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RECENT ADVANCES IN HUMAN PROTOZOAN PARASITES OF GASTROINTESTINAL TRACT

J.H. Cross

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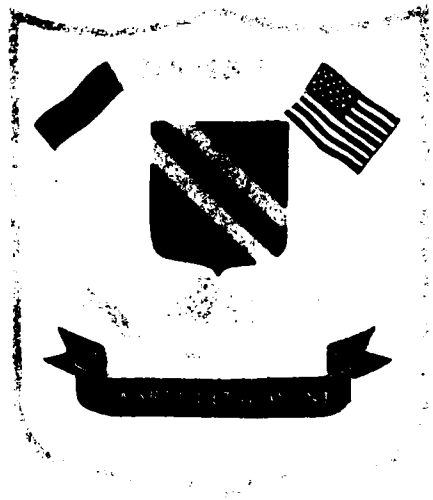

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Giardiasis continues to be a cause of diarrhea among various groups especially campers

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RECENT ADVANCES IN HUMAN PROTOZOAN PARASITES OF THE GASTROINTESTINAL TRACT*

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INTRODUCTION

Even with the increasing number of antiprotozoan drugs available today, intestinal protozoan infections are a continuing problem world-wide. It is clear that, like helminthic parasitosis, the protozoans remain with us in as great a number today as they were 50 years ago. The prevalence and distribution of the organisms remain constant; however, advances have been made in a better understanding of the biology of the parasites, the epidemiology, diagnosis, pathogenesis and treatment. Furthermore, protozoan parasites formerly considered of no human importance are being found to infect man and cause disease. In addition, there appears to be changes occurring in the prevalence and distribution of intestinal protozoan infections among various segments of the population. For example, more careful examinations of stools from patients suffering from travellers diarrhea is incriminating protozoa as the etiologic agent. There have also been an increasing number of reports of intestinal protozoan parasitoses among gay populations with and without the acquired immunodeficiency syndrome (AIDS) (Macher, 1984). On the other extreme, there was a Turkish report in which 14 out of 18 members of the national wrestling team were found to be infected with one or more of the intestinal protozoan parasites (Sahin, Kilic, Ozcan & Orhan, 1984).

This paper presents a review of recent advances on the biology of human protozoal parasites of the gastrointestinal tract and the epidemiology and pathogenesis of the diseases they cause; in most cases only papers published since 1980 were included.

FLAGELLATES

Although there are eight or more intestinal flagellates reported from humans, most do not cause disease; they are rarely reported and have scant mention in most textbooks. No reports could be found in the recent literature on *Retortamonas* sp. and *Enteromonas* sp. There was also a paucity of information on *Chilomastix mesnili, Trichomonas tenax, T. hominis* and *Dientamoeba fragillis*. In surveys conducted over a number of years in the Philippines we never found human feces containing *Retoromonas intestinalis, R. siniusis* or *Enteromonas hominis* (Cross & Bacaca-Sevilla, 1984). We occasionally found *T. hominis* in human stools by direct smear and several years before on Taiwan, we isolated the parasite in cultures from 46% of 224 stool specimens. These findings suggested that *T. hominis* may be more common than indicated by routine microscopic stool examinations. In later surveys in which stool cultures for intestinal protozoa were carried out, *T. hominis* was also recovered. There have been sporadic reports on the prevalence of *T. tenax* but evidence remains to be provided on the pathogenesis associated with infection with this organism.

Chilomastix mesnili is not an uncommon parasite of man and continues to be reported in prevalence surveys from around the world. In studies from the Philippines, the parasite was detected in formalized stool specimens by direct and after formalin-ether concentration methods in approximately 1% of over 30,000 stools examinations. The prevalence rates were equally distributed in all age groups and both sexes.

*The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the United States Department of Defense or the Uniformed Services University of the Health Sciences.

In some areas of the country, the parasite was not found but in other areas as many as 8% of the stools examined were positive for *C. mesnili* cysts or trophozoites (Cross & Bacaca-Sevilla, 1984).

Dientamoeba fragilis

Several aspects about this amoeboflagellate are puzzling. The means of transmission of the parasite has not been definitely established. Pinworm eggs are a suspected vehicle, but this has not been proven conclusively. Cystic forms of the parasite have been implied, but this too has not been proven (Colea, Silard, Panaitescu, Florescu, Roman & Caparru, 1983). Although prevalences are usually considered universally low, Millet, Spencer, Chapin, Garcia, Yatabe & Stewart (1983) reported infection rates of 76% in children and 56% in adults of a California semicommunal group. The pathogenicity of the parasite is also questionable and may be strain related. Most symptoms reported are diarrhea, abdominal pain and occasionally anal pruritis. There have also been several reports of eosinophilia associated with *D. fragilis* infections (Spencer, Chapin & Garcia, 1982), but this finding as well as the report of pruritis requires further investigation.

Giardia lamblia

Giardia lamblia and giardiasis have received a great deal of attention in recent years with numerous papers published on the epidemiologic, clinical, diagnostic and immunologic aspects of the parasitosis. The parasite continues to be reported world-wide with prevalence rates ranging from 2% to over 60%. In surveys conducted in the Philippines, 6% of over 30,000 stool examinations were positive. The rate was similar for both sexes with the highest rate (14%) in children below 10 years of age (Cross & Basaca-Sevilla, 1984).

Water-borne *G. lamblia* epidemics continue to be reported in the Western United States. In the state of Colorado, an outbreak of water-borne disease attributed to the parasite was reported in eight of 18 epidemics and at a resort in Colorado the attack rate was 42% among those who drank six or more glasses of water per day. Residents who had lived in the area for two or more years had a lower attack rate than short-term residents (Istre, Dunlop, Gaspard & Hopkins, 1984). In Montana, outbreaks of water-borne giardiasis were associated with heavy water run-off due to warm weather and volcanic ash fall (Weniger, Blaser, Gedrose, Lippy & Juranek, 1983). Other interesting reports were of the parasitosis being identified in 61% of children, 53% of mothers and 28% of fathers attending an infant and toddler swim class in Washington State (Harter, Frost, Grunenfelder, Perkins-Jones & Libby, 1984). Water-borne outbreaks of giardiasis have been associated with animals and, in a report by Sautter & Knights (1983), muskrats were considered a probable source.

Giardia lamblia is considered by Wright (1984) as the most important parasitic cause of travellers diarrhea. It is also being considered a sexually transmitted parasite among both heterosexuals and homosexuals who practice oral-anal sex. Furthermore, infections are being detected with increasing frequency in AIDS victims (Macher, 1984). In addition to causing intestinal disturbance, *G. lamblia* infection has been associated with allergic syndromes, such as chronic urticaria (Hamrick & Moore, 1983), retinal arteritis and iridocylitis in children and adults (Knox & King, 1982).

The ability to establish *G. lamblia* in continuous culture has led to the development of a number of immunodiagnostic tests for the parasitic infection. The indirect fluorescent antibody (IFA) test and enzyme-linked immunosorbent assay (ELISA) to detect antibodies of the parasite have been successfully used by Wittner, Maayan, Farrer & Tanowitz (1983), as well as others. Counter immunoelectrophoresis (CIE) has been used to detect antigen in feces (Craft & Nelson, 1982) and the ELISA has also been used for the detection of antigens in fecal samples (Ungar, Yooken, Nash & Quinn, 1984).

Following the report by Andrews & Hewlett (1981) that infant mice were protected from *Giardia muris* infection by milk from previously infected mice, Gillin, Reiner & Wang (1983) reported that *G. lamblia* was rapidly killed by normal human milk in vitro. The latter authors suggested that human milk may play a protective role in infants exposed to the parasite. In a further study, Gillin, Reiner & Gault (1985), hypothesized that killing of *G. lamblia* by fresh normal human milk depended upon bile salt stimulated lipase, which must be activated by bile salts. In their initial study, Andrews & Hewlett (1981) reported evidence for involvement of the immune response in controlling giardiasis by demonstrating that previously infected mothers were able to transfer protection to their offspring via breast milk containing anti-giardia IgA and IgG. In another study, Kaplan, Uni, Aikawa & Mahmoud, (1985) reported that both antitrophozoite IgG, secretory IgA and mouse phagocytic cells interacted in vitro to promote parasite clearance. Because both the humoral and cellular components are found in the lumen of the small intestine and in milk, the authors believed that these may represent a biologically relevant protective response against *G. lambia*.

Animal models in studies of *Giardia* spp. are receiving attention. Interesting information is being

obtained, such as those reported above in mice. It has been suggested that the Mongolian gerbil would be useful in epidemiologic studies for the determination of cyst viability and for the identification of the etiological agents (Faubert, Belosevic, Walker, MacLean & Meerovitch, 1983).

AMOEBAE

There are seven amoebae which are considered of importance to humans. Six inhabit the intestines and one is occasionally found in the mouth. Although most are considered non-pathogenic commensals, infection with any one of the intestinal forms indicates fecal contamination; if non-pathogenic protozoa are present there is also a possibility of infection with pathogenic protozoa. In preparation for this review no new or significant information was found on *Entamoeba coli, E. hartmanni, Endolimax nana* and *Iodamoeba beutschlii* except for survey findings. We found all of these parasites in Philippine populations with the prevalence rates of 21%, 3%, 9%, and 1%, respectively.

There have been occasional reports of surveys for *Entamoeba gingivalis*. In a survey of gingival scrapings stained by the Papanicolaou method in the United States, Dao, Robinson & Wong, (1983) recorded 59% of 113 dental patients and 32% of healthy controls as having the parasite. Suhsmann, Neuhold & Matejka (1985) in Australia examined material from the gingival sulcus of 90 normal and the pocket bottom of 120 patients with periodental disease. In the normal group, *E. gingivalis* was present in 26%, while in those with a pocket depth of 3-6 mm and above 6 mm, the organism was found in 40% and 50%, respectively. The organism did not seem to be casually involved in the inflammatory periodontal disease.

Entamoeba histolytica

Most of the recent publications on intestinal protozoa have been on *Entamoeba histolytica* and the number of reports and the diversity of subject matter has been enormous.

The prevalence of intestinal and extraintestinal amoebiasis remains variable throughout the world with rates ranging from less that 1% to over 50%. In the studies conducted in the Philippines an attempt was made to obtain the prevalence and distributions of the parasitosis by stool examination and by serologic testing (Cross & Basaca-Sevilla, 1984). Stool specimens were collected from nearly 20,000 people from all of the major islands and nearly 15,000 sera were obtained from the same people. The stools were preserved in 10% formalin and selected ones placed into polyvinyl alcohol (PVA). The specimens were examined microscopically by direct and after formalin-ether concentration and the PVA specimens were stained with trichrome stain. The indirect hemagglutination (IHA) test was used to test the sera using the axenic HK9 stain of the parasite for antigen. A titer equal to and greater than 1 to 128 was considered significant. The stool positive rate was approximately 6% and the seropositive rate was 5%. Amoebiasis is considered by most clinicians in the Philippines as one of the most important problems, but on many occasions it is felt that much of what is diagnosed as amoebiasis is due to an over-enthusiastic laboratory technician. Over-reporting of the disease is rampant. There are several reports of amoebiasis in 36 to 72 hour old infants based on single direct stool examination (Rife-Iledan & Concel, 1979). Attempts were made to confirm reports of amoebiasis in early infancy by a thorough bacterial, viral and parasitological examination of fecal specimens and by serology testing. The parasite was never found and the IHA and gel-diffusion serologic tests were negative. Rotovirus and certain bacterial pathogens were found, however. There are reports of the disease in infants of three weeks to a few months of age, but none in newborn infants. There was a recent report of a pseudo-outbreak of intestinal amoebiasis in California (CDC, 1985). A laboratory in Los Angeles received a report of 38 cases of enteric amebiasis over a four month period. The laboratory followed approved procedures for the collection and examination of the stools for protozoa and permanent slides were prepared from PVA fixed specimens stained with trichrome. The slides were subsequently examined by another laboratory and 36 were found to be negative for *E. histolytica*. This report shows that even in the best of laboratories over-reporting can also occur. In the California laboratory, 34 slides contained polymorphonuclear neutrophils and/or macrophages and two contained nonpathogenic protozoa.

There have been other interesting epidemiologic reports on amoebiasis in the United States. An outbreak of gastroenteritis was reported in a group of New York City's police and fire department scuba divers following practice dives in sewage-contaminated waters. Stool specimens from 55 divers were examined for intestinal pathogens and one person was found infected with *Campylobacter* sp. and 12 with parasites: five were *E. histolytica* and seven were *G. lamblia*. The scuba divers now practice in waters designated acceptable by the New York City Health Department (CDC, 1983).

A more serious outbreak of amoebiasis occurred in Colorado in 1982. Thirty-six cases occurred in persons who had colonic irrigation therapy at a chiropractic clinic between June 1978 and December 1980.

Ten persons required colectomy and six died. A total of 176 people had been seen at the clinic during the last four months of 1980; 80 had received colonic irrigation and 96, other forms of treatment. Twenty-one percent of those receiving colonic irrigation had bloody diarrhea compared to 1% in the non-irrigation group and 37% of the irrigation group who submitted specimens had evidence of amoebiasis on either stool examination or had positive serum titers. Only 2.4% of the non-irrigation group were positive. Tests of the colonic-irrigation machine after routine cleaning was found to be contaminated with fecal coliform bacteria (CDC, 1981).

During the past several years there have been increasing reports of *E. histolytica* infection among homosexual men. In a study in California, by stool examination and by questionnaire, a total of 855 persons were early participants in the study, but stools and complete questionnaires were received from only 508. Infection with *E. histolytica* was found in over 28.6% and the infection was correlated significantly with a prior history of syphilis or gonorrhea, with the number of sexual partners in the preceding 12-month period, and with the reported frequency of oral-anal sexual contacts. No relation was seen between the presence or chance of gastrointestinal symptoms and infection with *E. histolytica* (Markell, Havens, Kuritsubo & Wingerd, 1984). In earlier studies in England on the mobility patterns of isozymes from cultures of *E. histolytica* from homosexual males, Sargeaunt, Oates, MacLennan, Oriel & Goldmeier (1983) found that none of the isolates corresponded to the pathogenic zymodemes. Later, in Scotland, McMillan, Gilmour, McNeillage & Scott (1984) studied 35 *E. histolytica* infected homosexuals and 35 non-infected homosexual male controls for evidence of intestinal pathology. Isolates of the parasite were tested for isoenzymes and all were of the nonpathogenic zymodeme type I. There was no difference in the numbers of infected and non-infected homosexuals with gastro-intestinal symptoms, but the mean duration of symptoms was greater in those with amoebiasis. The rectal mucosa was abnormal in more persons with amoebiasis than in those without the parasitosis. There was no evidence of intestinal invasion in those with infection and none of the infected persons had significant *E. histolytica* antibody titers. The evidence presented, however, suggests that the parasite had some pathologic effect since proctitis was resolved following anti-amoebic treatment. It is known, however, that proctitis in homosexuals may resolve spontaneously. In homosexual men with AIDS there may be a range of bowel problems due to *E. histolytica* (Macher, 1984). It will be interesting to observe zymodemic patterns in *E. histolytica* isolates from AIDS homosexual patients. Is it possible for strains of the parasite with nonpathogenic zymodemes to be pathogenic in these immunocompromised hosts?

Isoenzyme analysis on cultured *E. histolytica* are showing that virulence of *E. histolytica* could be correlated with zymodeme distribution patterns by starch-gel electrophoresis. At least 18 zymodemes of *E. histolytica* are known (Sargeaunt, Baveja, Nanda & Anand, 1984) for the four enzymes used (malate dehydrogenase, hexokinase, phosphoglucomutase and glucophosphate isomerase.) The virulence judged by clinical criteria correlates with the position of a phosphoglucomutase band on starch-gel electrophoresis. In other studies, Mathews, Moss, Healy & Visvesvera (1983), showed that glucophosphate isomers may also differentiate invasive amoebae from non-invasive amoebae when the isoenzymes are separated by polyacrylamide gel electrophoresis. Sargeaunt and his co-workers have accumulated a great deal of information from various parts of the world and report seven zymodemes associated with clinical disease and tissue invasion. Gathiram & Jackson (1985) described two new zymodemes from Africa, one pathogenic and one, nonpathogenic. In a more recent paper, Sargeaunt (1985) cloned two isoenzymatically distinct strains representing zymodeme II and XIV and cultured them together. After culturing, three zymodemes were demonstrated: II, XIV and the third clearly different from the other two. The new zymodeme has been designated XX SAW LEE and since it consistently gives the same enzyme patterns, this suggests that it is pathogenic to man. These experiments reveal possible genetic exchange and questions arise regarding its origin and whether it was produced by asexual or a sexual process.

A study associating zymodeme patterns and serologic responses was conducted in South Africa and Jackson & Gathiram (1985) and showed that 94-100% of subjects with pathogenic zymodemes were strongly sero-positive compared with 2-4% of subjects with non-pathogenic zymodemes. The study suggests that pathogenic amoebae, even in persons without symptoms, are constantly in contact with host tissue and that the organisms are potentially capable of deeper tissue penetration.

Although isoenzyme electrophoresis has provided a great deal of information on pathogenic and non-pathogenic strains of *E. histolytica*, some investigators still prefer to use animal models. Gill, Ganguly, Mahajan, Bhushnurmath & Dilawari (1984) carried out histopathological studies in guinea pigs infected intracecally with *E. histolytica* and reported finding histological lesions which mimic human intestinal amoebiasis. In studies from Canada, Chadee & Meerovitch (1984) found the Mongolian gerbil a suitable animal model for both intestinal and hepatic ameobiasis. In a later study, Chadee, Smith & Meerovitch (1985) found that the severity of histologically demonstrable cecal lesions in gerbils positively correlated with the isoenzyme patterns characteristic of the more virulent *E. histolytica* strains.

A new experimental model for the production of early stages of invasive amoebiasis was recently

described by Anaya-Velazques, Martinez-Palomo, Tsutsumi & Gonzalez-Robeles (1985), which involves the washing out of the cecum contents of hamsters and guinea pigs and the formation of a closed loop. Trophozoites are introduced into the loop and lesions produced by virulent amoeba can be studied microscopically. Some investigators are also using tissue culture systems to study the pathogenesis of strains of *E. histolytica*. Martinez-Palomo, Gonzalez-Robeles, Chavez, Orozco, Fernandez-Castelo & Cervantes (1985) studied the effect of virulent strains on epithelial monolayers of cells by using time lapse microcinematography and transmission and scanning electromicroscopy. Finegold, Bracha, Wexler & Mirelman (1985) studied enterotoxins from extracts of *E. histolytica* and reported that soluble cell-free extracts of *E. histolytica* and serum-free media in which trophozoites were incubated were found to contain substances that caused rapid rounding up and detachment of tissue-cultured monolayers of mammalian cells (cytopathic activity) and induced fluid secretions in ligated intestinal loops in rats (enterotoxic activity).

A variety of serologic tests have been developed to detect antibodies associated with invasive amoebiasis. The most commonly used tests today are the IHA, ELISA, and gel-diffusion. These tests are acceptable for the detection of antibody but a more accurate immunodiagnosis could be made by demonstrating antigens from the parasite. One approach to this would be to use monoclonal antibodies to detect antigens in stools, serum or liver aspirates. Ungar, Yolken & Quinn (1985) have reported the value of ELISA in detecting *E. histolytica* in feces using both monoclonal antibody and rabbit antisera. The ELISA was said to be a simple, sensitive and specific diagnostic tool.

SPOROZOA

There have been many interesting developments among the sporozoans during the past decades. The life cycle of *Toxoplasma gondii* was determined as was that of *Isospora belli* and *Sarcocystis hominis*. During the past few years two sporozoans not previously considered important human pathogens have received considerable attention: *Cryptosporidium* sp. and *Blastocystis hominis*.

Isospora belli and *Sarcocystis hominis*

There have been only a few reports on *I. belli* and *S. hominis* during the past several years. In France, after the examination of 3,500 fecal samples by flotation and after ether concentration, 5 cases of *I. belli* were detected in patients coming from tropical areas and who had gastrointestinal symptons. Seventy cases of *S. hominis* were found also in persons returning from the tropics but they did not have symptons that could be associated with infection (Deluol, Mechali, Cenac, Savel & Coulaud, 1980). In California, *Isospora* enteritis was reported in three homosexual males (Forthal & Guest, 1984).

Cryptosporidium

Although cryptosporidiosis has been known for many years, human infection was not reported until recently. The majority of the first reports in humans were in immunocompromised persons, especially persons with AIDS. These reports were followed by others on infections in immunocompetent persons from various parts of the world. We reported detecting the parasite in the feces of 2.6% of 735 children with diarrhea seen in a Manila hospital (Cross, Alcantara, Alquiza, Zaraspe & Ranoa, 1985) and reports of children with diarrhea and passing oocysts have also been made from Brazil (Weikel, Johnston, de Sousa & Guerrant, 1985) and elsewhere in Latin America. *Cryptosporidium* oocysts have also been found in sputum of an adult male with AIDS (Miller, Wasserheit, Kirihara & Coyle, 1984). The finding of *Cryptosporidium* oocysts in the feces of a hospital intern with diarrhea who was responsible for the care of an AIDS patient has become of great concern (Koch, Phillips, Aber & Current, 1985) since it suggested that the parasite can be transmitted person to person in the hospital environment.

It is quite apparent from the myriad of reports that *Cryptosporidium* is a ubiquitous parasite. It is found in both immunocompetent and immunocompromised persons, adults and children. It may be acquired from animals and from humans. It may be an occupational hazard in persons working with animals and has been shown to be a cause of travellers diarrhea as well as other types of gastrointestinal disturbances.

Blastocystis hominis

Blastocystis hominis for several decades was considered a harmless yeast found in the feces of persons with and without diseases. Zierdt and his associates found the organisms interesting and after a series of studies established that it was not a yeast but a protozoan belonging to the sporozoa. In later studies it was shown to be a parasite of man capable of causing disease (Zierdt, 1983). The increasing numbers of reports are clearly incriminating *B. hominis* as pathogenic. Ricci, Toma, Furland, Casell & Ginnin (1984) reported diarrhea in two patients with only *B. hominis*. Taylor, Echeverria, Blaser, Pitarangsi, Blacklow, Cross &

Weniger (1985) found the parasite in 7% of 35 American Peace Corps members with diarrhea and Vannatta, Adamson & Mullican (1985) presented a patient with recurrent diarrhea who had large number of *B. hominis* in his stools. Like *Cryptosporidium*, *B. hominis* should be considered as a cause of diarrhea, especially in travellers.

CILIATE

Although *Balantidium coli* is found everywhere in pigs, human infections are rare. In studies in the Philippines, it was found only occasionally (Cross & Basaca-Sevilla, 1984). There was a case report from Italy in which *B. coli* was found on a cervicovaginal smear of a patient who was in daily contact with pigs but did not have intestinal balantidiasis (Rivasi & Giannotti, 1983). Two cases of balantidial diarrhea were reported from Spain, a fatal case was reported from Venezuela in which the parasite was found in the intestines and lung, and in Ecuador, *B. coli* was found to be the cause of bloody-mucoid diarrhea and hepatic abscess.

SUMMARY

An attempt was made in this report to present an update on the recent development on intestinal protozoan infections in humans. Except for a few historical references the review covers the period from 1980 to the time of writing, mid-1985. The emphasis was on the more important parasites and an effort made to cover the latest developments in their biology, epidemiology and pathogenesis. During preparation of this paper I was impressed with the plethora of papers published on some parasites and the paucity of reports on others. There are an increasing number of papers on *Cryptosporidium* sp. and the interest in the organisms should continue. Furthermore, it will be of interest to follow the association between *Blastocystis hominis* and disease. These are essentially new protozoan parasites of man, and one wonders how many more intestinal protozoan parasitosis are still waiting to be found. Like the *Cryptosporidium* sp., it may be a matter of finding the right diagnostic technique to detect the unknown organism.

Giardiasis continues to be a cause of diarrhea among various groups especially campers who are drinking untreated water and *G. lamblia* as well as *E. histolytica* are being found more frequently in homosexuals with and without AIDS. The ability to predict virulence in strains of *E. histolytica* by enzyme patterns is intriguing but some skeptics still prefer the older test for virulence by cecal scoring in animals. New animal models are being evaluated and new techniques applied to the study of pathogenic protozoa. In the future the use of new biotechnological methods will most certainly lead to a better understanding of intestinal protozoa as well as of other parasitic organisms.

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