# Detection of Entamoeba histolytica and Giardia lamblia in cases of diarrhea among children and adults

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# Abstract

*Entamoeba histolytica* and *Giardia lamblia* are very important causes of diarrhea due to parasitic diseases. Their prevalence varies in different areas of the world. In the present study, a trial to evaluate the role of both organisms in cases of diarrhea among children and adults was conducted. An enzyme-linked immunosorbent assay (ELISA) was used for detection of *Entamoeba* species and compared it with microscopic examination. Regarding *Entamoeba* direct wet examination was positive in 21.1% of samples, concentration method was positive in 22.2% and ELISA was positive in 16.7%. Sensitivity of ELISA was found to be 72.2% and specificity was found to be 97.2 %. So, it was found that ELISA is a more specific test than a sensitive test. Direct examination for *Giardia lamblia* gave positive results in 12.4% of samples and 14.4% by concentration method. ELISA test is expensive compared to the conventional microscopic examination, but, it yields objective results and do not require experienced microscopists so can be recommended for survey studies.

Key words: *Entamoeba histolytica / Entamoeba dispar* antigen – *Giardia lamblia* - microscopy - ELISA.

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# List of abbreviations

- ALA = Amoebic liver abscess.
- ATP = Adenosine tri-phosphate.
- CF = Complement fixation.
- CIE = Counterimmunoelectrophoresis.
- CME = Conventional microscopic examination.
- CRP = Cysteine-rich surface protein.
- CT = Computerized tomography.
- DNA = Deoxy ribonucleic acid.
- dsRNA = double-stranded ribonucleic acid.
- E/M = Electron microscope.
- *E. coli = Entamoeba coli.*
- ECM = Extracellular matrix.
- *E. dispar = Entamoeba dispar.*
- EDTA = Ethylenediamine tetra acetic acid.
- E-ELISA = *Entamoeba*- ELISA.
- *E. hartmani = Entamoeba hartmani.*
- *E. histolytica = Entamoeba histolytica.*
- EIA = Enzyme immunoassay.
- ELISA = Enzyme-linked immunosorbent assay.
- *E. moshkovskii = Entamoeba moshkovskii.*
- ER = Endoplasmic reticulum.
- GalNAc = Galactose N-acetyl D-galactosamine.
- Gal-GalNAc = Galactose and Galactose N-acetyl D-galactosamine.
- GIT = Gastrointestinal.
- GLV = *Giardia lamblia* virus.
- HIV = Human immuno-deficiency virus.

- HK = Hexokinase.
- HSP = Heat shock protein.
- IE = Immunoelectrophoresis.
- IFA = Indirect fluorescence assay.
- IgA = Immunoglobulin A.
- IgE = Immunoglobulin E.
- IgG = Immunoglobulin G.
- IgM = Immunoglobulin M.
- IHA = Indirect hemagglutination assay.
- IMS-IFA = Immunomagnetic Separation coupled with Immunofluorescence.
- Kb = Kilo base.
- kDa = Kilo Dalton.
- Mb = Mega base or Million base.
- MIF = Macrophage inhibition factor.
- MRI = Magnetic resonance imaging.
- nm = Nano meter.
- PAMPs = Pathogen-associated molecular patterns.
- PCR = Polymerase chain reaction.
- RBCs = Red blood cells.
- RNA = Ribonucleic acid.
- r-DNA = Ribosomal- deoxy ribonucleic acid.
- SPA = Surface proteolytic activity.
- Th1 = T helper 1.
- μm = micro meter.

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# *Chapter 1*

# INTRODUCTION AND AIM OF WORK

# Introduction

Humans are hosts to nearly over 70 species of protozoa, some derived from our primate ancestors and some are acquired from the domesticated animals or animals that came in contact with us during our relatively short history on Earth. Thereafter, the history of human parasitology proceeded along two lines, the discovery of a parasite and its subsequent association with disease and / or the recognition of a disease and the subsequent discovery that it was caused by a parasite (Cox, 2002).

*Entamoeba histolytica* and *Giardia lamblia* are micro-aerophilic protists, which have long been considered models of ancient pre-mitochondriate eukaryotes that cause dysentery and diarrhea respectively. Each is a single cell protist with a motile trophozoite stage and an immotile cyst stage (Samuelson, 2002).

The parasitic protozoa were not possible to be recognized because of their small size until the invention of the microscope. The study of parasitic protozoa only really began two centuries later, following the discovery of bacteria and the promulgation of the germ theory by Pasteur and his colleagues at the end of the 19th century (Cox, 2002).

Regarding amoebae humans harbor nine species of intestinal amoebae, of which only one, *Entamoeba histolytica*, is a pathogen. Most infections are asymptomatic, but some strains of *Entamoeba histolytica* can invade the gut wall, causing severe ulceration and amoebic dysentery characterized by bloody stools. If the parasites gain access to damaged blood vessels, they may be carried to extraintestinal sites anywhere in the body (Bray, 1996).

The protozoan *Giardia lamblia* that causes giardiasis is the most commonly diagnosed flagellate in the intestinal tract. There is still debate over the appropriate classification and nomenclature of *Giardia* species.

The *intestinalis* group infects a variety of mammals (including humans), birds, and reptiles (Marshall et al., 1997)

Most infected individuals show few or no signs of infection, they act as unaffected carrier, but in some, particularly children, there may be malabsorption, diarrhea, and abdominal pain (Cox, 2002).

The diagnosis of giardiasis and intestinal amoebiasis is primarily based on microscopical detection of the organism in stool, but it has a low sensitivity and depends on the skill of experienced microscopist. The recently developed enzyme-linked immunosorbent assays (ELISAs) for detection of the specific antigens in stool hold the potential to become an efficient diagnostic technique (Schunk et al., 2001).

# Aim of work

This study aims at detection of *Entamoeba histolytica* parasite and / or *Giardia lamblia* parasite in patients having diarrhea. Also, to evaluate and compare the diagnostic value of direct microscopy versus antigen detection using enzyme linked immunosorbent assay technique (ELISA) in case of *Entamoeba* infection.

# *Chapter 2*

# REVIEW OF LITRATURE