

Intestinal Protozoan Infestation and Systemic Illness

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The gastrointestinal tract is the largest organ of immune surveillance in the body, home to two-thirds of the total lymphocyte population.¹ Intestinal lymphocytes manifest a variety of responses which depend upon their CD phenotype, histologic location and communication with other effector cells. Stimulation of intestinal immune response networks by lumen-dwelling microbes may produce a variety of systemic responses that are independent of gastrointestinal symptoms.² Immunologic hypersensitivity to *Giardia lamblia* has been shown to provoke asthma^{3 4}, urticaria^{5 6 7 8 9 10 11}, arthritis (reference 11 +^{12 13 14}; ¹⁵ and uveitis¹⁶. Hypersensitivity reactions may occur in the absence of digestive complaints (refs 6, 10, 15). In none of these cases was the mechanism of hypersensitivity known; eosinophilia was a feature in only two cases (refs 10,11)). A high frequency of pre-existing atopic disease occurs in patients with chronic giardiasis^{17,18} and may be a factor in susceptibility to infection.

Immunologic mechanisms other than hypersensitivity reactions may also be associated with chronic giardiasis. In humans, chronic giardiasis has been associated with deficiency of secretory IgA¹⁹ and with impaired macrophage cytotoxicity²⁰, characteristics that may predispose to systemic illness. Athymic mice chronically infected with *Giardia muris* do not develop mucosal damage²¹. Gillon *et al.* have proposed that the release of enteropathic lymphokines by intraepithelial T cells is the cause of the intestinal injury in chronic giardiasis²². In humans, the severity of malabsorption observed with chronic giardiasis is more closely related to the presence of intraepithelial lymphocytes and the antibody titer to *Giardia* cyst antigen than to the estimated parasite burden²³. A normal

immunologic response to the parasite may be necessary to avoid chronic infection but also creates much of the tissue damage associated with chronic giardiasis.

Giardia may provoke systemic illness by non-immunologic mechanisms. *G. lamblia* can cause intestinal protein loss without producing diarrhea²⁴. Specific micronutrient deficiencies have also been described in chronic giardiasis. Low levels of carotene and folate²⁵ and abnormal vitamin A, folic acid and vitamin B12 absorption (ref 24) occurs in a large minority of patients with chronic symptoms. Nutritional deficiency associated with chronic giardiasis may add to the burden of illness. Bacterial overgrowth of the small bowel has been described in giardiasis and may contribute to malabsorption (ref 24 +²⁶. Colonization of the jejunum with *Candida albicans* was reported in 30% of patients with giardiasis and was absent in controls²⁷. A role for intestinal candidosis in provoking systemic illness has been debated for a quarter century (review in ref # 2). Some strains of *G. lamblia* contain double-stranded RNA viruses²⁸. The role of *Giardia* as a vector for viral infection requires further study.

Galland et. Al. conducted a two-year retrospective study of 218 patients who presented to our medical clinic with a chief complaint of chronic fatigue (ref 18). *G. lamblia* infection was identified in 61 patients. The symptoms of patients with and without giardiasis, are shown in Table 1. All patients with giardiasis and 86% of patients without giardiasis complained of digestive symptoms, but these were generally mild. The most interesting difference between the two groups lies in the positive association between giardiasis and

symptoms such as myalgia, muscle weakness, flu-like feelings, sweats and adenopathy. In fact, 61% of fatigued patients with giardiasis had been diagnosed elsewhere as suffering from chronic fatigue immune dysfunction syndrome (CFIDS), compared to only 19% of fatigued patients without giardiasis. Cure of giardiasis resulted in clearing of fatigue and related 'viral' symptoms (myalgia, sweats, flu-like feelings) in 70% of cases, some palliation of fatigue in 18%, and was of no benefit in 12%. The association between intestinal protozoa and chronic fatigue in patients without prominent digestive complaints may not be limited to giardiasis. In an unpublished presentation, Galland reported that 80% of patients with a diagnosis of CFIDS who were infected with the protozoan *Blastocystis hominis* showed significant improvement of fatigue associated with treatment that cleared the protozoa from stool specimens.²⁹

Chronic infestation with *Entamoeba histolytica*, another common protozoan parasite, has been associated with autoimmune phenomena, including the appearance of antibodies to colonic epithelial cells³⁰ and the development of ulcerative colitis after cure of amebic colitis³¹. Extra-intestinal autoimmune reactions to intestinal amebiasis include a case of antiphospholipid antibody syndrome with deep vein thrombosis and pulmonary embolism³² and development of symmetrical polyarthritis very similar to rheumatoid arthritis (RA)^{33 34 35}. Singh et al.³⁶ measured amoebic antibody levels in 41 Indian patients with a primary diagnosis of RA, 35 age- and sex-matched healthy volunteers, 162 hospital inpatients and 26 patients with other arthritides. Amoebic antibodies were elevated in 39% of RA patients and 0-11% of the various control groups. Only two patients with RA had experienced recent diarrheal disease. These authors suggest that an

excessive and prolonged antibody response to *Entamoeba histolytica* or other enteric organisms may contribute to joint inflammation in RA.

Galland (ref 15) described a patient with rheumatoid-like arthritis and antinuclear antibodies whose arthritis went into rapid and complete remission upon treatment of *G. lamblia* infection with metronidazole. Relapse occurred when the patient acquired *Entamoeba histolytica* during a trip to Egypt; remission occurred slowly following treatment of amoebiasis. Diarrhea, polyarthritis and circulating antinuclear antibodies developed in a United States serviceman heavily infested with *Endolimax nana*, an allegedly non-pathogenic ameba³⁷ Metronidazole rapidly reversed all abnormalities. The reported cases of amoebic arthritis may represent a variant of parasitic rheumatism, an inflammatory polyarthropathy produced by circulating antigen-antibody complexes³⁸. The presence of autoantibodies, however, is not characteristic of parasitic rheumatism, and suggests other mechanisms of immune dysfunction: either a pre-existing disease is exacerbated by intercurrent amoebic infection or amoebic infection itself provokes autoimmunity, perhaps mediated by the action of immune response genes (ref 23). Reiter's Syndrome (arthritis, uveitis and urethritis) has been reported as a complication of infection with two other intestinal protozoa, *Cryptosporidium*³⁹ and *Cyclospora*.⁴⁰ *Cyclospora cayatenensis* has also provoked Guillain-Barre syndrome, a severe autoimmune neuropathy.⁴¹

Entamoeba histolytica contains a soluble lectin which is mitogenic for T lymphocytes⁴²

⁴³. T helper cell activation by this lectin may induce HIV replication *in vivo*. A soluble

Entamoeba histolytica protein, although not mitogenic itself, induced HIV replication in tissue culture of lymphocytes obtained from three out of seven men with chronic HIV infection⁴⁴. Infection with *E. histolytica* and other parasites may promote the development of AIDS in HIV-infected individuals^{45 46}. Epidemiologic evidence associates pre-existing intestinal protozoan infection with the appearance of Kaposi's sarcoma among homosexual men in the United States.⁴⁷ Although the influence of treating intestinal protozoan infection on the course of HIV infection has not been systematically studied, treatment of intestinal helminth infestation decreases the HIV viral load among African patients with AIDS.⁴⁸ Synergism between intestinal parasites and other lymphotropic retroviruses has been advanced as an explanation for the pathogenesis of Burkitt's lymphoma⁴⁹ and adult T cell leukemia/lymphoma⁵⁰

Protozoan infection is usually diagnosed by stool examination, however, comparison of stool microscopy with duodenal aspiration has consistently shown that stool may fail to contain identifiable parasites even at the height of acute giardiasis.^{51 52}Collecting multiple specimens over several days increases the sensitivity to 85-90%⁵³. Laboratories that specialize in tropical medicine or parasitology are more likely to find organisms in stool specimens than are general or hospital laboratories [reference to be supplied]. Some authors have suggested empirical treatment for intestinal parasites in high risk groups, such as immigrants to the United States from Asia, the Middle east, sub-Saharan Africa, Eastern Europe, Latin America and the Caribbean and have justified this on a cost effective basis, given the safety of current medical therapies.⁵⁴ A similar case might be

made for treating chronically ill patients at high risk for parasitic infection because of residence, travel, sexual practices or the context in which illness occurred.

Numerous naturally occurring substances have anti-protozoan activity. The most extensively studied is *Artemisia annua* (sweet Annie or qinghao), a plant that yields the lactone artemisinin (qinghaosu) which is the basis for a new class of anti-malarial compounds widely used in Asia and Africa.⁵⁵ Artemisinin is thought to owe its anti-protozoan effects to its content of endoperoxides and to kill parasites through oxidation. Its activity, at least in the treatment of Simian malaria, is enhanced by co-administration of cod liver oil and diminished by co-administration of vitamin E (reference to follow). Artemisinin has low toxicity. In addition to its antibiotic activity, it stimulates macrophages, an important component of the immune response to protozoan infestation.⁵⁶ Artemisinin may induce abortion if given during pregnancy.

The alkaloid berberine can be extracted from the roots of several plant species, notably *Berberis aquifolium* (Oregon grape), *Hydrastis Canadensis* (goldenseal) root, and *Coptis chinensis* (goldthread). Berberine has protostatic and protocial activity against *E. histolytica*, *G. lamblia* and *B. hominis*.^{57 5859} It has shown benefit in the treatment of giardiasis in children⁶⁰

Allium sativum (garlic) and *Juglans nigra* (black walnut) have a long history of use as antimicrobials. Allicin inhibits growth of *E. histolytica* in culture⁶¹ and may be responsible for the antimicrobial activity of garlic.⁶²

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Title

*Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in *hymenolepiasis nana* and *giardiasis*.*

Source

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Abstract

*The effect of serial dilutions of crude garlic (*Allium sativum*)*

extract on adult Hymenolepis nana was studied to detect the minimal lethal concentration which was found to be 1/20. A. sativum was tried in the treatment of 10 children infected with H. nana and 26 infected with G. lamblia as 5 ml crude extract in 100 ml water in 2 doses per day, or commercial preparation (0.6 mg capsules) 2 capsules twice/day for 3 days. A sativum was found to be efficient, safe and shortens the duration of treatment. The possible mode of action of A. sativum and the correlation between the clinical and parasitological findings were discussed.

The intestinal bacterial milieu may be important in the treatment of protozoan infestation, especially for colonic organisms like *E. histolytica*. Pathogenic strains of *Entameba histolytica* are able to evade lysis by both classical and alternative pathways of complement. Intestinal bacteria, *E. coli* in particular, are necessary for complement resistance and for amebic virulence⁶³. Mirelman has suggested that ingested bacteria lower the redox potential within the parasite and allow the amebae to escape destruction by oxidative enzymes⁶⁴ He has reported that one can reversibly change the zvmodeme patterns of *Entameba histolytica* isolates from non-pathogenic to invasive by culturing

amebae with the gut flora of patients who have either invasive disease or no symptoms.⁶⁵

⁶⁶. Optimal treatment of protozoan infection requires not only the administration of antimicrobial substances, but strategies aimed at enhancing the function of intestinal resistance factors like secretory IgA and phagocyte function and creating a bacterial milieu that is not parasite friendly.

Table 1 Systemic symptoms of CFS patients

	With giardiasis (%) (N = 63)	Without giardiasis (%) (N = 157)
Depression	61	41
Muscle weakness	46	19
Headache	41	36
Sore throat	41	11
Lymphadenopathy	36	8
Arthralgia	36	27
Myalgia	34	18

- ¹ Targan, R. S., Kagnoff, F. M., Brogan, M. D. and Shanahan, F. (1987) Immunologic mechanisms in intestinal disease. *Annals of Internal Medicine*, 106, 854-870.
- ² Galland L. The Effect of Systemic Microbes on intestinal Immunity. Post-Viral fatigue Syndrome, R. Jenkins and J. Mowbray, editors. John Wiley & Sons Ltd. London. 1991. pp 405-430.
- ³ Fossati, C. (1971) Manifestazioni broncopulmonari in corso di infestazione da *Giardia lamblia*. *Revista Iberica de Parasitologia*, 31, 283-298.
- ⁴ Lopez-Brea, M., Sain, Z. T., Camarero, C. and Baquero, M. (1979) *Giardia lamblia* associated with bronchial asthma and serum antibodies, and chronic diarrhoea in a child with giardiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73, 600.
- ⁵ Harris, R. H. and Mitchell, J. H. (1949) Chronic urticaria due to *Giardia lamblia*. *Archives of Dermatology and Syphilology*, 59, 587-589.
- ⁶ Wilhelm, R. E. (1958) Urticaria associated with giardiasis lamblia. *Journal of Allergy*, 28, 351-353.
- ⁷ Webster, B. H. (1958) Human infection with *Giardia lamblia*. *American Journal of Digestive Diseases*, 3, 64-71.
- ⁸ Dellamonica, P., Le Fichoux, X., Monnier, B. and Duplay, H. (1976) Syndrome dysenterique et urticaire, au cours d'une giardiase. *Nouvelle Presse Medicale*, 5, 1913.
- ⁹ Weisman, B. L. (1979) Urticaria and *Giardia lamblia* infections. *Annals of Allergy*, 49,91.
- ¹⁰ Kennou, M. F. (1980) Skin manifestation of giardiasis. Some clinical cases. *Archives de Institut Pasteur de Tunis*, 51, 257-260.
- ¹¹ Farthing, M. J. G., Chong, S. K. F. and Walker-Smith, J. A. (1983) Acute allergic phenomena in giardiasis. *Lancet*, 2, 1428.
- ¹² Goobar, J. P. (1977) Joint symptoms in giardiasis. *Lancet*, 1, 1010-1011.

¹³ Woo, P. and Panayi, G. S. (1984) Reactive arthritis due to infestation with *Giardia lamblia*. *Journal of Rheumatology*, 11, 719.

¹⁴ Shaw, R. A. and Stevens, M. B. (1987) The reactive arthritis of giardiasis. A case report. *Journal of the American Medical Association*, 258, 2734-2735.

¹⁵ Galland, L. (1989) Intestinal protozoan infection is a common unsuspected cause of chronic illness. *Journal of Advancement in Medicine*, 2, 529-552.

¹⁶ Carroll, M. E., Anast, B. P. and Birch, C. L. (1961) Giardiasis and uveitis. *Archives of Ophthalmology*, 65, 775-778.

¹⁷ Chester, C. A., Macmurray, F. G., Restifo, M. D. and Mann, O. (1985) Giardiasis as a chronic disease. *Digestive Disease and Sciences*, 30, 215-218.

¹⁸ Galland, L., Lee, M., Bueno, H. and Heirnowitz, C. (1990) *Giardia lamblia* infection as a cause of chronic fatigue. *Journal of Nutritional Medicine*, 2, 27-32.

¹⁹ Vinayak, V. K., Kij, K., Venkateswarlu, K., Khanna, R. and Mehta, S. (1987) Hypogammaglobulinemia in children with persistent giardiasis. *Journal of Tropical Pediatrics*, 33, 140-142.

²⁰ Smith, P. D., Gillin, F. D., Spira, W. M. and Nash, T. E. (1982) Chronic giardiasis: Studies on drug sensitivity, toxin production, and host immune response. *Gastroenterology*, 83, 797-803.

²¹ Roberts-Thompson, I. C. and Mitchell, G. F. (1978) Giardiasis in mice 1. Prolonged infections in certain mouse strains and hypothyroid (nude) mice. *Gastroenterology*, 75,

42-46.

²² Gillon, J., Althamery, D. and Ferguson, A. (1982) Features of small intestinal pathology (epithelial cell kinetics, intraepithelial lymphocytes, disaccharidases) in a primary *Giardia muris* infection. *Gut*, 23, 498-506.

²³ Solomons, N. W. (1982) Giardiasis: nutritional implications. *Reviews of Infectious Diseases*, 4, 859-869.

²⁴ Sherman, P. and Liebman, W. L. (1980) Apparent protein-losing enteropathy associated with giardiasis. *American Journal of Diseases of Children*, 134, 893-894.

²⁵ Brasitus, T. A. (1983) Parasites and malabsorption. *Clinics in Gastroenterology*, 12, 495-511.

²⁶ Tompkins, A. M., Wright, S. G., Draser, B. S. and James, W. P. T. (1978) Bacterial colonization of jejunal mucosa in giardiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 72, 33-36.

²⁷ Naik, S. R., Rau, N. R., Vinajak, V. K., Narayanna, V. A., Zunzurwade, S., Sehgal, S. C. and Talwar, P. (1978) Presence of *Candida albicans* in normal and in *Giardia lamblia* infected human jejunum. *Annals of Tropical Medicine and Parasitology*, 72, 493-494.

²⁸ denHollander, N., Riley, D. and Befus, D. (1988) Immunology of *Giardia*. *Parasitology Today*, 4, 124-133.

²⁹ "Patient-centered diagnosis and treatment for chronic fatigue syndrome", annual scientific session of the American Academy of Environmental Medicine, Virginia beach, Virginia, October 7, 1994.

³⁰ Salem, E., Zaki, S. A., Moneim, W. A., Kadry, S., Eisa, H. and Ezz, F. A. (1973) Antoantibodies in amoebic colitis. *Journal of the Egyptian Medical Association*, 56, 113-118

³¹ Sturgess I, Greenfield SM, Teare J, O'Doherty MJ. (1992). Ulcerative colitis developing after amoebic dysentery in a haemophiliac patient with AIDS. *Gut*. 33, 408-410.

³² Korkmaz C, Harmanci E, Metintas I, Gulbas Z . (2001). Antiphospholipid syndrome associated with intestinal amoebiasis. *Scandinavian Journal of Infectious Disease* 33,:938-40

³³ Zinneman, H. H. (1950) Ten cases of amebiasis with arthritic complaints. *American Journal of Digestive Diseases*, 17, 343-344.

³⁴ Rappaport, E. M., Rossieu, A. X. and Rosenblum, L. A. (1951) Arthritis due to intestinal amebiasis. *Annals of Internal Medicine*, 34, 1224-1231.

³⁵ Kasliwal, R. M. (1970) Correlation between amoebic colitis and rheumatoid arthritis. *Journal of the Association of Physicians of India*, 18, 739-743.

³⁶ Singh, I. P., Das, S. K., Sharma, P., Dutta, G. P. and Agarwal, S. S. (1985) Antibodies to *Entamoeba histolytica* in patients with rheumatoid arthritis. *Tropical Gastroenterology*, 6, 141-144

³⁷ Burnstein, S. L. and Liakos, S. (1983) Parasitic rheumatism presenting as rheumatoid arthritis. *Journal of Rheumatology*, 10, 514-515.

³⁸ Bocanegra, T. S., Espinoza, L. R., Bridgeford, P. H., Vasey, F. B. and Germani, B. F. (1981) Reactive arthritis induced by parasitic infestation. *Annals of Internal Medicine*, 94, 207-209.

³⁹ Cron RQ, Sherry DD (1995). Reiter's syndrome associated with cryptosporidial gastroenteritis. *Journal of Rheumatology* 22, 1962-3

⁴⁰ Connor BA, Johnson EJ, Soave R. (2001) Reiter syndrome following protracted symptoms of Cyclospora infection. *Emerging Infectious Diseases* 7, 453-4

⁴¹ Richardson RF Jr, Remler BF, Katirji B, Murad MH. (1998), Guillain-Barre syndrome after Cyclospora infection. *Muscle nerve*. 5, 669-671.

⁴² Chen, Z. C., Herrmann, F., Koleski, F. and Diamantstein, T. (1985) Mitogenic factor for T inducer/ helper cells in *Entamoeba histolytica* extracts. *Acta Academiae Medicinae Wuhan*, 5 (4), 213-216.

- ⁴³ Petri, W. A. and Ravdin, J. I. (1986) Treatment of homosexual men infected with *Entamoeba histolytica*. *New England Journal of Medicine*, 315, 393.
- ⁴⁴ Croxson, S., Mildran, D., Mathews, H. and Poiez, B. J. (1988) *Entamoeba histolytica* antigen-specific induction of Human Immunodeficiency Virus replication. *Journal of Clinical Microbiology*, 26, 292-294.
- ⁴⁵ Pearce, R. B. and Abrams, D. (1987) *Entamoeba histolytica* in homosexual men. *New England Journal of Medicine*, 316, 690-691.
- ⁴⁶ Archer, D. L. and Glinsman, W. H. (1985) Enteric infections and other co-factors in AIDS. *Immunology Today*, 6, 292-294.
- ⁴⁷ Abrams DI. (1990). The relationship between Kaposi's sarcoma and intestinal parasites among homosexual males in the United States. *Journal of the Acquired Immune Deficiency Syndrome* ;3 Suppl 1:S44-6
- ⁴⁸ Wolday D, Mayaan S, Mariam ZG, Berhe N, Seboxa T, Britton S, Galai N, Landay A, Bentwich Z. (2002). Treatment of intestinal worms is associated with decreased HIV plasma viral load. *Journal of the Acquired Immune Deficiency Syndrome* 31, 56-62
- ⁴⁹ Burkitt, D. P. (1983) The discovery of Burkitt's lymphoma. *Cancer*, 51, 1777-1786.
- ⁵⁰ Tajima, K., Tominaga, S., Shimizu, H. and Suchi, T. (1981) A hypothesis on the etiology of adult T-cell leukemia/ lymphoma. *Gann*, 72,684-691.
- ⁵¹ Rosenthal, P. and Liebman, W. M. (1980) Comparative study of stool examinations, duodenal aspiration, and pediatric Entero-Test for giardiasis in children. *Journal of Pediatrics*, 96, 278-279.
- ⁵² Kamath, K. R. and Murugasu, R. (1974) A comparative study of four methods for detecting *Giardia lamblia* in children with diarrheal disease and malabsorption. *Gastroenterology*, 66, 16-21.

- ⁵³ Gillon, J. (1984) Giardiasis: Review of epidemiology, pathogenetic mechanisms and host responses. *Quarterly Journal of Medicine*, 53, 29-39.
- ⁵⁴ Muenning P, Pallin D, Sell RL et al (1999). The cost effectiveness of strategies for the treatment of intestinal parasites in immigrants. *New England Journal of Medicine*, 340, 773-779.
- ⁵⁵ Hien TT, White NJ. (1993) Qinghaosu. *Lancet*. 341, 603-608.
- ⁵⁶ Tang W, Eisenbrand G. (1992) Chinese Drugs of Plant Origin. Springer-Verlag, Berlin, 159-174.
- ⁵⁷ Kaneda Y, Tori N, Tanaka T, Aikawa M. (1991) In vitro effects of berberine sulfate on the growth and structure of *Entamoeba histolytica*, *Giardia lamblia* and *Trichomonas vaginalis*. *Annals of Tropical Medicine and Parasitology*. 85, 417-425.
- ⁵⁸ Subbaiah TV, Amin AH. (1967) Effect of berberine sulfate on *Entamoeba histolytica*. 215:527-528.
- ⁵⁹ Yang LQ, Singh M, Yap EH, et al (1996). In vitro response of *Blastocystis hominis* against traditional Chinese medicine. *Journal of Ethnopharmacology*. 55, 35-42.
- ⁶⁰ Gupte S. (1975). Use of berberine in treatment of giardiasis. *American Journal of Diseases of Childhood*. 129, 866.
- ⁶¹ Mirelman D, Monheit D, Varon S (1987). Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle in garlic extract (*Allium sativum*). *Journal of Infectious Diseases*. 156, 243-244.
- ⁶² Wright CW, Phillipson JD, (1987). Natural products and the development of selective antiprotozoan drugs. *Phytotherapy Research*. 4, 1127-139.
- ⁶³ Wittner, M. and Rosenbaum, R. M. (1970) Role of bacteria in modifying virulence of *E. histolytica*. Studies of amoebae from axenic cultures. *American Journal of Tropical Medicine and Hygiene*, 19, 755-761.
- ⁶⁴ Gitler, C. and Mirelman, D. (1986) Factors contributing to the pathogenic behavior of *Entamoeba histolytica*. *Annual Reviews of Microbiology*, 40, 237-261.
- ⁶⁵ Mirelman, D., Bracha, R., Chayen, A., Anst-Kettis, A. and Diamond, L. S. (1986) *Entamoeba histolytica*: effect of growth conditions and bacterial associates on isoenzyme patterns and virulence. *Experimental Parasitology*, 62, 142-148.
- ⁶⁶ Mirelman, D. (1987) Effect of culture condition and bacterial associates on the zymodemes of *Entamoeba histolytica*. *Parasitology Today*, 3, 37-40.