THE PUERTO RICAN STRAIN OF ENDAMEBA HISTOLYTICA

COMPARISON OF THE DIAGNOSTIC VALUE OF DIRECT SMEAR EXAMINATION AND CULTIVATION WITH PATHOGENICITY TEST *

By HILDRUS A. POINDEXTER
From the Department of Bacteriology, Preventive Medicine and Public Health, School of Medicine, Howard University, Washington, D. C.

During the summer of 1932 the author, while in Puerto Rico, made the following investigations with the object of ascertaining diagnostic values by means of various test methods on the Puerto Rican strain of *Endameba histolytica.*

Five hundred and sixty-four separate stool examinations were made involving the same number of individuals, each specimen receiving only one examination. This consisted of one complete microscopic examination of a smear, both unstained and stained with iodine solution, and by examination after cultivation for 48 hours at 37.5°C.

Results of pathogenicity tests on the excysted *Endameba histolytica* from some patients are also included.

The medium was essentially the same as that previously used by Poindexter(1): the base consisted of slants of Boeck-Drobohlav egg medium and of liver infusion agar prepared according to the method of Cleveland and Sanders(2). The liquid part of the medium consisted of one part of fresh inactivated serum to 9 parts of Ringer's or Locke's solution. Rabbit serum was chiefly used, although small quantities of human or monkey serum were also utilized from time to time; the results from the three different sera were identical. Ringer's solution was used with the sera much more frequently than Locke's, because it retarded the rapid reproduction of a common contaminant, viz: a form of yeast, and a closely associated fungus of the genus *Monilia,* culturally and morphologically resembling *Monilia sitophila,* the growth of which Locke's solution appeared to encourage to the extent of its obscuring the endamebae and rendering identification difficult.

* This work was done at the School of Tropical Medicine, San Juan, Puerto Rico, under a special grant from the Central Education Board of the Rockefeller Foundation.
Received for publication April, 1933.

Endamoeba coli was the most common intestinal protozoan found. Of 564 specimens examined, 215 were identified by microscopic means. By cultural methods, *E. coli* was shown to be present in 45 of the 349 specimens which microscopic examination had declared negative, thus giving a total of 260 positives for *E. coli.* Of the 215 demonstrated by microscopic analysis 52 were found positive by culture.

E. histolytica was found positive in 66 of the 564 specimens examined by smear, four additional being found by culture among those negative by microscopic means. Fifty-three of the 66 positive were also demonstrated by culture.

In only one case—one showing clinical symptoms—submitted by a practicing physician, were active amebae encountered. The amebae were typical representatives of *E. histolytica,* both as to type of locomotion and the presence of red blood corpuscles. This stool was cultivated and the active endamebae were injected into a puppy.

Occasionally a specimen was examined which contained cysts of *Iodameba bütschlii* and *Endolimax nana,* neither of which was cultivated. Many of the fresher specimens contained active *Trichomonas intestinalis,* which grew readily in culture. A few specimens contained the cysts of *Giardia lamblia,* which in two cases excysted in culture and grew for 8 and 10 days respectively. Trophozoites were in one fresh specimen, and cysts of *Chilomastix mesnili* were occasionally seen, which in three cases excysted and grew for 11, 13, and 16 days respectively. *Embadomonas intestinalis* was observed in 2 cultures, but was not observed in the original smear examination before cultivation.

EXPERIMENTAL

Marín(3) reported the results of experiments with the Puerto Rican strain of endamebae which is indistinguishable morphologically from *E. histolytica.* His results seem to show that the endamebae were not pathogenic for kittens. Using the already accepted test animals for the pathogenicity of *E. histolytica*—kittens—and in accordance with the recent results of Faust(4)—puppies—we decided to test the pathogenicity of the vegetative endamebae obtained from cyst-containing specimens.

Cultures of *E. histolytica* which had excysted in tubes and

were actively motile were used to infect the animals. When the cultured endamebae were reproducing at the rate of 8 to 10 active trophozoites per high power field in 24 hours subcultures, the contents of several of these tubes were combined and 20 cc. of the suspension, while still at 37.5°C., were injected into the proximal part of the colon and distal part of the ileum. This was done by etherizing each kitten and passing a flexible rubber catheter to a depth of about 6½ inches. This was calculated to reach the cecum. A warm pipette containing 20 cc. of the active trophozoites was then connected with the outside end of the catheter and the animal being suspended by the tail, the material was permitted to run in by gravity. No pressure was used to force it into the colon. The etherized state of the animal and the large amount of the material employed gave us reason to believe that some of the material passed even beyond the ileocecal valve into the ileum. After all of the material had run in, the catheter was withdrawn and the rectum plugged with cotton while the animal was still under ether. The next day (24 hours later), the plug was removed by pulling the string which remained attached to it.

Eleven kittens were similarly infected, each animal receiving the trophozoites cultured from the cyst of a different individual. Three of the animals died, one 9, one 18, and one 21 days after the infection. On the 7th day active trophozoites were found in the stools of 10 kittens, and in 5 of these during the entire period of observation.* Active amebae could be demonstrated in the 3 kittens that died, up to the time of death and in the scrapings from the mucosa of the colon after death. These 3 kittens had a diarrheal, but only an occasionally dysenteric, stool.

Macroscopically only 2 of the 3 kittens that died showed the type of ulcerations usually observed in kittens dying of experimental amebiasis. In those 2, the lesions resembled those described by Rees(5) in kittens that were killed during the early diarrheal stage of the infection before actual dysentery began.

The microscopic sections of the colon, cecum and rectum showed areas of hyperemia, but only in one of the kittens

* The period of observation was interrupted on the 29th day of the experiment.

was definite invasion of the mucosa by the endamebae observed. These lesions were in the cecum.

Five puppies were injected in a similar way, except that they were not etherized. The catheter was gently passed into the rectum while an assistant held them. The rectal plugs were removed after 24 hours. Trophozoites appeared in the stool of numbers 1, 2 and 5 on the 7th, 8th and 13th days, respectively, of the infection, and continued for various lengths of time, later disappearing without causing the death of any one of them. Only one of the animals seemed really ill.

DISCUSSION

One complete stool examination of 564 individuals shows 12.4 per cent positive for *E. histolytica* and 46 per cent positive for *E. coli*. It is believed that the percentage of positives would have been increased by repeated examinations of stools from these same individuals at short intervals.

While the percentage of positives for *E. histolytica* by direct smear examinations alone and by cultivation were essentially the same, the combined methods gave a slightly higher percentage. When only the cysts are present in the stool, it is easier for the less expert laboratorian to make a differential diagnosis of *E. histolytica* from a culture (after excystation) than from the direct smear examination alone. We therefore feel that cultural methods should be more generally used, not as substitutes for the direct method, but as a useful supplement.

Concerning the experimental results: the percentage of infection of the kittens in Puerto Rico with the Puerto Rican strain of *Endameba histolytica* is not significantly different from the percentage of infestation of kittens in various parts of the United States, similarly infected with strains isolated in the United States. However, the percentage of death is less and the lesions of the colon, cecum, and rectum are very much less marked.

We believe that this observation will be a step toward the explanation of the fairly high carrier incidence in the Island, with a relatively low percentage of clinical cases of amebiasis. The explanation seems to lie within one of the three following categories:

(*a*) The endamebae of Puerto Rico, though morphologi-

cally and in some respects culturally resembling *E. histolytica,* are not really the pathogenic species that cause acute amebiasis.

(*b*) The people, being long in contact with the specific protozoa as well as the high percentage of infestation with the closely related protozoa, have developed some degree of specific as well as group immunity, which together with the category given below may offer an explanation, and

(*c*) The dietary habits of most of the people of the Island, which consist of a high carbohydrate diet (the kittens and puppies used in this experiment were fed on this type of diet) tend to decrease the activity of the endamebae without retarding their rate of development or interfering with the life cycle. The endamebae living in the lumen of the intestinal tract can obtain plenty of readily absorbable carbohydrates. Experimentally Rees (6) observed that the starch-fed *E. histolytica* in culture are more sluggish than those growing in a similar media but free from starch. We used Slade's rice starch or Ralston's whole wheat flour and our experiments coincide with his observation. Rees (6) states that because of this sluggishness of the *E. histolytica,* they may be voided before they can invade the mucosa. This, he thought, accounted for the so-called loss of pathogenicity for kittens of certain starch-fed strains, as well as the apparent lack of pathogenicity of *E. histolytica* from liver abscess, these endamebae having ingested large amounts of glycogen.

In the light of these observations we believe that the endamebae multiply in the proximal part of the lumen of the colon, without invading the mucosa. They encyst as they pass down and appear in the stools chiefly as cysts.

The high percentage of infection is related to the sanitary practices and personal hygiene of the locality.

Our experimental technique with puppies was not identical with that of Faust(4). The preliminary preparation of his dogs as well as his method of observation were different.

SUMMARY AND CONCLUSION

Cultural and microscopic examination of Puerto Rican strains of *E. histolytica* reveal a high positive correlation, yet we believe the cultural method as described to be superior in the interests of accurate diagnosis, because in doubtful cases

E. histolytica may be more easily differentiated from *E. coli* in the vegetative stage than in the encysted stage.

The Puerto Rican strain of the vegetative *E. histolytica* shows less pathogenicity for kittens fed on a high carbohydrate diet, than the United States strain injected into the kittens of the same age fed on a high protein diet.

Puppies fed on a high carbohydrate diet appeared to tolerate the infection, even though some of them showed active trophozoites in the stools for several days.

I wish to express my appreciation to Doctor George W. Bachman, Director of the School of Tropical Medicine, his Faculty and Technical Staff; to the various members of the Department of Sanitation; to the University Hospital, the Presbyterian Hospital and the Clínica Miramar, for their valuable assistance in providing me with the necessary apparatus and material for this study.

BIBLIOGRAPHY

1. POINDEXTER, H. A. The Puerto Rico Journal of Public Health and Tropical Medicine, 7: 417. 1932.
2. CLEVELAND, L. R. and SANDERS, E. P. Arche F. Protistenk. 70: 223. 1930.
3. MARIN, R. A. The Puerto Rico Journal of Public Health and Tropical Medicine. 6: 429. 1931.
4. FAUST, E. C. The Puerto Rico Journal of Public Health and Tropical Medicine. 6: 391. 1931.
5. REES, C. W. Arch. Path. 7: 1. 1929.
6. REES, C. W. Journal of Parasitology. 15: 131. 1928.