A Study of the Intestinal Parasites of the Monkeys of the Santiago Island Primate Colony[1]

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I

SOME EPIDEMIOLOGICAL OBSERVATIONS ON THE INTESTINAL FAUNA OF THE PRIMATES ON SANTIAGO ISLAND

THE first part of this study was undertaken in quest of those etiological factors responsible for recurrent mild epidemics of diarrhea and dysentery among the monkeys of the free-range primate colony, located on Santiago Island. The chief symptoms of some of these animals which died had been diarrhea and dysentery, or diarrhea only. Members of the Flexner group of dysenteric bacilli had also been isolated from some of the cases, yet this organism had not been considered fully explanatory of the condition. The question therefore arose as to whether animal parasites alone, or in association with some other organisms, might not be the etiological factor, or factors. With that possibility in mind, the writer decided to determine the intestinal fauna of the primates on the island for the purpose of evaluating the significance of any parasites found in relationship to these epidemics. The prevalence and pathogenicity of any histolytica-like Endamoebae were of special interest.

Santiago Island Primate Colony. Santiago Island is a small islet of some thirty-seven acres off the southeast coast of Puerto Rico and near the port of Humacao, set aside as a free-range reservation for approximately 450 monkeys of all ages and of two species, together with a few apes. More than 430 members of this colony belong to the species *Macaca mulatta*, and constitute the remainder and offspring of 409 specimens collected in India and brought to Puerto Rico by Dr. C. R. Carpenter[2] in 1937, at which time the animals were released on the island. A group of thirteen gibbons, *Hylobates*

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lar, was at the time confined in large, sanitary enclosures pending their disposal.

Determination of the intestinal fauna of the monkeys. During a four-month study period in the summer of 1940, 151 fecal specimens from 122 different monkeys were collected and examined. About 20 percent of these animals had two or more fecal examinations at intervals averaging about three weeks. The method of obtaining these specimens was as follows:

The animals were lured with fruit into large wire enclosures and trapped without physical harm. They were then placed in cages until a fecal specimen could be procured, this being later collected in a properly labeled container bearing the identification number of the monkey. In most cases, a preliminary microscopic examination was carried out on the island, while the specimen was fresh, and then rechecked in the laboratory of the School of Tropical Medicine by the concentration method, or other special technique. The purpose of the preliminary examination was the detection of the trophozoites of certain protozoa which would not be found after a twelve-hour delay, or after twenty-four hours of refrigeration. The examination of each specimen consisted of:

1. A microscopic examination of fecal films, unstained, stained separately with iodine, and with a 2 percent copper sulphate solution. Another microscopic examination of the feces followed concentration.

2. A culture of each specimen in Locke-egg-serum (rabbit) medium of Boeck and Drbohlav was incubated at 37.5° C. for twenty-four hours, and subcultural, twenty-four and forty-eight hours later. This last step was necessary in certain cases because some of the Endamoeba cysts were slow in ex-cysting.

3. A microscopic examination of these cultures and permanent stains by the phospho-tungstic acid-hematoxylin methods were made on all cultures showing amoebae which resembled *Endamoebae histolytica.*

The examination was not as complete as that made by Faust *et al*,[3] therefore it is not claimed that all the different parasites in each specimen were found. However, the culture method employed did increase the percentage of positives. The total of 151 fecal

3. Ernest Carroll Faust, Willi Sawitz, John Tobie, Vada Odom, Charles Peres, and David R. Linicome, "Comparative Efficiency of Various Technics for Diagnosis of Protozoa and Helminths in Feces," *J.Parasitology*, XXV (1939), 241.

examinations revealed 448 intestinal parasites (exclusive of certain free-living flagellates and nematodes observed in specimens contaminated by soil), thus giving an average of about three parasites per fecal examination. In 117 (97 percent) of the 122 animals examined, one or more species of parasites were revealed in a single fecal examination, but repeated examinations showed that 99 percent of all monkeys examined harbored one or more species. Not over one percent showed only one species, while 18 percent harbored five or more. The largest number was found in monkey No. 119, an eight-year-old female of the *Macaca mulatta* group. This animal, which did not appear sick, gave the following results on repeated fecal examinations:

Balantidium simile
Strongyloides papillosus
Physaloptera sp.
Endamoeba histolytica
Endamoeba coli
Chilomastix sp.
Giardia lamblia
Trichomonas hominis
Hexamita sp.

As the 122 animals examined represented all ages from two months to over fifteen years and, since they were caught at random from all parts of the island, the writer believes that these findings are fully representative of the intestinal fauna of the primate colony.

Table 1 denotes the protozoa found in the fecal examinations of

TABLE 1

Protozoa Detected in 122 Monkeys by Fecal Examination

Species of Parasite	*Number of Animals Examined*	*Number Positive*	*Percentage Positive*
Balantidium simile	122	106	87
Endamoeba coli	122	74	61
Endamoeba histolytica	122	35	29
Iodamoeba keuneni	122	16	13
Endolimax nana	122	18	15
Giardia lamblia	122	11	9
Chilomastix sp.	122	9	7
Trichomonas hominis	122	39	32
Other intestinal flagellates exclusive of *Bodo caudatus*	122	9	7

the 122 monkeys. There is a significant difference between the low incidence of *Endolimax* infection in the macaques of this series and the high incidence reported by Hegner[4] in chimpanzees.

A review of the observations of da Cunha and Muñiz[5] with regard to the *Balantidium* most commonly found in monkeys showed that it differed from *Balantidium coli* in conjunction, cultural reaction, and intracellular morphology. The *Balantidium* in this series appeared to be *Balantidium simile*. The *Iodamoebae* found resembled the species *Iodamoeba williamsi* in some specimens and the species *Iodamoeba keuneni* in others.

Table 2 lists the helminths found in the 122 animals. In three cases, ova resembling those of *Protospirura muricola* were seen.

TABLE 2

Helminths Detected in 122 Monkeys by Fecal Examination

Species of Parasite	*Number of Animals Examined*	*Number Positive*	*Percentage Positive*
Oesophagostomum bifurcum	122	32	26
Strongyloides papillosus	122	42	34
All other helminths	122	10	8

There was also found in a male aged eight years, monkey No. 152, a mite resembling Pneumonyssus. The nymph of this mite is occasionally found in the stools of monkeys, according to Weidman,[6] and Chandler and Ruhe.[7] The stool from this same monkey was fluid and tarry, containing *Balantidium simile* and *Chilomastix sp.*

Table 3 shows the prevalence of six of the most important parasites encountered in these monkeys, which have been classified here according to age groups determined on the basis of their dentition.

Chief sources of infection and reinfection. There is evidence to indicate that the carrier state for most of the parasites listed in Table 3 was temporary. A comparison established between nineteen members of the species *M. mulatta*, kept at the School of Tropical Medicine in sanitary cages that were cleaned daily, and nineteen

4. Robert Hegner, "Intestinal Protozoa of Chimpanzees," *Am.J.Hyg.*, XIX (1934), 480.
5. Aristides Marques da Cunha and Julio Muñiz, "Conjugação e Endomixis em ciliados do genero Balantidium," *Mem.Inst.Oswaldo Cruz*, XXXII (1937), 75.
6. F. D. Weidman, "*Pneumonyssus foxi*, nov. sp., an Arachnoid Parasite in the Lung of a Monkey (Macacus rhesus)," *J.Parasitology*, II (1915–16), 37.
7. W. L. Chandler and D. S. Ruhe, "*Pneumonyssus caninum* N.S.P., a Mite from the Frontal Sinus of the Dog," *J.Parasitology*, XXVI (1940), 59.

TABLE 3

Prevalence of the Six Different Intestinal Parasites in Members of the Santiago Island Primate Colony, According to Age Groups

Ages in Years	*B. simile*			*E. histolytica*			*E. coli*			*G. lamblia*			*O. bifurcum*			*S. papillosus*		
	Number Examined	*Number Positive*	*Percent Positive*	*Number Examined*	*Number Positive*	*Percent Positive*	*Number Examined*	*Number Positive*	*Percent Positive*	*Number Examined*	*Number Positive*	*Percent Positive*	*Number Examined*	*Number Positive*	*Percent Positive*	*Number Examined*	*Number Positive*	*Percent Positive*
0– 3	33	25	76	33	9	27	33	15	45	33	2	6	33	14	42	33	21	64
4– 6	23	21	91	23	4	17	23	11	48	23	2	9	23	4	17	23	5	22
7–12	44	35	80	44	17	40	44	24	55	44	3	7	44	8	18	44	8	18
Over 12	22	20	91	22	3	14	22	20	91	22	2	9	22	5	23	22	11	50
Totals and averages	122	101	83	122	33	27	122	70	57	122	9	7	122	31	25	122	45	37

free-range animals of comparable age and of the same species, living in the colony on Santiago Island, gave the results shown in Table 4.

TABLE 4

A Comparison of Parasitic Infection between Two Groups of M. mulatta, Nineteen in Clean Cages and Nineteen Free Ranging

	Percentages Positive for					
	B. simile	*E. histol.*	*E. coli*	*G. lamblia*	*O. bifurcum*	*S. papillosus*
GROUP I (Cages in School of Tropical Medicine)	47	5	31	16	16	21
GROUP II (Free ranging on Santiago Island)	84	37	47	26	42	32

These differences appear significant in spite of the probable errors associated with the observations based on such small numbers. The writer believes, however, that the carrier stage would be relatively transient under proper conditions of hygiene and sanitation and that, barring reinfection, these animals would eliminate the parasites within a reasonable length of time. In line with this belief, the intestinal parasites of twelve members of the species *M. mulatta*, whose stools had been examined while living free on the island, were compared with the findings of these same animals after they had been kept in sanitary cages for more than two months at the School of Tropical Medicine. Table 5 shows the results.

TABLE 5

Comparison of Findings in Stools of Twelve Monkeys under Free Ranging Conditions and after Two Months' Confinement in Sanitary Cages

	Percentages Positive for					
	B. simile	*E. histol.*	*E. coli*	*G. lamblia*	*O. bifurcum*	*S. papillosus*
GROUP II (Free ranging on Santiago Island)	83	25	33	25	25	33
GROUP I (Caged at School of Tropical Medicine)	58	8	17	17	17	8

In a further attempt to determine the most probable sources for these infections, a parasitological study was made of the soil, water, and particles of food collected in twenty-three feeding and drinking stations and sleeping places of these monkeys. Samples of soil included not only specimens from around the feeding stations, but also from under the trees, where the animals slept at night, and from the storm shelters to which they went during strong winds or inclement weather. Such specimens were submitted to microscopic examination, with and without stain, and before and after concentration. Cultures and further microscopic studies were made for Endamoebae and other protozoa.

After excluding the free-living soil nematodes, flagellates, and amoebae, three intestinal parasites were found present in significant percentages in soil and water samples. *Balantidium simile* cysts and trophozoites were present in 48 percent of all specimens examined, and in 61 percent of the specimens of soil. *B. simile* was present in only 9 percent of the receptacles that held drinking water. Forty-two percent of the *B. simile* organism appeared to be viable at the time the samples were examined; 18 percent were apparently undergoing disintegration. These observations suggest that the chief source of *B. simile* reinfection was from food contamination by fecally polluted soil around the feeding stations. Viable larvae of *Strongyloides* were found in 13 percent of all the specimens examined. *Oesophagostomum bifurcum*, as ova or larvae, was found in 17 percent of the specimens. All positive *O. bifurcum* came from soil or from contaminated food.

It was not possible to cultivate histolytica-like Endamoebae from any of these specimens, but amoebic cysts, morphologically different from those of the free-living amoebae, were found in 30 percent of the specimens, and were more common in soil specimens than in those of water.

However, the soil, fecally polluted with histolytica-like Endamoebae, the Balantidia, and to some extent, the Strongyloides, was a menace only from day to day. Scavenger crabs, lizards, the heat of the sun with its dessicating effect, and even the biology of the soil, all contributed to render it relatively free of protozoa within twelve to twenty-four hours, reducing the *Strongyloides* and *Oesophagostomum* to a nondangerous level within the same length of time. Reinfection, however, offset much of the natural effects of the agents named above.

The fact that monkeys are heavily parasitized in their wild

habitat, even before they become closely associated with man, was shown by Hegner and Chu[8] in 1930. Observations tend to confirm this finding, the high incidence being due, in a large measure, to the animals' feeding habits, whereby contaminated hands and feet add to the already contaminated food that has fallen upon polluted soil. Notwithstanding, this same high incidence, without marked symptoms, suggests an equilibrium between host and parasite, comparable to the high carrier incidence of certain parasites of man when living under similar insanitary environmental conditions. Continuous contact between host and parasite over many decades with the resultant infection immunity has contributed to this apparent equilibrium.

SUMMARY AND CONCLUSION

The Santiago primate colony offered exceptional opportunities for the study of many phases of animal ecology under a minimum alteration of the normal habitat. The monkeys in this colony were heavily parasitized with protozoa, the most important being *Balantidium simile* and a histolytica-like Endamoeba. They were also heavily infected with helminths, the most important of which were *Oesophagostomum bifurcum* and *Strongyloides papillosus*. The monkey to all appearances possessed a degree of immunity to most of these intestinal protozoa and helminths, but the frequent opportunities for reinfection served to keep the incidence of parasitism high. The chief method of spreading the organisms was, to all intents, associated with their insanitary feeding habits.

II

COMPARATIVE STUDY OF THE PATHOGENICITY OF ENDAMOEBAE ISOLATED FROM MAN AND FROM MONKEYS

Inasmuch as the foregoing observations suggest that the parasitization of these animals by certain organisms appears transient, only when opportunities for reinfection were reduced, it was considered desirable to determine which, if any, of the parasites detected were sufficiently pathogenic to justify the expenditure which would be necessary to reduce materially the chances for reinfection. Individual or group medication of monkeys, at free range on the island, would be difficult; the construction of special sanitary feeding places, expensive.

8. R. Hegner and H. J. Chu, "A Comparative Study of the Intestinal Protozoa of Wild Monkeys and Man," *Am.J.Hyg.*, XII (1930), 68.

Three common parasites were therefore selected for their study from the viewpoint of pathogenicity, viz., the histolytica-like Endamoebae, the Balantidia, and the Strongyloides. In this article only the first receive consideration.

Histolytica-like Endamoebae were isolated from the stools of the monkeys by cultivation in Locke-egg-serum medium of Boeck and Drbohlav, in infusion agar, and Locke's serum as used by Cleveland and Collier.[9] Similarly, *E. histolytica* were isolated from chronic and carrier cases of human specimens obtained from the University Hospital of the School of Tropical Medicine, and from one case of acute symptomatic amoebiasis with the syndrome of blood and mucus in the stool and active *E. histolytica* trophozoites present therein.

The trophozoites from these three different sources were morphologically similar after cultivation and were injected rectally into comparable sets of kittens and puppies for the pathogenicity test. The seventeen kittens utilized varied in weight from .52 to 1.4 kg.; the ten puppies, from 1.3 to 4.5 kg. Before commencing the experiment, the stools of these kittens were examined by direct smear, before and after concentration, by the sedimentation method commonly used with the aid of a sieve of different mesh, by the zinc sulphate method of Faust *et al*[10] and by cultures for intestinal protozoa. Forty-one percent of these kittens harbored *Toxocara cati;* 18 percent were infected with hookworm. The eggs of *Platynosoma concinnum* were found in the stools of 29 percent, while nematode larvae of the genus *Strongyloides* appeared in 12 percent and *Capillaria* ova in the stool of one kitten.

Attempts were made to rid these animals of their helminth infections before utilizing them. Hexylresorcinol was therefore given, which anthelmintic expelled most of the parasites. Ten days after treatment an examination was again made of the stool of each treated kitten. Those that were free of helminths, or showed only occasional ova, were considered satisfactory for the experiment.

Each kitten was also examined for intestinal protozoa. *Isospora felis* was present in 35 percent; Endamoebae in two of them, though these did not resemble *E. histolytica*. Flagellates were also found in several of the stools. As it was difficult to rid the kittens of all flagellates, it was decided to use these animals for the experiment in

9. L. R. Cleveland and J. Collier, "Various Improvements in Cultivation of *Entamoeba histolytica*," *Am.J.Hyg.*, XII (1930), 606.

10. Faust, Sawitz, Tobie, Odom, Peres, and Linicome, *op. cit.*

spite of the presence of intestinal flagellates, chiefly trichomonads, in some of them.

The kittens were experimentally infected by passing a soft, rubber, flexible catheter through the anus, until its tip reached at least the transverse colon. While the animal, under medium sodiumamytal anesthesia, was held suspended head down, an irrigation syringe containing a suspension of a twenty-four-hour culture of active trophozoites was connected with the catheter. A purse string suture of twenty-four-hour soluble catgut was then placed around the anal opening in a manner suggested by Dr. Juan Bacigalupo,[11] and the anus closed after withdrawal of the catheter. This procedure prevented expulsion of material for twelve to twenty-four hours, without further handling of the animal. It was found more satisfactory than the anal plugs, or tampons, used previously.

Kittens Injected with Endamoebae Isolated from Monkeys

Kitten No. 1: Endamoeba culture from monkey No. 149. Precystic Endamoebae persisted for fifteen days; no active trophozoites, no blood or mucus present. Animal sacrificed fifty-one days later. Autopsy revealed no gross or microscopic lesions of amoebiasis.

Kitten No. 2: Endamoeba culture from monkey No. 104. Nonactive Endamoebae found for eight days; no blood or mucus. Animal sacrificed after forty-six days. Gross and microscopic examinations negative for amoebiasis in intestinal tract or liver.

Kitten No. 3: Endamoeba culture from gibbons B and M. Animal developed severe diarrhea after injection with occasional, not very active trophozoites present, containing red blood cells; also large number of pus cells, giving bacillary infection picture; *B. dysenteriae* isolated. Animal died on eighth day. Autopsy revealed intestinal lesions of profuse catarrhal inflammation of lower part of small intestine and entire colon; no discrete ulceration. Endamoebae present mostly in lumen and superficial part of intestinal wall; none below muscularis mucosa.

Kitten No. 4: Endamoeba culture from monkey No. 34. No active Endamoebae seen for thirty-one days, after which animal was sacrificed. Autopsy revealed no lesions of intestinal tract, but marked infiltration with equal proportions of mononuclear and polymorphonuclear cells in region of cecum and transverse colon; occasional nonulcerative pyogenic zones with, and some without, Endamoebae, which were rounded trophozoites without red blood corpuscles, not extending beyond pyogenic zones. Solitary glands of intestinal tract showed

11. Juan Bacigalupo, "Personal Communication during a Visit to His Laboratory." Bueno Aires, Argentina, 1940.

increased inflammatory activity with tendency toward superficial ulceration but not submucosal extension. Sections of liver disclosed acute inflammatory reaction and some necrosis; no Endamoebae in spite of considerable microscopic pathology.

Kitten No. 5: Endamoeba culture from monkey No. 47. Showed blood and mucus on seventh day with occasional, sluggish Endamoebae without ingestion of red blood corpuscles; active trophozoites few in relation to the symptoms which continued in varying degree until the nineteenth day. No blood, mucus, or Endamoebae found for next forty-two days. Animal sacrificed sixty-one days after injection. Autopsy negative for amoebic pathology.

Kitten No. 6: Endamoeba culture from monkey No. 116. Showed no symptoms of acute amoebiasis; sacrificed after fifty-one days. Autopsy revealed no evidences of amoebiasis for intestines and liver, though nematodes were attached to walls of small intestine instead of those of colon; inflammatory reaction at places of attachment and, in some instances, recent petechial hemorrhagic areas.

Results. A total of 147 sections from these six kittens was studied microscopically. Gross and microscopic studies of both sections and tissues revealed almost no evidence of pathogenicity for kittens. If pathogenicity for kittens can be considered a criterion of their virulence for man, the Endamoebae isolated from these monkeys are not pathogenic for human beings.

Kittens Injected with E. Histolytica Isolated from Human Carriers

Kitten No. 7: *E. histolytica* culture from human carrier, M. M. No blood, mucus, or trophozoites observed until forty-two days after injection. Animal died eleven days after onset of dysenteric symptoms and fifty-three days after injection. Trophozoites of *E. histolytica* present at time of death. Autopsy, twelve hours after death, showed only superficial lesions of large intestine, which were not sufficient to warrant death. No Endamoebae seen in any lesions. Study of sections of intestine and abdominal lymph nodes revealed superficial ulcerations in different areas of colon; some necrotic changes in the solitary nodes of intestinal tract, and macrophage infiltration in region of nodes, or ulcerated areas. Macrophage reactions extended into muscular walls of intestines, but with no evidence of perforation or peritonitis. Some lymph nodes and sections of liver showed inflammatory reaction and beginning toxic degeneration.

Kitten No. 8: *E. histolytica* culture from human carrier, O.P.D. A224. No dysentery, though nonmotile Endamoebae occasionally seen for first three weeks; none thereafter. Animal sacrificed after sixty-one days. Autopsy showed no evidence of amoebiasis.

Kitten No. 9: *E. histolytica* culture from human carrier, No. 481. No dysenteric symptoms; no trophozoites found, though repeated examinations revealed occasional rounded, nonmotile form of Endamoebae which grew in culture. Animal sacrificed after forty-sixth day. Autopsy showed no gross lesions of intestines or liver; microscopic studies negative.

Kitten No. 10: *E. histolytica* culture from human carrier, H. O. Diarrhea developed on ninth day, continuing for thirteen days, animal dying twenty-two days after injection. Nonmotile Endamoebae present up to six days before death; mucus occasionally seen without blood. Autopsy revealed extensive catarrhal reaction with loss of epithelium for large areas in distal part of mucosa of large and small intestines. Circumscribed ulcers not present. Occasional section of large intestine showed trophozoite in ducts of intestinal glands, or crypts between two glands. Cellular response predominantly microphagic. Liver revealed areas of early cellular necrosis with macro- and microphage infiltration; no abscesses, no Endamoebae.

Kitten No. 11: *E. histolytica* culture from human carrier, J. M. No symptoms of amoebiasis; animal sacrificed fifty-six days after injection. Autopsy findings all negative for amoebiasis both in gross and microscopic observations.

Kitten No. 12: *E. histolytica* culture from human carrier, O.P.D. A327. Endamoebae in pre-cystic stage observed throughout forty-six days; small amounts of mucus but no blood nor active trophozoites containing red blood corpuscles present. Numerous Blastocystis and Monilia present. Animal sacrificed after forty-sixth day. Autopsy showed areas of hyperemia of mucous and submucous layers with some cellular infiltration below muscularis mucosa; no distinct ulcerations; Endamoebae free in lumen and in crypts of large intestine; liver not involved.

Results. A total of 211 microscopic sections from six kittens was studied. Two of these animals died, while the remaining four were sacrificed. In the light of Kessel's[12] observations, and those of many others on experimental amoebiasis in kittens, the autopsy findings herein reported do not support a conclusion of experimental amoebiasis as the dominant and significant pathology. There is a suggestion that Endamoebae, isolated from human carriers, were not virulent for kittens, or that these particular kittens showed exceptional resistance. The transient diarrheas might have been due to endotoxin, released by autolysis of the organisms in the injected material.

12. John F. Kessel, "Amebiasis in Kittens," *Am.J.Hyg.*, VIII (1928), 311.

Blood differentials made on some of these animals are recorded in Table 6.

TABLE 6

Blood Counts of Experimental Animals Injected with Cultures from Various Sources

Animal Number	Date	Organism	Source	Polys. Percent	Juveniles Percent	Lymphocytes Percent.	Monocytes Percent	Eosinophils Percent	Basophils Percent	Schilling Index
Kitten 7	42 days after injection	*E. histolytica*	Human carrier	41	7	38	8	5	1	1/6
10	11 days after injection	*E. histolytica*	Human carrier	64	9	19	7	1	...	1/7
	16 days after injection	*E. histolytica*	Human carrier	46	23	18	10	3	...	1/2
16	During diarrhea	*E. histolytica*	Cases showing symptoms	58	7	15	17	1	Myelocytes 2	1/9
Puppy 1	24 days after injection	Histolytica-like Endamoebae	Monkey	50	10	14	14	12	...	1/5
2	Before injection	Histolytica-like Endamoebae	Monkey	67	4	21	6	2	...	1/17
	25 days after injection	Histolytica-like Endamoebae	Monkey	75	8	11	3	2	Basophils 1	1/9
4	Before injection	Histolytica-like Endamoebae	Monkey	48	11	15	20	5	1	1/6
7	During diarrhea	*E. histolytica*	Human carrier	67	4	23	6	..	...	1/17
8	At injection	*E. histolytica*	Human cases with acute amoebiasis	50	10	16	13	11	..	1/5
	59 days after injection	*E. histolytica*	Human cases with acute amoebiasis	45	12	30	8	5	..	1/4
1	At injection	*B. simile*	Monkey	71	6	14	4	5	...	1/12
2	5 days after injection	*B. simile*	Monkey	72	3	12	2	11	...	1/24
3	Before injection	*B. simile*	Monkey	74	11	8	3	4	...	1/7
3	5 days after injection	*B. simile*	Monkey	48	3	30	18	1	...	1/16
4	Before injection	*B. simile*	Monkey	65	6	13	9	2	...	1/11
	12 days after injection	*B. simile*	Monkey	57	7	10	18	8	...	1/8
5	Before injection	*B. simile*	Monkey	49	3	30	11	7	...	1/18
		B. simile	Monkey	59	5	18	14	4	...	1/12

Kittens Injected with Cultures of E. Histolytica from a Human Case Showing Symptoms at the Time the Isolation Was Made

Kitten No. 13: *E. histolytica* culture from young made adult, F. E. Diarrhea developed on sixth day continuing for about eight days, during which time trophozoites and pre-cysts appeared; blood and mucus absent. Diarrhea stopped, to start again after thirteen days, continuing intermittently for next eighteen. Animal sacrificed forty-five days after injection. Autopsy showed marked diffuse hyperemia in many sections of colon, even in lower small intestine, near ileocecal valve. Numerous superficial ulcers present in colon, but only two extended below muscularis mucosa. No extension of Endamoebae beyond craters of ulcers. Cellular infiltration predominantly mononuclear in character; liver not involved.

Kittens Nos. 14 and 15: Cultures from patient previously mentioned. Course of illness comparable in most instances to that of No. 13. Sacrificed fifty-one and sixty-three days after injection. Pathology not sufficient to suggest that either animal would have died from amoebiasis with syndrome usually present in kittens infected with virulent *E. histolytica*.

Kittens Nos. 16 and 17: Cultures of *E. histolytica* from adult, isolated during acute exacerbation of recurrent attacks of dysentery. Diarrhea developed in both animals with blood, mucus, and occasional, practically inactive trophozoite without red blood corpuscles. Animals sacrificed forty-nine and fifty-four days, respectively, after injection. Autopsy showed scattered superficial ulceration of colon, predominantly in cecum. Endamoebae found in crypts and between ducts; occasional Endamoeba found deep in tissues, even at level of muscularis mucosa. Marked cellular infiltration present with microphages and macrophages in about equal proportions. Liver not involved.

Results. A total of 167 sections was studied from this last set of kittens, which had been injected with cultures from active symptomatic human cases. The Endamoebae in this group possessed some ability to invade the intestinal walls of the kittens. There was evidence that the presence of Endamoebae in both colon and cecum was significantly associated with intestinal irritation, ulcerations, and acute dysentery. However, the virulence of this organism seemed insufficient to produce fatal results in kittens by intracolonic injection.

Differential blood count made for kitten No. 16 is recorded in Table 6.

Puppies Injected with Cultures of Histolytica-like Emdamoebae Isolated from Monkeys

Puppy No. 1: Culture of Endamoebae from monkey No. 149. No symptoms of amoebiasis; animal sacrificed twenty-five days after injection. Hookworm present. Autopsy revealed neither gross nor microscopic lesions of experimental amoebiasis.

Puppy No. 2: Culture of Endamoebae from monkey No. 104. No evidence of amoebiasis. Animal sacrificed twenty-five days after injection. Autopsy findings negative for amoebiasis, both by gross and microscopic study of intestines, liver, and regional lymph nodes.

Puppy No. 3: Culture of Emdamoebae from monkey No. 34. No symptoms of amoebiasis noted for fifty-six days, but pre-cysts occasionally seen in stools. Animal sacrificed on fifty-sixth day. Autopsy revealed no pathology suggestive of amoebiasis.

Puppy No. 4: Culture of Endamoebae from monkey No. 47. Animal developed diarrhea on twenty-second day which continued for twenty-seven more. Endamoebae present in stools, but no blood, mucus, or trophozoites containing red blood corpuscles. Animal sacrificed on fiftieth day. Autopsy revealed massive edema of large intestine with thickening of walls and petechial hemorrhagic areas scattered throughout. No definite ulcers present. Microscopic study of hemorrhagic areas showed cellular reaction of acute inflammation but no Endamoebae.

Results. A total of 217 sections from intestines, liver, and other tissues in region of the cecum of these puppies was studied microscopically. No symptoms or pathology attributable to amoebiasis were noted.

Differential blood counts made on three of these animals are recorded in Table 6.

Puppies Injected with E. Histolytica Isolated from Human Carriers

Puppy No. 5: Culture of *E. histolytica* from human carrier, M. M. No symptoms of amoebiasis; animal sacrificed on fiftieth day. Autopsy revealed only scattered areas of hyperemia and cellular infiltration in large intestine. Polymorphonuclear leukocytes were predominant cells. No Endamoebae seen.

Puppy No. 6: Culture of *E. histolytica* from human carrier, O.P.D. A224. No evidence of amoebiasis; animal sacrificed on thirty-ninth day. Autopsy negative for lesions of amoebiasis by gross and microscopic observations.

Puppy No. 7: Culture of *E. histolytica* from human carrier, A481. Animal developed fluid stools on eleventh day, continuing for six more. Slightly

motile trophozoites seen on three occasions. Animal sacrificed on forty-second day. Autopsy showed only small hyperemic areas of colon, but no ulcers. Microscopic picture was one of polymorphonuclear leukocyte infiltration with some thickening of walls. Endamoebae not found in section of intestines or liver.

Results. Ninety-four microscopic sections were studied from puppies Nos. 5, 6, and 7.

Blood differential made of puppy No. 7 is recorded in Table 6.

Puppies Injected with E. Histolytica from Human Cases with Symptoms of Acute Amoebiasis

Puppy No. 8: Culture of *E. histolytica* from human case, F. E. Occasional pre-cystic form of Endamoebae found in stools. Animal sacrificed sixty-one days after injection. Clinical history and findings negative.

Puppies Nos. 9 and 10: Culture of *E. histolytica* from same individual as indicated above. Animals sacrificed on fiftieth and sixtieth days. Autopsy findings comparable to those in No. 8, except for a few small areas of hyperemia.

Results. Seventy-nine microscopic sections were studied from animals Nos. 8, 9, and 10. Cultures of histolytica-like Endamoebae of human origin, either in the carrier or acute stages, failed to cause frank amoebiasis in the young dogs employed in these series.

Blood differential made of puppy No. 8 is recorded in Table 6.

DISCUSSION

The morphological similarity existing between certain Endamoebae found in the intestines of these monkeys and the *E. histolytica*, isolated from human amoebiasis, has caused some speculation as to the danger of these animals acting as reservoirs for Endamoebae pathogenic to man. Experiments herein, however, do not support such a probability.

The high incidence of *E. histolytica* cysts in the stools of people who live in many tropical countries, and who show little or no clinical symptoms of amoebiasis, has been known for a long time. There are at least four possible explanations for this, viz., the microörganism is not pathogenic; the diet of the people renders a normally pathogenic organism temporarily nonpathogenic; the people have developed an "infection immunity," which operates as long as they are in continuous contact with the sources of reinfection, or their resistance is kept reasonably high; the invasive power of the organism undergoes attenuation under the climatic influence of long

tropical residence. The works of Siler,[13] Benítez and Morales Otero,[14] James, Nicol, and Shute,[15] Poindexter,[16] Boyd, Carr, and Rozeboom,[17] Wenrich,[18] and Pottenger,[19] have given varying amounts of support to one or more of these theories.

So far as the pathogenicity for kittens and puppies is concerned, the *E. histolytica* recently isolated from individuals with active symptoms of amoebiasis are more virulent than the organisms isolated from carriers, or from monkeys. There is a possibility that the more actively growing parasites, such as are present in active symptomatic cases, elaborate metabolic substances which promote tissue damage by the combined action of increasing the permeability of the intestinal epithelium and the invasive power of the motile organism. The work of Menkin[20] suggests this last possibility.

CONCLUSIONS

1. The histolytica-like Endamoebae, isolated from the monkeys of the Santiago Island Primate Colony, are not sufficiently pathogenic for kittens and puppies, when experimentally infected through intracolonic injections, to be considered a source of infection or danger to man.

2. Cultures of *E. histolytica*, isolated from human carriers and tested in Puerto Rico, are only mildly pathogenic for kittens and puppies, when experimentally infected through intracolonic injections.

3. Cultures of *E. histolytica*, isolated from active symptomatic cases of amoebiasis, are more pathogenic for kittens and puppies than morphologically similar Endamoebae, isolated from chronic carriers, or from monkeys.

4. Young dogs appeared to be refractory to all amoebae, regardless of their origin.

5. There does not seem to be any justification, therefore, for large expenditures to prevent transient infection or reinfection.

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