencing clinical colitis of some type or other, and they are often not present in specimens from dysenteric stools. When they are present, however, it is usually safe to assume that the amoeba concerned is *E. histolytica.*

The zinc sulphate-centrifugation method of separating *E. histolytica* cysts from stools was tested out by us on 50 stools some time before we left for Burma. Its disadvantages were as follows: (1) It was time consuming; (2) it did not reveal any *E. histolytica* infections we had not previously spotted by the smear method; (3) in the iodine the floated cysts of *E. histolytica* often presented a puzzling appearance with one side deeply indented and with indistinct, distorted nuclei; (4) the angle-centrifuge, which was the only centrifuge of small size available for us to take on the expedition, often failed to bring the cysts to the surface for looping off onto a slide. The method of zinc sulphate levitation without centrifugation had the disadvantage that often so much detritus rose to the surface that it interfered with both levitating and looping off the cysts.

SUMMARY

An intestinal parasite survey was made of 435 U. S. troops operating in India. The micsroscopic examination of one stool from each soldier by the smear method only revealed an incidence of *Endamoeba histolytica* of 29 percent, which was very much higher than that of any other intestinal protozoan. Only 2 cases of worm infestation were

uncovered. A discussion of practical problems arising during microscopic examination of fecal smears follows the parasite report.

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