# Evaluation of Trichrome-PLUS™ Stain

## A New Permanent Stain and Procedure for Intestinal Parasites in Fecal Specimens




### Abstract

*Heavy metal based, low-viscosity polyvinyl alcohol (LV-PVA, ZNPVA, Z-PVA, PVA-Hg and modified PVA) have been widely used by clinical laboratories to perform permanent stained smears from fecal specimens to demonstrate the presence of intestinal parasites. Regulatory, environmental concerns and costs have forced laboratories to consider alternative fixatives for these procedures. We compared the Trichrome-PLUS™ stain permanent stained smears, taken from the concentrated fecal fixed specimen using the Proto-fix™ fixative with CONSED™ Sedimentation Reagent (viewed as wet mount), at the Parasitology Center, Inc. (PCI) in Arizona. Test results of the same concentrated specimens were compared using the wet preparation against the permanent stained smear. The number and identity of parasite species, fungi, blood cells and crystals were identical in all 61 tests compared. The intensity of infection measured on a scale of 1 (rare) to 4 (many) was identical in 75% of the 61 samples tested. Differences in intensity levels, in the remaining 25% of the specimens, were variable by only one degree of intensity. Our data suggests that the Trichrome-PLUS™ stain provides an ideal system to obtain a permanent record of findings, using the Proto-fix™ fixative and CONSED™ Sedimentation Reagent.*


### 1. Introduction

Schaudinn's fixative and low-viscosity polyvinyl alcohol *(PVA)* fixatives which contain heavy metal bases *(i.e. mercuric chloride, zinc sulfate or copper sulfate)* have been used to preserve stool specimens for the recovery of intestinal parasites utilizing permanent stained smears without concentration techniques *(Brooke and Goldman, 1949, Garcia et al., 1979, 1983; Garcia and Voge, 1980; Horen, 1981, Isenberg, 1992; Melvin and Brooke, 1982).* PVA fixative *(a plastic resin with a modified Schaudinn's fixative)* is used to prepare permanent smears for viewing protozoan cysts and trophozoites stained with Wheatley's *(1951)* trichrome stain *(Committee on Education, 1977; Garcia et al., 1979; Garcia and Bruckner, 1997; Isenberg, 1992; NCCLS, 1997).* Formalin, a toxic carcinogen, is a fixative used to preserve and view helminth eggs, larvae and protozoan cysts utilizing a wet preparation following a formalin-ethyl acetate concentration procedure *(NCCLS, 1997).* Mercuric chloride must be disposed of through controlled disposal companies. Zinc sulfate *(contained in Zn-PVA, Z-PVA, and several single-vial parasitology fixatives)* and zinc sulfide are controlled hazardous materials as per the Clean Water Act and the Resources Conservation and Recovery Act.

To meet the challenges of regulatory controls, technology advances *(i.e. EIA and PCR procedures)*, and cost containment demands of clinical laboratories, single-vial fixatives have been introduced into the diagnostic marketplace. We evaluated one of these single-vial fixatives, Proto-fix™ *(Alpha-Tec Systems, Inc.; P.O. Box 5435, Vancouver, WA, USA 98668-5435)* to compare the value of the permanent stained smear in correlation to the concentrated wet preparation slide.

The Proto-fix™ is an environmentally safe, non-flammable, parasitology fixative, free from all heavy metals. It is suitable for EIA procedures *(i.e. Giardia and Cryptosporidium)*, direct fluorescent antibody *(DFA)* stains, special stains *(Microsporidium spp., Cryptosporidium stains, etc.)*, and PCR testing. The newly developed Trichrome-PLUS™ Stain provides a rapid permanent stain from the concentrated fecal fixed specimen using the Proto-fix™ fixative and CONSED™ Sedimentation Reagent. The superiority of the Proto-fix™ and CONSED™ system over the formalin-ethyl acetate concentration method has been documented by Allen and Frankel. *(1997, Am. Soc. Microbiol. Meet., Helen, GA)* and Amin *(2000).* The ease of concentrating the specimen, diagnostic clarity of both parasites and the background, and the increased detection and identification of larger numbers of protozoa, helminth eggs and larvae have also been demonstrated *(Allen and Frankel, loc. cit., 1997; Amin, 2000; Jensen et al., 2000).*

This paper is a follow up on a recent work by Amin *(2000)* evaluating the performance of the Proto-fix™/CONSED™ system.

### 2. Material and Methods

Human fecal specimens were collected in Proto-fix™ fixative supplied in mailable kits throughout the United States under physicians' orders and submitted

to the Parasitology Center, Inc. in Arizona for processing and identification. All specimens were first processed and stained in CONSED™ as described by Amin *(2000)* and briefly summarized as follows. Preserved specimens were filtered to remove debris. One ml. of filtered specimen was retained, mixed with 8 ml. of CONSED™ solution and vortexed for 10 s. Four ml. of ethyl acetate were added, the vial recapped, shaken, and vortexed. The specimen was centrifuged for 5 min. at 600xg. The three upper layers of ethyl acetate, fatty and fecal debris, and reagent were removed leaving the fecal plug undisturbed. CONSED™ Diluting reagent was added to the plug *(3-5 drops)* and mixed with the pellet using a wooden applicator stick. The pellet was transferred to a microscopic slide to make a wet mount. The wet mount was scanned using light microscopy at 10X, then at 40X and 100X *(oil)* for definitive diagnosis.

The procedure for Trichrome-PLUS™ using CONSED™ concentrated fixed specimens produced by the above procedure is summarized as follows: the CONSED™ concentrated fixed specimen was spread evenly over a slide coated with ATS Slide Coating Solution; the specimen was chopped using an applicator stick, and spread out. Excess fluid was allowed to drain, and slides to dry for 10 minutes. The following steps were used for Trichrome-PLUS™ staining and stain removal and draining after each step: 1 minute in 70% alcohol *(ATS#033-59)*, 2-4 dips in distilled water, 4 minutes in Trichrome-PLUS™ stain *(ATS #041-11)*, 2 dips in distilled water, 2-4 dips in Trichrome-PLUS™ decoloizer *(ATS #041-15)*, 5 dips in 100% alcohol *(ATS #033-03)*, 5 dips in 100% alcohol, and 1 minute in 100% alcohol. Proceed to examine the specimen after it has properly dried or add the following 2 steps if using coverslip: 5 dips in xylene *(ATS #033-42)* or PRO-Clear™ *(ATS #033-36)* and 3 minutes in xylene or PRO-Clear™, remove and coverslip. With this preparation, only light microscopy at 100X *(oil)* was used for diagnosis.

Only one observer *(OA)* made the diagnosis of wet and permanent mounts, using the same fecal plug of each specimen tested.

## 3. Results

*Table 1* summarizes the results obtained using the Proto-fix™ preserved specimens, concentrated with the CONSED™ Sedimentation Reagent method and those stained with Trichrome-PLUS™ Stain. The Trichrome-PLUS™ Stain procedure is a new and the only method available to obtain a permanent record of specimens originally diagnosed using the Proto-fix™/CONSED™ wet mount preparations. The number and identity of parasite species, fungi, white and red blood cells, and crystals, seen on the permanent stained smear were identical in all 61 samples compared to the concentrated wet preparation slide, as displayed in *Table 2*. The intensity of infection, measured on a scale of 1 [rare], 2 [few], 3 [moderate] and 4 [many] was identical in 46 (75%) of the 61 samples tested. The intensity levels of the remaining 15 *(25%)* varied by only one degree of intensity. Of the 61 specimens tested, 10 *(16%)* were singly or multiply infected with parasites, 41 *(67%)* specimens contained fungi *(e.g. Candida and/or other yeast)*, 12 *(20%)* specimens contained blood cells, and 2 *(3%)* specimens contained Charcot-Leyden crystals. Other cells and particles of no pathological significance were not reported.

**Table 1.**
Parasites, WBC's, RBC's, fungi and crystals identified by the Trichrome-PLUS™ Stain vs. the CONSED™ Sedimentation wet preparation slide.

| No. (%) of identified parasites, cells and crystals | | |
|---|---|---|
| | CONSED™, n(%) | Trichrome-PLUS™, n(%) |
| No Ova or Parasites | 51 (83.6%) | 51 (83.6%) |
| Fungi or yeast | 40 (65.6%) | 41 (67.2%) |
| WBC's | 12 (19.7%) | 12 (19.7%) |
| RBC's | 10 (16.4%) | 10 (16.4%) |
| *Blastocystis hominis* | 6 ( 9.8%) | 6 ( 9.8%) |
| *Entamoeba histolytica* | 1 ( 1.6%) | 1 ( 1.6%) |
| *Entamoeba hartmanni* | 1 ( 1.6%) | 1 ( 1.6%) |
| *Giardia lamblia* | 1 ( 1.6%) | 1 ( 1.6%) |
| *Entamoeba coli* | 1 ( 1.6%) | 1 ( 1.6%) |
| *Endolimax nana* | 2 ( 3.3%) | 2 ( 3.3%) |
| *Strongyloides stercoralis* | 1 ( 1.6%) | 1 ( 1.6%) |
| Charcot / Leyden crystals | 2 ( 3.3%) | 2 ( 3.3%) |

**Table 2.** Actual specimen comparison between results obtained using the Proto-fix™ preserved specimens, concentrated with the CONSED™ Sedimentation Reagent method vs. the Trichrome-PLUS™ Stained smears using the same concentrated pellet.

| Sample # | USA patient history (Date tested) | Results using Protofix/CONSED | Results using Trichrome-PLUS |
|---|---|---|---|
| 1 | 39 year old male from Maryland with no symptoms (7/17/99) | No ova/parasites | No ova/parasites |
| 2 | 74 year old female from Wisconsin with no symptoms (8/1/99) | No ova/parasites<br>Yeast (few) WBC (few) | No ova/parasites<br>Yeast (few) WBC (rare) |
| 3 | 28 year old female from Texas with history of travel, IBS and Lyme (7/20/99) | No ova/parasites | No ova/parasites |
| 4 | 56 year old male from Maryland who previously tested positive at PCI and treated with antibiotics (7/17/99) | No ova/parasites | No ova/parasites |

**Table 2.** *Continued*

| Sample # | USA patient history (Date tested) | Results using Protofix/CONSED | Results using Trichrome-PLUS |
|---|---|---|---|
| 5 | 45 year old female from Iowa with no prior infection; routine check (7/21/99) | No ova/parasites | No ova/parasites<br>Yeast (rare) |
| 6 | 45 year old female from California with chronic sinusitis and gas; no travel history (7/22/99) | No ova/parasites | No ova/parasites |
| 7 | 45 year old female From California with low energy, travel to France, 1998 (7/20/99) | *Blastocystis hominis* (few)<br>Yeast (few)<br>RBC (rare) | *Blastocystis hominis* (few)<br>Yeast (few)<br>RBC (rare) |
| 8 | 32 year old female from California with food sensitivities, IBS, travel to Europe, parasites 1995-97, (10/5/99) | No ova/parasites<br>Yeast (few)<br>RBC (few) | No ova/parasites<br>Yeast (few)<br>RBC (few) |
| 9 | 30 year old male from Georgia with no travel or clinical history (7/26/99) | No ova/parasites<br>Yeast (few) WBC (few) | No ova/parasites<br>Yeast (few) WBC (moderate) |
| 10 | 35 year old female from Maryland who traveled to Mexico, 1995 (7/25/99) | No ova/parasites<br>WBC (few) | No ova/parasites<br>WBC (few) |
| 11 | 25 year old male from Maryland with chronic fatigue (10/28/99) | No ova/parasites | No ova/parasites |
| 12 | 52 year old male from Iowa; no history provided (7/31/99) | *Entamoeba histolytica* (few)<br>*E hartmanni* (few)<br>Yeast (few) | *Entamoeba histolytica* (few)<br>*E hartmanni* (rare)<br>Yeast (few) |
| 13 | 70 year old female from Maryland, no clinical or travel history given (7/26/99) | *Giardia lamblia* (many)<br>Yeast (rare) RBC (rare) | *Giardia lamblia* (moderate)<br>Yeast (few) RBC (few) |
| 14 | 49 year old female from California who had Giardia in 1990 and was successfully treated (8/4/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 15 | 55 year old female from New Mexico with poor digestion, bloating, and loose stool, travel to Europe, 1995 (8/2/99) | No ova/parasites<br>Yeast (few)<br>Bacteria overgrowth | No ova/parasites<br>Yeast (few)<br>Bacteria (many) |
| 16 | 32 Year old female from Iowa with fatigued eyes and being treated (7/26/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 17 | 7 year old male from Ontario with no travel history but with food allergies and sensitivities (9/23/99) | No ova/parasites | No ova/parasites |
| 18 | 9 year old from Ontario with no travel history but with eczema and asthma (91/3/99) | No ova/parasites<br>RBC (rare) | No ova/parasites<br>RBC (few) |
| 19 | 37 year old female from New Jersey who is always tired with low back pain and foreign travel (9/23/99) | No ova/parasites<br>Yeast (few)<br>RBC (few) | No ova/parasites<br>Yeast (rare)<br>RBC (few) |
| 20 | 52 year old female from Maryland with Crohn's disease (8/3/99) | *Blastocystis hominis* (few)<br>Yeast (few), RBC (few) | *Blastocystis hominis* (rare)<br>Yeast (few)' RBC (few) |
| 21 | 82 year old male from South Dakota with no travel history but with hard stool, alternating with diarrhea (9/23/99) | No ova/parasites<br>Yeast (few), Charcot Leyden crystals (rare) | No ova/parasites<br>Yeast (few), Charcot Leyden crystals (few) |
| 22 | 49 year old male from Washington who traveled to Chile in 1990 and is fatigued (8/3/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 23 | 51 year old male from Colorado with cancer, lymphoma and back and leg pain (7/29/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 24 | AAB 3rd Q; #1 (9/28/99) | *Strongyloides stercoralis* | *Strongyloides stercoralis* |
| 25 | AAB 3rd Q; #2 (9/28/99) | No ova/parasites | No ova/parasites |
| 26 | MB 3rd Q; #3 (9/28/99) | *Endolimax nana*<br>*Entamoeba coli* | *Endolimax nana*<br>*Entamoeba coli* |
| 27 | 12 year old from Ottawa with allergies, asthma, chemical, sensitivities (9/23/99) | No ova/parasites<br>*Candida* (many) | No ova/parasites<br>*Candida* (many) |
| 28 | 60 year old female from Washington; low energy after successful parasite treatment (8/26/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 29 | 39 year old female from Ontario with fatigue, chemical, and food sensitivities (9/23/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |

**Table 2.** *Continued*

| Sample # | USA patient history (Date tested) | Results using Protofix/CONSED | Results using Trichrome-PLUS |
|---|---|---|---|
| 30 | 34 year old female from California with poor digestion, nausea, and history of treatment for *Candida* (7/21/99) | No ova/parasites<br>*Candida* (moderate) | No ova/parasites<br>*Candida* (moderate) |
| 31 | 44 year old female from California who had mucus, diarrhea, and was treated with antibiotics, herbs, and supplements (7/23/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 32 | 17 year old male from Vermont with history of foreign travel and ulcerative colitis (8/8/99) | No ova/parasites<br>WBC (few) | No ova/parasites<br>WBC (few) |
| 33 | 29 year old female from Michigan with numerous food allergies and mental problems (9/13/99) | No ova/parasites | No ova/parasites |
| 34 | 36 year old female from California with constipation and foreign travel to Nepal (9/15/99) | *Blastocystis hominis* (rare)<br>Yeast (few), RBC (rare) | *Blastocystis hominis* (rare)<br>Yeast (few), RBC (few) |
| 35 | 54 year old female from Texas with poor elimination and dysplasia who is medicated (9/21/99) | No ova/parasites | No ova/parasites |
| 36 | 39 year old male from Massachusetts with irregular bowel movements and no travel history (9/12/99) | No ova/parasites | No ova/parasites |
| 37 | 64 year old female from Georgia with gastro-intestinal problems who is herbally medicated (9/2/99) | *Blastocystis hominis* (few)<br>Yeast (few) | *Blastocystis hominis* (few)<br>Yeast (few) |
| 38 | 56 year old female from California with soft stools and travel in Mexico (9/15/99) | No ova/parasites | No ova/parasites |
| 39 | 42 year old female from California with chronic fatigue and no travel history (9/22/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (rare) |
| 40 | 45 year old female from California with no travel history but with skin inflammation and poor digestion (10/25/99) | No ova/parasites<br>Yeast (few)<br>WBC (few), RBC (few) | No ova/parasites<br>Yeast (rare)<br>WBC (few), RBC (few) |
| 41 | 47 year old female from New Mexico with gut pain, headaches, gas and nausea (8/24/99) | No ova/parasites<br>*Candida* overgrowth | No ova/parasites<br>*Candida* (many) |
| 42 | 34 year old female from Pennsylvania with digestive and nervous imbalances; no foreign travel; medicated (10/25/99) | No ova/parasites<br>*Candida* (many) | No ova/parasites<br>*Candida* (many) |
| 43 | 45 year old female From Minnesota with bloating and soreness, but no travel history or previous infections (10/25/99) | No ova/parasites<br>Yeast (many) | No ova/parasites<br>Yeast (many) |
| 44 | 5 year old child from California with bloating and weight gain (8/3/99) | No ova/parasites<br>Yeast/*Candida* (few) | No ova/parasites<br>Yeast/*Candida* (few) |
| 45 | 55 year old female from Colorado who travels to Mexico, has ulcerative colitis, and is medicated (10/25/99) | No ova/parasites<br>Yeast (few)<br>WBC (few) | No ova/parasites<br>Yeast (few)<br>WBC (rare) |
| 46 | 55 year old male from New York who traveled to India and has gas and fatigue (8/12199) | *Blastocystis hominis* (moderate)<br>Yeast (moderate) | *Blastocystis hominis* (few)<br>Yeast (few) |
| 47 | 43 year old female from Arizona with poor digestion, fatigue, and mental confusion (9/14/99) | No ova/parasites<br>Yeast (moderate) | No ova/parasites<br>Yeast (many) |
| 48 | 13 year old male from Colorado who had Blastocystis infection and was vomiting and treated with Flagyl (9/16/99) | No ova/parasites<br>Yeast (moderate) | No ova/parasites<br>Yeast (moderate) |
| 49 | 36 year old female from California with many allergies and swelling in face and hands (9/28/99) | No ova/parasites<br>Yeast (few), Fungi (many)<br>WBC (rare) | No ova/parasites<br>Yeast (few), Fungi (many)<br>WBC (rare) |
| 50 | 53 year old female from New Mexico with sore esophagus and gas (9/24/99) | No ova/parasites | No ova/parasites |
| 51 | 52 year old femaie from Illinois with no travel or clinical symptoms (10/25/99) | No ova/parasites<br>Yeast (rare) | No ova/parasites<br>Yeast (rare) |
| 52 | 29 year old female from Connecticut with GI problems and fatigue who traveled to the Caribbean 1996-1997 (10/25/99) | No ova/parasites<br>Yeast (moderate)<br>RBC (rare) | No ova/parasites<br>Yeast (moderate)<br>RBC (rare) |
| 53 | 30 year old female from California who traveled in 1995 to Germany and India and was treated for parasites; retesting (10/7/99) | No ova/parasites<br>Yeast (few)<br>WBC (few) | No ova/parasites<br>Yeast (few)<br>WBC (few) |

**Table 2.** *Continued*

| Sample # | USA patient history (Date tested) | Results using Protofix/CONSED | Results using Trichrome-PLUS |
|---|---|---|---|
| 54 | 46 year old female from Illinois with low energy and bloating and undated travel history (8/12/99) | No ova/parasites<br>Yeast (moderate) | No ova/parasites<br>Yeast (moderate) |
| 55 | 40 year old female from New York with chronic diarrhea and mucus and travel history in Mexico and England (8/12/99) | No ova/parasites<br>Charcot-Leyden crystals (Moderate), WBC (few)<br>Yeast (moderate) | No ova/parasites<br>Charcot-Leyden crystals (Moderate), WBC (rare)<br>Yeast (moderate) |
| 56 | 62 year old female from California with recent travel to Africa and digestive upsets (8/16/99) | No ova/parasites<br>WBC (rare) | No ova/parasites<br>WBC (rare) |
| 57 | 20 year old female from New Mexico who traveled to Mexico 1998-1999 and had gas, bloating, and loose stools and was treated (8/2/99) | No ova/parasites<br>Yeast (few) | No ova/parasites<br>Yeast (few) |
| 58 | 47 year old female from Arizona with itching and muscle pain but no history of foreign travel (10/20/99) | No ova/parasites<br>Yeast (moderate)<br>WBC (few) | No ova/parasites<br>Yeast (moderate)<br>WBC (few) |
| 59 | 30 year old female from California with chronic fatigue and indigestion who traveled to Mexico, Bali, Fiji, etc. (9/7/99) | *Blastocystis hominis* (few)<br>*Endolimax nana* (few) | *Blastocystis hominis* (few)<br>*Endolimax nana* (few) |
| 60 | 38 year old female from Colorado with no clinical or travel history (10/12/99) | No ova/parasites | No ova/parasites |
| 61 | 64 year old female from California with fatigue, overweight, and fatigue, overweight, and high blood sugar with extensive sugar with extensive travel history (9/6/99) | No ova/parasites<br>*Candida* (moderate)<br>WBC (few)<br>RBC (rare) | No ova/parasites<br>*Candida* (moderate)<br>WBC (rare)<br>RBC (rare) |

*Specimens nos. 24, 25, 26 are those supplied by American Association of Bioanalysts (AAB) Proficiency Testing for the third quarter, 1999 with a score of 100% correct results as tested and reported on 9/28/99.*

## 4. Discussion

The gold standard for permanent stained fecal smears is specimens fixed in a PVA solution with a heavy metal *(mercuric chloride, zinc sulfate or copper sulfate) (Garcia and Bruckner, 1997; Garcia and Voge, 1980).* These fixatives do not allow for adequate concentration procedures due to the viscosity of the PVA within the reagent. As such, the smears are prepared directly from the diluted sample *(diluted 1:4 – specimen to fixative)* by extracting a few drops of the diluted specimen and preparing a smeared slide. The correlation of this type of smear to the formalin concentration preparation is quite poor. The formalin concentration procedure also has limitations in that it does not fix all trophozoites well and may not fix *S. stercoralis,* resulting in missing the protozoan trophozoites for identification as well as missing the juvenile nematodes. Therefore, results of the PVA *(metal)* fixative must be combined with the formalin concentration wet preparation to give a complete ova and parasite result. The value of a single vial fixative with a very low viscosity *(i.e. without plastics such as PVA)* allows for a concentrated specimen to be used for both the wet preparation and the permanent stained slide. This makes the permanent stained slide a true representation of the sample and allows for a real correlation between the concentrated wet preparation and the concentrated permanent stained smear.

The superiority of the Proto-fix™ preserved specimens, concentrated with the CONSED™ Sedimentation Reagent method has already been established *(Allen and Frankel, 1997, loc. cit.; Amin, 2000; Jensen et al., 2000).* The Trichrome-PLUS™/CONSED™ system allows the production of a permanent smear made from the concentrated preparation produced by the Trichrome-PLUS™/ CONSED™ thus keeping a permanent record of the complete concentrated sample by utilizing the fixatives, reagents and stains listed above. However, this objective *(a permanent record of a concentrated sample)* can only be accomplished if the microscopic screening of the specimens produced by both methods *(stained smear and wet prep)* match.

In conclusion, this investigation has demonstrated that the Proto-fix™ preserved specimens, concentrated with the CONSED™ Sedimentation Reagent method and stained Trichrome-PLUS™ Stain, is a reproducible method that allows for a real correlation between the concentrated wet preparation and the concentrated permanent stained smear. It is less labor-intensive and simpler to perform than the combined PVA fixative and formalin concentration procedures. Our results suggest that the Proto-fix™ parasitology fixative when combined with the CONSED™ and Trichrome-PLUS™ Stain is the ideal system for providing a permanent stained record of fecal specimens for intestinal parasitology examinations. ◆

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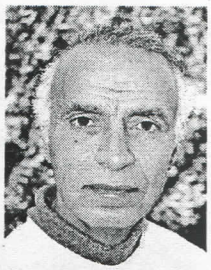
## ABOUT THE AUTHOR



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sex, address, foreign travel, symptoms, past infections and treatments, and household contacts with parasite or other infections. Patients failing to provide the above information included most of those testing positive for *Giardia lamblia* and *DiEntamoeba fragilis* who thus had to be excluded from consideration.

One fresh specimen or two preserved specimens collected on two separate days were provided by local or mostly out-of-state patients, respectively. Specimens were routinely processed using a formalin-ethyl acetate concentration-sedimentation procedure and examined as wet mounts stained in Lugol's iodine. Positive results were quantified (average number of organisms per high power field on a scale of 1 to 4), entered on computerized patients' records, and forwarded to referring physicians for treatment and follow-up.

## Results and Discussion

A random sample of 644 patient records who collected specimens in June, July, and August, 1996 was selected for this cross-sectional study. Samples were carefully screened, and 5 major species of protozoans were considered for this study.

## Prevalence and Host Associations in Study Population

The prevalence of infection of all species was 58.7% (*Table 1*). This is considerably higher than the 20.0% reported for the United States[28]. The patient population was made up of ill patients with predisposing factors and frequent infections. Prevalence was more comparable to that reported from Texas/Mexico (42.9%)[58], but markedly lower than others from Mexico (71.7%)[24] or South America (80.4%, 83%)[9, 5]. Of all 378 infected patients, 271 (71.7%) and 107 (28.3%) were singly and multiply infected, respectively. These proportions agree with Canadian pediatric studies showing 85% single infections[27], but contrasts with the considerably more prevalent multiple infections in South America (65.8%)[9] and Africa (72.7%)[11]. Of all infected patients, 50 (13%) were asymptomatic in all categories of single and multiple infections (*Table 1*).

The sex ratio of the examined patients was in favor of females (1 M: 1.91). The proportion of females was, however, even higher than males in the infected category (1:2.31) including those with single (1:2.15) and multiple infections (1:2.45) compared to the non-infected (1:1.56) (*Table 1*). This suggests that females are more likely to be relatively more frequently and heavily infected than males. Similar observations were noted in other studies from Canada[48] and Africa[11]

The age of the patient population varied between 0 and 78, but the overall average in each of the infection categories was comparable (early 40s) except for occasional differences attributable to small sample size. While patients were generally infected at a very young age, only adults appear to be infected by certain parasites, e.g., *Cyclospora cayetanensis*, and no young children were multiply infected.

### *Blastocystis hominis*

*Blastocystis hominis* is well represented in the patient population with similar prevalences among symptomatic and asymptomatic patients of all ages in single (8.3%, 8.9%) and multiple infections (8.5%, 7.1%), respectively (*Table 1*). This is considerably greater than in the United States in 1994 (2.6%; state diagnostic laboratories) prevalence[28], but markedly lower than in Chile (61.8%)[54] and Argentina (43%)[5]. The United States prevalence was greater in the 1960s (18%) and varied between 4 and 12% by 1990[61].

Approximately half the *B. hominis* infected patients were asymptomatic and the other half had enteric and extra-intestinal symptoms (*Table 1*). Other studies have also reported symptom dichotomy in *B. hominis* infections[50, 55, 62]. It appears that separate "strains" of *B. hominis* have distinct pathogenic abilities. Canadian "strains" seem to be primarily non-pathogenic[48] while those of some Middle Eastern countries appear to be pathogenic[40]. It is proposed that *B. hominis* is a species complex including taxa of distinct genetic identity and pathogenicity. This is supported by Boreham and colleagues[6] who identified two immunologically distinct "demes" of *B. hominis* with different DNA content and by Mueller[37] who characterized 4 serologically different "groups" of *B. hominis* using immunodiffusion assay. Reports describing the relationship between the concentration of *B. hominis* in fecal specimens and symptomology[50, 55] may have actually been dealing, at least in part, with "strain" differences. No such relationship could be definitely established in the IPD study.

Symptomatic patients infected with *B. hominis* alone (n = 44) as well as those with concurrent *Entamoeba* spp. infections (n = 45) experienced a number of enteric symptoms including most frequently bloating (34%), diarrhea (39%), flatulence (43%), constipation (16%), cramps (18%), and maldigestion/malabsorption (14%). These symptoms and the less frequent ones (bleeding, irritable bowel, leaky gut, mucus) are similar to the less comprehensive and unquantified ones reported by others associated with *B. hominis* infection[21, 40, 45]. Haralabidis[21] also reported vomiting and bloody stool.

Extra-intestinal symptoms associated with single *B. hominis* infections are rarely reported. The IPD patients more frequently suffered allergies (14%), fatigue (39%), nausea (14%), nervous/sensory disorders (23%), skin disorders (16%), pain (18%), and muscle problems (9%). Other less prevalent extra-intestinal symptoms included fever, headache, immune deficiency, insomnia, and weight changes. Some of these symptoms could be related to *B. hominis* metabolic byproducts being toxic to the body particularly of immune-compromised patients. Evidence of the invasion of extra-intestinal sites by *B. hominis*, e.g., the synovial fluid[31] and vagina[59] have been reported. Krech[30] reported only 10 of 56 German *B. hominis* patients having extra-intestinal symptoms and Haralabidis[21] listed depression, nausea, dizziness, weight loss, anorexia, pruritus, and insomnia as extra-intestinal symptoms of 16 Greek *B. hominis* patients.

### *Cyclospora cayetanensis*

Thirteen patients (2%) from Arizona (6), California (5), Massachusetts (1), and North Carolina[1] were infected with *C. cayetanensis*. They included only 3 males and were all older adults (30-62 years) (*Table 1*). This is the first report of *C. cayetanensis* from Arizona. Specimens received in laboratories in Chicago, Burlington (Massachusetts), and the United Kingdom revealed lower prevalences of 0.5%, 0.3%, and 0.1%, respectively[60, 42, 12], but rates are considerably higher in endemic areas, e.g., Peru, reaching 18%[43].

The fact that 3 of the 13 patients were asymptomatic documents the wide range of pathogenicity of *C. cayetanensis* which was observed to vary between short lived subclinical self-limited cases to those causing long term overt clinical disease which may be intermittent in nature [23]. An example of the latter condition is that of an IPD middle age male (not among the above 13 cases) who tested positive for *C. cayetanensis* on February 19, March 26, and September 25, 1996. His test results were negative on April 4 and 11, 1996. His infection was moderate to heavy, and his symptoms were relatively

consistent and included fatigue, cramps, headache, and fibromyalgia. Intermittent shedding of *Cyclospora* in stool was also reported by Soave[51]. Asymptomatic *C. cayetanensis* cases are rarely reported by other observers[32, 18].

Enteric symptoms of patients included bloating (30%), diarrhea (20%), cramps (10%), and flatulence (20%). Enteric symptoms reported by other observers invariably include diarrhea, but constipation, malabsorption, villus blunting, and vomiting were also reported[18, 51].

Extra-intestinal symptoms included anemia (10%), fatigue (30%), headache (10%), muscle weakness/aches (10%), nausea (10%), depression (10%), and pruritus (20%). Additional symptoms reported by other observers[4, 23, 51, 60] include anorexia, decreased appetite, fatigue, fever, and weight loss.

## Entamoeba coli

Fifty (7.8%) patients of all ages were singly infected with *E. coli* and 9 (1.4%) more concurrently with *E. hartmanni* (*Table 1*). This is twice as high as the 1994-1995 United States and Canadian prevalence rates of 4.2%[28, 27], but considerably lower than those in Texas, Mexico, and South America of 24.8-30.8% [5, 9, 24, 58].

*Entamoeba coli* is routinely dismissed as a harmless commensal of the intestinal tract. This is confirmed by the fact that 3 patients with single *E. coli* infections were asymptomatic. However, most patients of all ages (n = 47) had enteric and extra-intestinal symptoms (*Table 1*). The most common intestinal symptom was diarrhea (40%), but bloating (26%), constipation (13%), cramps (32%), flatulence (30%) and irritable bowel (13%) were also frequent. These symptoms are comparable to those in patients concurrently infected with *E. hartmanni*, another presumed non-pathogen. Other significant, but less common intestinal symptoms included bleeding and maldigestion. Reports incriminating *E. coli* as a pathogenic agent causing enteric symptoms are rare. They invariably refer to persistent diarrhea, particularly in children, and occasionally to more severe symptoms[13, 56].

Symptomatic patients infected with *E. coli* singly or concurrently with *E. hartmanni* also experienced extra-intestinal symptoms the most common of which was fatigue (36%, 44%). Other less common symptoms include allergies, headache, nausea, depression/lack of concentration/irritability, joint/lower back pain, respiratory and skin disorders, and weight changes, among other symptoms.

The full spectrum of enteric symptoms and the extra-intestinal symptoms associated with *E. coli* infections is reported here for the first time. The clinical implications of these findings may need further consideration. However, it is clear that *E. coli* is often not a harmless commensal particularly in immune compromised patients as other observations from the DEL (unpublished) suggest.

## *Entamoeba hartmanni*

Fifty-three (8.2%) patients of all ages were singly infected with *E. hartmanni* and 9 (1.4%) more concurrently with *E. coli*. It was not reported in the United

**TABLE 1. Prevalence of protozoan infections in stool samples of 644 symptomatic and asymptomatic patients, by age and sex examined between June and August, 1996 at DEL-IPD.**

| Categories | Number and | prevalence (%) | of infections | Patient sex ratio | Patient age range (mean) | Patient age range (mean) |
|---|---|---|---|---|---|---|
| | Patients with symptoms | Patients without symptoms | Total patients | 1M:FF | Males | Females |
| **Single infections** | 233 (43.8) | 38[b] (33.9) | 271 (42.1) | 1:2.15 | 3–77 (40.4) | 0–67 (40.9) |
| *Blastocystis hominis* | 44 ( 8.3) | 10 ( 8.9) | 54 ( 8.4) | 1:1.45 | 6-65 (39.7) | 5–63 (39.4) |
| *Cyclospora cayetanensis* | 10 ( 1.9) | 3 ( 2.7) | 13 ( 2.0) | 1:3.33 | 30–45 (36.7) | 31–62 (44.3) |
| *Entamoeba coli* | 47 ( 8.8) | 3 ( 2.7) | 50 ( 7.8) | 1:2.57 | 3–73 (46.0) | 6–61 (43.0) |
| *E. hartmanni* | 44 ( 8.3) | 9 ( 8.0) | 53 ( 8.2) | 1:1.65 | 13–68 (40.9) | 1–57 (39.2) |
| *E. histolytica* | 88 (16.5) | 11 ( 9.8) | 99 (15.4) | 1:2.67 | 2–77 (38.1) | 0-67 (40.9) |
| **Multiple infections** | 95 (17.8) | 12 (10.7) | 107 (16.6) | 1:2.45 | 6–73 (42.9) | 9–78 (43.9) |
| *E. histolytica* and other *Entamoeba* spp. | 27 ( 5.1) | 1 ( 0.9) | 28 ( 4.3) | 1:2.50 | 28–67 (48.1) | 9–78 (42.2) |
| *E. coli* and *E. hartmanni* | 9 ( 1.7) | | 9 ( 1.4) | 1:3.50 | 44–60 (52.0) | 31–57 (42.4) |
| *B. hominis* and *Entamoeba* spp. | 45 ( 8.5) | 8 ( 7.1) | 53 ( 8.2) | 1:2.31 | 6–54 (34.9) | 16–70 (44.9) |
| **Other combinations** | 14[a] ( 2.6) | 3[c] ( 2.7) | 17 ( 2.6) | 1:2.40 | 44–73 (56.4) | 27–65 (44.5) |
| Total infected | 328 (61.6) | 50 (44.6) | 378 (58.7) | 1:2.31 | 3–77 (41.1) | 0–78 (41.8) |
| Not infected | 204 (38.4) | 62 (55.4) | 266 (41.3) | 1:1.56 | 1–77 (40.3) | 1–76 (43.1) |
| Total examined | 532 (100.) | 112 (100.) | 644 (100.) | 1:1.91 | 1–77 (40.7) | 0–78 (42.3) |

[a]Involving *Blastocystis hominis, Cyclospora cayetanensis, Cryptosporidium parvum, DiEntamoeba fragilis, Entamoeba hartmanni,* E. histolytica, Giardia lamblia, Iodamoeba butschlii.

[b]The 38 asymptomatic patients included 2 each with single *Cryptosporidium parvum* infection.

[c]Involving *B. hominis, C. cayetanensis, C. parvum, E. coli, E. hartmanni.*

States[28] or Canada[27] in 1994 or 1995, respectively, and was rare in Australia (0.52%)[36] and India (1.37%)[53]. Its distribution is clearly not homogeneous. It was prevalent (35%) in endemic Mexico City[47], but absent elsewhere in Mexico, e.g., Chihuahua[58] and Tijuana[24].

Like *E. coli*, *E. hartmanni* is also considered a harmless commensal. This fact is supported by the findings of 9 asymptomatic patients singly infected with *E. hartmanni* (*Table 1*). However, more singly infected patients (n = 44) had symptoms including bloating (23%), cramps (23%), diarrhea (32%), flatulence (23%), and irritable bowel (9%). Extra-intestinal symptoms comparable to those associated with *E. histolytica* infections are also noted and commonly included allergies (11%), nausea (9%), nerve system (18%) and skin (16%) disorders, pain (11%), and respiratory difficulties (16%).

Early studies incriminated *E. hartmanni* as an intestinal pathogen until it was considered a small race of *E. histolytica* in 1959[8] and its distinct isoenzyme patterns were reported in 1979[46]. The fact that *E. hartmanni* is now recognized as a species separate from *E. histolytica* does not mean that both cannot produce symptoms; they do. Similar observations may be overlooked or not reported by others influenced by the notion that *E. hartmanni* can only be non-pathogenic. Only Marquardt and Demaree, Jr.[33] noted that *E. hartmanni* may cause "mild symptoms of enteritis." The early European studies suggested that *E. hartmanni* and *E. histolytica* are interchangeable forms associated with variable pathologies and stresses. While these studies have not been sufficiently confirmed by subsequent studies[15], we often observe at IPD that patients treated for *E. histolytica* retest positive with *E. hartmanni* a few weeks later.

### *Entamoeba histolytica/ Entamoeba dispar*

Ninety-nine (15%) patients were singly infected with *E. histolytica /E. dispar* and 28 (4%) more concurrently with *E. coli* and/or *E. hartmanni*. These include 4 and 1 presumptive *E. histolytica* patients with Charcot-Leyden crystals, respectively. Charcot-Leyden crystals are byproducts of the breakdown of eosinophils attributed tissue invading parasitic infections usually including *E. histolytica* [3]. All ages were affected, but multiple infections were not noted in children less than 9 years old (*Table 1*).

The overall 1994 prevalence in the United States is reported between 0.9%[28] and 4%[29]. In northern California, 11.9% of patients were infected[21]. Prevalence rates of 7.6-8.7% are more commonly reported, e.g., in Venezuela[9], Mexico[23], and India[53]. Despite its cosmopolitan distribution, *E. histolytica /E. dispar* is often not reported in studies yielding heavy and frequent infections of other intestinal protozoans such as those from Canada[27], Texas/Mexico[58], and Argentina[5].

Of the 99 and 28 single and concurrent infections of *E. histolytica /E. dispar*, 11 and 1 were asymptomatic (*Table 1*). The organisms identified from asymptomatic patients could be assigned to *Entamoeba dispar* Brumpt, 1925 as separated from the morphologically identical, but invasive *E. histolytica* Schaudinn, 1903 based on DNA, RNA, and clinical epidemiology differences[14, 46]. The IPD results, however, show a predominance of symptomatic over asymptomatic cases in contrast to the contentions of others[29, 25]. A definitive relationship between symptomology and the species of *Entamoeba* involved is, however, far from certain. Newton-Sanchez *et al.*[39], using RFLP, found that 78% of 48 stool samples of non-dysenteric but microscopically Amoeba positive Mexican children were positive for *E. histolytica* and *E. dispar* or only *E. histolytica* and 22% were positive for *E. dispar* only. In Bangladesh, the prevalence of *E. histolytica* and *E. dispar* antigens was approximately equal in urban children with diarrhea, whereas in rural asymptomatic children *E. dispar* was 7 times more common[20].

The most common enteric symptoms included diarrhea (48%), cramps (34%), constipation (26%), bloating (24%), and flatulence (24%). Malabsorption and irritable bowel were also noted in 10% and 9% of the patients. Least frequent symptoms included leaky gut, mucus, and vomiting. All symptoms are characteristic of enteric amoebiasis and have been thoroughly discussed by Kelsall and Ravdin[29].

The most frequently observed extra-intestinal symptoms were fatigue (27%) and nausea (15%). Other significant symptoms included allergies (11%), nervous system and skin disorders (10%), pain (9%), weight changes, mostly loss (8%), and insomnia (7%). Observed respiratory and nervous system symptoms were similar to those noted by others[29].

### Multiple Infections

A total of 107 (16.6%) of examined patients were concurrently infected with more than one species of intestinal protozoans. Most of these infections involved *B. hominis* and *Entamoeba* spp. (53 patients) and *E. histolytica /E. dispar* and *Entamoeba* spp. (28). All concurrent infections occurred in older patients and none involved very young children. Exposure time sufficient for the establishment of multiple infections appear to be necessary.

Symptoms of multiply infected patients were comparable to those of component single infections. In concurrent infections of *E. histolytica /E. dispar* and other *Entamoeba* spp., however, the frequency of major intestinal symptoms (including bleeding and mucus) was markedly higher compared to those in single component species infections. If a cumulative effect is involved in the above situation, this may not be the case in concurrent infections with *B. hominis* and *Entamoeba* spp.

### Other Combinations

Concurrent infections involving other combinations (17 patients) involved 14 symptomatic patients (*Table 1*) that showed high frequency of cramps (71%), diarrhea (50%), bloating (36%), and flatulence (21%) as well as of fatigue (36%), nausea, and nervous and skin disorders (29%). It is hard to assign these symptoms to any one or more component species because of their diversity (see *Footnote a, Table 1*).

### Non-infected Patients

A total of 266 (41.3%) patients were not infected of whom 204 had symptoms (*Table 1*) including mostly diarrhea (31%), cramps (30%), flatulence (24%), and bloating (24%) as well as fatigue (34%), nervous (19%) and skin (13%) disorders, and allergies (12%), among other less frequent symptoms. Some of these symptoms may have been caused by nonprotozoan infections. Mendis and colleagues[35] found that yeast infections were etiologically associated with diarrheal stools in 6% of children and 19% of adults. The association of rotaviruses and adenovirus to diarrhea in the same study was 6% and 3%, respectively. Of the 204 uninfected patients with symptoms, 165 (80.1 %) were infected with yeast that was dividing in 71 patients (an indication of pathology[2]) and had heavy presence in 27 patients. The same patient population was also infected with many other fungal infections including 166 patients infected with *Candida* of whom 16 were heavily infected. Other causal agents of symptoms are suspected, but are yet to be identified. Some of these may include enterobacterial agents,

such as, *Salmonella* or *Shigella*.

Some of the symptoms, however, may have been parasite related. For example, the major symptoms of uninfected patients are somewhat similar to but usually less frequent or intense than those observed in *E. histolytica* and Giardia lamblia infections[15]. It is possible that, in some cases, the intermittent and uneven distribution of cysts and trophozoites of those two parasites[34, 49, 15] as well as of *C. cayetanensis* (this paper), may have caused false negative results. The sensitivity of 1 versus 3 fecal examinations was estimated to be 73.0% and 89.6% positive for *E. histolytica*, and 88.1% and 98.1% for *G. lamblia*[19].

The uninfected IPD population of patients have also included previously medicated former *E. histolytica* and *G. lamblia* patients who may have subsequently developed recurrent invasive infections related to a high background rate of undiagnosed abdominal symptomology[26]. The 204 uninfected IPD population included 42 (21%) patients with recent history of treatment mostly with metronidazole usually for amoebiasis and occasionally for *Giardia* infections.

## Foreign Travel

The population of patients under study is a well traveled one. Over half report foreign travel within the last 5 years. More symptomatic patients (56%) traveled outside the United States compared to asymptomatic patients (49%). More infected than uninfected patients traveled among those with symptoms (59% vs. 50%) and those without (56% vs. 44%). Clearly, foreign travel appears to be associated with higher prevalence of intestinal infections. In general, three categories of geographical regions with different risk levels are identified in symptomatic patients as follows. The first category includes Mexico and Europe with the highest travel prevalence of (22%, 24% in symptomatic patients). The second category includes East Asia, Central and South America, and the Caribbean (12%, 12%, 10%). The third category includes all other geographical regions: Africa (2%), Australia/New Zealand (4%), Canada (5%), Indian Subcontinent (8%), Middle East (5%), Hawaii (4%). The pattern in asymptomatic patients was comparable except that travel prevalences were relatively lower particularly in non-infected patients. Place associated variables such as environmental sanitation, cultural practices, drinking water supplies, and socioeconomic status of visited countries as well as duration of stay and extent of partaking of native life are often related to traveler's diarrhea, among other symptoms, in third world countries. However, this does not explain the similarity between the high prevalence among Mexico and European travelers. High infection rates are, however, noted in some European countries including Switzerland[52], Germany[30], and Romania[7].

Protozoan infection parameters in South America[5, 9, 38, 45, 54] were markedly higher than those reported in European studies (above), yet prevalence in travelers to the latter region was lower.

The following paragraphs dealing with specific parasite species association with geographical regions traveled will be confined to symptomatic patients. The same trend applies to asymptomatic patients except that sample sizes are small or sometimes non-existent.

*Blastocystis hominis* infected symptomatic patients (singly or concurrently with *Entamoeba* spp.) most frequently traveled to Mexico, Europe, East Asia, and Central and South America (18% to 36%). Studies from Switzerland[52], Argentina[5], and Chile[54] report *B. hominis* prevalence of 17-19%, 43%, and 62%, respectively.

Among the 44 symptomatic patients singly infected with *B. hominis*, 18 had very heavy infections of whom 16 traveled to the Indian subcontinent (Nepal, India) (37%), Europe (37%), Mexico (37%), east Asia (Thailand, Indonesia) (31%), Central and South America (31%), Caribbean (25%), Canada (6%), Hawaii (6%), Middle East (6%), and Australia (6%). Some travelers visit more than one country/region.

Patients traveling to Nepal return with severe *B. hominis* infections that are not easily resolved even after 2 years of treatment. A case control study among expatriates and tourists in Kathmandu, Nepal implicates *B. hominis* as the cause of traveler's diarrhea in 56 of 189 (30%) patients with diarrhea[50]. Among the 45 symptomatic patients concurrently infected with *B. hominis* and *Entamoeba* spp. 17 had very heavy infections of *B. hominis* of whom 14 traveled to Mexico (35%), Europe (35%), India (18%), Australia (18%), Central and South America (12%), Canada (12%), Fiji (6%), Middle East (6%) and Thailand (6%).

*Cyclospora cayetanensis* infected symptomatic patients traveled most frequently to Europe (30.0%) and Mexico (20.0%). All Berlin and colleagues[4] patients were reported to have traveled in Mexico or Thailand and other endemic foci (Nepal, Haiti, Peru) were reported by Soave[51]. Two IPD patients with heavy *C. cayetanensis* infections recently traveled to Mexico, Austria, and Italy.

*Entamoeba* coli infected symptomatic patients who traveled most frequently to Mexico (30%), Europe (23%), Caribbean (15%), and Central and South America (13%). Available studies from some of these regions show *E. coli* prevalence of only 4% in Romania[7], but of 29% in Mexico[24], 25% in Venezuela[9], and unspecified high prevalence in Chile[54] and in Brazil[38]. Concurrently infected *E. coli* and *E. hartmanni* symptomatic patients visited Europe and Canada frequently.

*Entamoeba* hartmanni infected symptomatic patients traveled most frequently to Mexico (20%) and Europe (18%). Studies reporting the distribution of *E. hartmanni* are rare, but Sargeaunt and colleagues[47], report up to 35% prevalence in Mexico City. Asymptomatic *E. hartmanni* patients traveled frequently to Mexico (22%), Europe (44%), and Caribbean (33%).

*Entamoeba* histolytica infected symptomatic patients traveled most frequently to Mexico (18%) and Europe (25%) and somewhat less frequently to East Asia (11%) and the Caribbean (15%). Symptomatic patients concurrently infected with *E. histolytica /E. dispar* and *Entamoeba* spp. traveled most frequently to Mexico (15%), Europe (15%) and Indian subcontinent (11%). Most asymptomatic *E. histolytica* patients (27%) traveled to the Caribbean. Mexico, Indian subcontinent, Central and South America are recognized endemic areas of *E. histolytica* and travelers to and immigrants from these areas are at particular risk[29]. A study of the occurrence of *Entamoeba* spp. cysts on vegetables commercially traded in metropolitan Sao Paulo, Brazil[41] incriminated lettuce among other fresh vegetables, as vehicles of transmission. Unpublished records of IPD patients traveling to Mexico lead to the same conclusion. The agricultural practice of fertilization with night soil is believed to be largely responsible for this observation. IPD travelers to Europe, however, appear to be at an equally high risk for infections with *E. histolytica* as well as all other infection categories. This geographical region needs to be studied more closely before an explanation may be provided.

## Household Contacts

More infected than uninfected patients lived with infected household contacts. This was evident in symptomatic

patients (8% vs. 4%). The difference between the higher prevalence of 16% and 15% among asymptomatic patients was, however, less dramatic. Patients living with infected household contacts appear to have a greater chance of being asymptomatic (15%) than symptomatic (7%). The impact of infected household contacts on the increased risk of infection with fecal-oral transmitted intestinal protozoans have been demonstrated by other observers[11,54]. Family outbreaks of Blastocystosis have been reported[19] and individual family members of IPD index cases infected with *B. hominis* or *Entamoeba* spp. often test positive with the same pathogen(s), when infected (unpublished).

## Fungal Associations

Heavy presence of common yeast and *Candida* was frequently associated with subnormal levels of bacteria as well as with *Entamoeba* spp. infections in single and/or concurrently infected symptomatic patients. Observations of individual immune compromised and fatigued IPD patients[1] provide examples of the latter association particularly between *Candida* and *E. histolytica* infections. The prevalence of *E. histolytica* infection in immune compromised patients (37%) was higher than in the immune competent (24%). It is conceivable that the effect of *E. histolytica* on host immune depression[29] would allow the establishment of superimposed opportunistic fungal infections in those patients. Gonzalez-Mendoza and associates[16, 17] documented such a relationship between *E. histolytica* and *Candida albicans*. A similar relationship involving common yeast is likely. In addition, yeast can cause intestinal pathology[35] particularly when budding[2] indicating a potential source for a systemic infection especially in the immunosuppressed patient[15].

## Previous Parasitic Infections

Of 532 symptomatic patients, 138 (26%) had recent (within prev. 5 years) parasitic infections compared to 13 of 112 (11.6%) asymptomatic patients. Of the above 138 symptomatic patients, 98 (71%) had existing infections and 40 (29%) did not. These findings suggest a relationship between symptomatic infections and history of pre-existing gastrointestinal illnesses. The same relationship has been incriminated as a factor in the etiogenesis of traveler's diarrhea[10].

In the 328 infected symptomatic patients, *E. histolytica, G. lamblia*, and *B. hominis* were the most common pathogens (8%, 5%, 5%, respectively) in previous infections, irrespective of existing infection. The adverse effect of these three pathogens on host immune system[29, 44, 57, 62] would render hosts more susceptible to new infections compared to those with no history of previous infections. Amin[1] found immune compromised patients to be more susceptible to parasitic infections than the immune competent.

## Acknowledgments

I would like to thank my associates Cindy Cordery, Karim Amin, and Scott Cagle for their help with the many varied and demanding tasks at the DEL-IPD. ♦

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