Reliability of ELISA in Detection of Giardia Specific Antigen 65 (GSA 65) versus Microscopy in Single Stool Sample

Thesis

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(GSA [illegible]

65)

[illegible]

ABSTRACT

Giardia lamblia is one of the most common causative agents of diarrheal illness throughout the world and Egypt. The importance of accurate diagnosis of patients with chronic giardiasis lies in the fact that children may suffer from elongated periods of profound malaise, diffuse epigastric pain, abdominal discomfort and malabsorption. For laboratory diagnosis, the most utilized method is microscopic examination of fecal samples, but the immunoenzymatic method is also available. The aim of this work was to verify the advantages and drawbacks of immunoassaying versus microscopy for diagnosing *G. lamblia*, when a single fecal sample is analyzed.

Samples were examined using direct wet smear, formol ether concentration technique and ELISA. The ProspecT ELISA kit was used for detecting *G. lamblia*-specific antigen (GSA-65), in accordance with the manufacturer's instructions. Results were expressed by both spectrophotometry and visual scale.

Results: The ELISA test was positive even when a significant proportion of corresponding samples examined by microscopy were negative. This trend was statistically significant ($p < 0.001$).

Conclusion: There is an increasing demand for diagnostic testing for *G. lamblia* with a priority being placed on obtaining results in an efficient and timely manner. The ELISA test is of great benefit in chronic misdiagnosed cases. When comparing test costs, all aspects should be considered. The high morbidity and ineffective empirical treatment that accompanies misdiagnosis, ultimately lead to higher cost on the health care sector and the whole community.

Key words: Giardiasis - *Giardia lamblia* – Coproantigen - ELISA

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List of Abbreviations

(s)IgA	: Secretory immunoglobulin A
ADI	: Arginine deiminase
Anti-GSA 65	: *Giardia* Specific Antigen 65 antibodies
DFA	: Direct fluorescent-antibody assay
EIA	: Enzyme immunoassay
ELISA	: Enzyme-linked immunosorbent assay
G. lamblia	: Giardia lamblia
GFP	: Green fluorescent protein
GSA 65	: *Giardia* Specific Antigen 65
IGS	: Intergenic spacer
IIF	: Indirect immunofluorescence
IMS-IFA	: Immunomagnetic separation coupled with immunofluorescence
kDa	: Kilodaltons
M cells	: Microfold cells
MIF	: Merthiolate-Iodine-Formaldehyde fixative
NPV	: Negative predictive value
O&P	: Ova and parasite
O.D.	: Optical Density
OCT	: Ornithine carbamoyl transferase
PCR	: Polymerase chain reaction
PPV	: Positive predictive value
PVA	: Polyvinyl alcohol
RFLP	: Restriction fragment length polymorphism
RT	: Reverse transcription
S.D.	: Standard Deviation
SAF	: Sodium Acetate Acetic Acid Formalin
SAGE	: Serial analysis of gene expression
SDB	: Specimen dilution buffer
TMB	: 3,3',5,5' Tetramethylbenzidine
tpi	: Triose phosphate isomerase gene
VSP	: Variant surface protein

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INTRODUCTION

Giardia lamblia is one of the most common causative agents of epidemic and endemic diarrheal illness throughout the world. It is a microaerophilic protist with a motile trophozoite stage and an immotile cyst stage **(Adam, 2001)**. Giardiasis is caused by ingestion of cysts. The infective dose could be as low as 10 cysts to cause clinical disease. Ingestion of more than 25 cysts results in a 100% infection rate. After ingestion of cysts, excystation, trophozoite multiplication, and colonization of the upper small bowel occur **(Pennardt, 2006)**.

Giardia lamblia, also known as *G. duodenalis* or *G. intestinalis*, was originally identified by Antonie van Leeuwenhoek, the inventor of the microscope, in the 1600s. Interestingly, it was associated by him with his own loose stools. The first good illustrations of *Giardia* are those of Vilém Lambl in 1859. Although it was the first protozoan parasite described, its role as a pathogenic organism was not recognized until the 1970s, after community outbreaks and after the appearance of the disease in travelers returning from endemic regions **(Cox, 2002)**.

This organism has a worldwide distribution and is a major cause of epidemic childhood diarrhea in developing countries where prevalence rates vary from 4-42%. It is the most common gut parasite in the United Kingdom and United States, and infection rates are especially high in Eastern Europe. There are fewer foodborne outbreaks documented than waterborne outbreaks **(Smith et al., 2007).**

Groups most at risk for infection include children mainly, travelers, homosexual men and individuals with immunoglobulin deficiency states.

Many infected subjects are asymptomatic, and most infections are self-limited. However, chronic infections, marked by chronic diarrhea, steatorrhea and malabsorption occur and may last for weeks to months **(Cash, 2006)**.

For accurate diagnosis of Giardiasis by direct microscopy, at least 3 stool samples taken at 2-day intervals should be examined and this might be very difficult especially in developing countries. A single microscopic examination for stool samples detects only 50-70% of infected patients due to the intermittent shedding of cysts and trophozoites **(Rosoff and Stibbs, 1986)**. Several commercially available tests to detect *Giardia* antigen in the stool exist. These utilize either an immunofluorescent antibody (IFA) assay or a capture enzyme-linked immunosorbent assay (ELISA) against cyst or trophozoite antigens. These tests have a sensitivity of 85-98% and a specificity of 90-100% thus, are more sensitive than stool examination **(Pennardt, 2006)**.

Giardia Specific Antigen 65 (GSA 65) is a *Giardia* antigen that is produced in abundant quantities by the *Giardia lamblia* protozoa and it is possible to find it in stool specimens without visible signs of cysts or trophozoites **(Rosoff and Stibbs, 1986 and Rosoff et al., 1989)**. GSA 65 and its antibodies do not cross-react with other enteric parasites **(Addiss et al., 1991 and Rosoff and Stibbs, 1986)** and is stable to most routine procedures used to collect and store stool specimens **(Rosoff et al., 1989, Schieven and Hussain, 1990 and Addiss et al., 1991)**.

Aim of Work

This study aimed to verify the efficacy, advantages and drawbacks of immunoassaying versus microscopy for diagnosing *Giardia lamblia*, when single fecal samples are analyzed. The *Giardia* antigen detected using ELISA for diagnosis was GSA 65.

REVIEW OF LITERATURE

Taxonomy

Taxonomical Classification (Kofoid and Christiansen, 1915)

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora (flagellated protozoan)
Class	Zoomastigophora
Order	Diplomonadida
Family	Hexamitidae
Genus	*Giardia*
Species	*lamblia*

Morphology and Life Cycle

Giardia lamblia exists in two forms: Trophozoite (Fig. 1) and cyst (Fig. 2) forms. Man is infected by ingesting cysts in contaminated water or food. Thereafter, excystation occurs in the proximal small intestine releasing trophozoites that multiply by simple binary fission and colonize the small inetstine. The trophozoite exhibits a characteristic pear or tear-drop shape with bilateral symmetry. It is typically 12-15 µm long, 5-10 µm wide and 2-4 µm thick. Characteristic features of the stained trophozoite include: two nuclei with central karyosomes, fibrils (axonemes) running the length of the parasite and median bodies. The large karyosome and lack of peripheral chromatin gives the nuclei a halo appearance. Axonemes are formed from the proximal regions of the flagellae within the body of the trophozoite. The median bodies are a pair of curved rod-shaped structures which lie posterior to the nuclei. At the ultrastructural level the median bodies contain an array of microtubules and their function might be involved with the ventral disk and its formation **(Elmendorf et al., 2003)**.

The ventral disk, not always visible by light microscopy, occupies two-thirds of the anterior end of the ventral surface and mediates attachment of the parasite to the intestinal epithelium. Ultrastructurally, it has striations which are the result of microtubules and a unique cytoskeletal element called microribbons. Each microribbon is associated with a microtubule and the combined microtubule-microribbon structure is arranged in concentric rows. A major component of microribbons is proteins called giardins (beta-giardins). The outer rim of the ventral disk,

called the lateral crest, contains components of the actin-myosin cytoskeleton **(Gosh et al., 2001)**.

Trophozoites possess four pairs of motile flagellae. Three pairs emerge from the dorsal surface (anterior, posterior-lateral, caudal) and one pair emerges from the ventral surface. Trophozoites exhibit a distinctive erratic twisting motion, sometimes compared to that of a falling leaf. However, they are predominantly found attached to epithelial cells of the small intestine (especially the duodenum and jejunum) and are rarely found in stools, except in the cases of severe diarrhea. The trophozoite absorbs nutrients from the intestinal lumen via pinocytosis. The trophic stage is characterized by an asexual replication where both nuclei divide at about the same time and cytokinesis restores the binucleated state. Each daughter cell receives one copy of each nucleus. Both nuclei appear equal in regards to gene expression and other properties **(Benchimol, 2004)**.

As an alternative to replication the trophozoite can encyst. The parasite rounds up, detaches from the intestinal epithelium, and secretes a cyst wall. Encystation can also be carried out in vitro. Optimal induction of encystment is obtained by depriving the trophozoites of bile at pH 7 followed by an exposure to high concentrations of bile at pH 7.8. The lack of bile at neutral pH mimics the conditions under the mucus blanket adjacent to the intestinal epithelial cells, whereas exposure to high concentrations of bile at more alkaline pH is analogous to the intestinal lumen. These studies highlight the extent to which *Giardia* has adapted to life within the gastrointestinal tract. After cyst wall formation the parasite undergoes one round of nuclear division without cytokinesis resulting in four nuclei. These four nuclei are usually located at the

anterior end of the cyst. The flagellae and ventral disk are lost as the cyst matures, but the axonemes and median bodies persist. The cysts are oval shaped, measuring 11-14 µm in length and 6-10 µm wide. The cyst wall is well-defined and is often set apart from the cytoplasm of the parasite. The cysts are passed in the faeces and can survive for up to three months under appropriate temperature and moisture. Mature cysts are infective, thus completing the life cycle (Fig. 3) **(Jarroll et al., 2001)**.

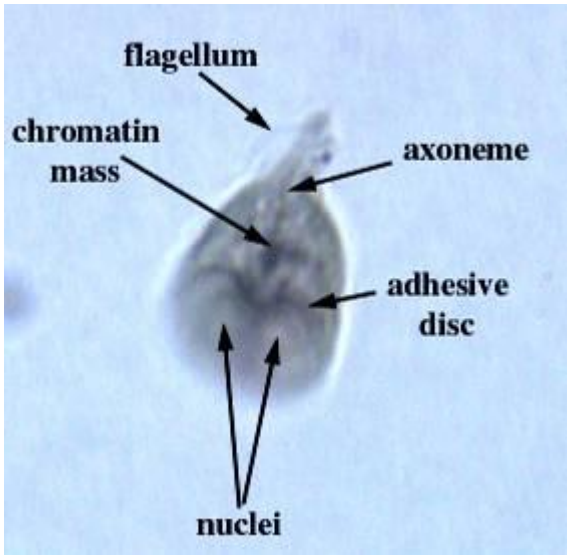

Fig. 1: *Giardia lamblia* trophozoite

Available at: **www.umanitoba.ca/.../dick/z346/giardiahome.html**

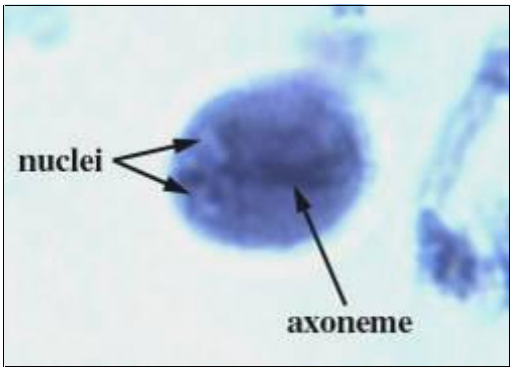

Fig. 2: *Giardia lamblia* cyst

Available at: **www.umanitoba.ca/.../dick/z346/giardiahome.html**