

Title Entamoeba histolytica – The causative agent of clinical amoebiasis

Name Peter George Sargeaunt

This is a digitised version of a dissertation submitted to the University of Bedfordshire.

It is available to view only.

This item is subject to copyright.

ENTAMOEBA HISTOLYTICA – THE CAUSATIVE AGENT OF CLINICAL AMOEBIASIS

by

PETER G. SARGEAUNT

A thesis submitted to the:

Department of Biology and Health Science
Faculty of Science, Technology and Design,
UNIVERSITY OF LUTON

In partial fulfilment of the requirements for the degree of:

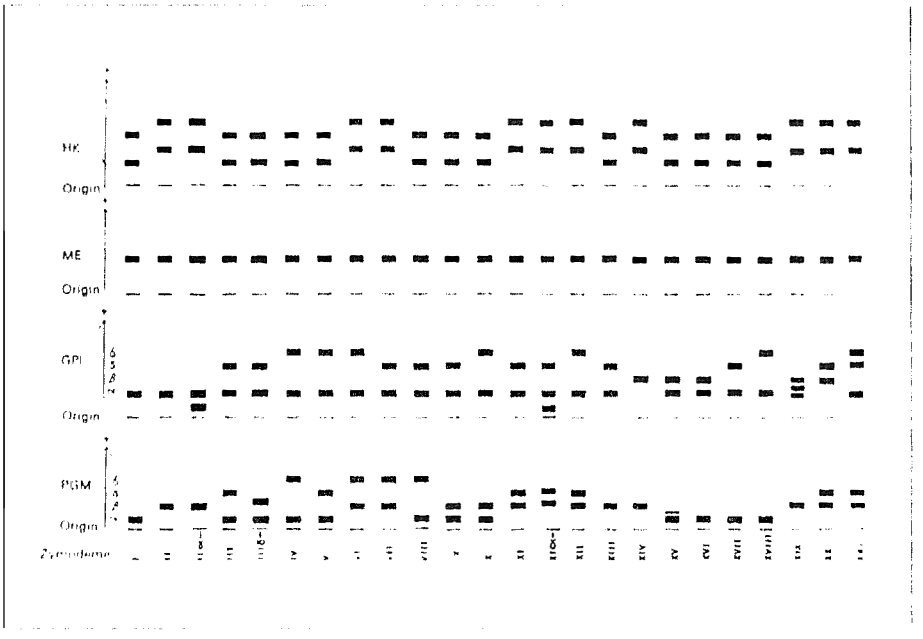
Degree of Doctor of Philosophy by Publication

Kept at enquiry desk
UNIVERSITY OF LUTON
LIBRARY
3402936845
574.5249
SAR
Reference only
July 2000

University of Luton,
Park Square,
Luton,
Bedforshire.
LU1 3JU

ABSTRACT

Entamoeba histolytica causing clinical amoebiasis, can, if left untreated, result in fatal consequences. This parasite has a global distribution with a high incidence in tropical countries. Many infections with the parasite do not produce disease. Because the parasite appeared to have two types of presentation in the host Brumpt (1925) raised the hypothesis that two organisms were responsible. His suggestion was neglected until in 1978, and subsequently, **Sargeaunt**, using thin layer starch gel electrophoresis, demonstrated that the parasites iso-enzymes produced different patterns for the two states of infection. Numerous communities throughout the world have benefited from this discovery and vast amounts of drugs are no longer required to treat spurious infections. The two types of infection are now correctly recognised as *Entamoeba histolytica* - causing clinical amoebiasis and *Entamoeba dispar* (Brumpt, 1925), a harmless intestinal commensal.



Zymodemes of *Entamoeba histolytica* identified using EC 5319 glucose phopshatase isomerase (GPI); EC 11140 L-malate: NADP + oxidoreductase (oxaloacetate decarboxylating) (ME); EC 2751 phosphoglucomutase (PGM); and EC 2711 hexokinase (HK).

A zymodeme is a population of amoebae differing from similar populations in the electrophoretic mobility of certain specified enzymes. The markers for pathogenicity are the absence of the α - band together with the presence of the β band in PGM. Advanced bands in HK confirm the PGM results. The only exception is zymodeme XIII which lacks advanced HK bands.

Sargeaunt 1990

FRONTISPIECE

DEDICATION

To XIRT.

AUTHORS DECLARATION

I declare that this thesis is my own work (*as further specified on page 46 of this thesis*). It is being submitted for the degree of Doctor of Philosophy by Publication at the University of Luton. It has not been submitted before for any degree or examination in any other University

Peter Sargeaunt

July 1st, 2000

CONTENTS

SECTION	Page
Abstract	II
Frontispiece	III
Acknowledgements	IV
Authors Declaration	V
Contents	VI
Corrigenda	VIII
1.0 Foreword	1
2.0 Introduction	3
2.2 The Parasite	6
2.2.1 Trophozoite.	6
2.1.2 Cyst.	6
2.3 Morphology in Tissues	14
2.4 Intestinal Infection	15
2.5 Pathology Of Liver Infections	17
2.6 Epidemiology	18
3.0 Discussion of published works submitted	19
4.0 Summary	26
5.0 References	28

SECTION	Page
Published work submitted for doctorate.	**39**
List of published work submitted for doctorate	40
List of published works used for the candidature specifying the percentage of work carried out by the candidate	44
List of Laboratories where the research was conducted	45
Copies of published work submitted for thesis	48
Co-authors forms together with supporting letters.	

CORRIGENDA

1.0 FOREWORD

Towards the end of the 1800's a Russian physicist examined a patient at post mortem and discovered ulcers in his intestine. Microscopic examination of material from the ulcers and faeces, revealed small motile organisms (parasites) inside which were red blood cells. He recognised these objects as amoebae. These amoebae were later named *Entamoeba histolytica*. From that time many scientists, particularly on the European continent, began producing discussions on the clinical picture given by examination of subjects that showed by microscopic examination of their faeces those amoebae that the Russian physician had first seen.

As knowledge of the parasite increased, it was observed that it was distributed world wide and particularly prevalent in tropical countries. The amoeba was seen to have a resistant form (in its life cycle) which was known as a cyst. This resistant form, in moist warm conditions, remained viable until ingested with contaminated food and drink. The cyst then travelled into the intestine where conditions were suitable for it to transform into the vegetative amoeba. As an amoeba in the intestine it has the ability to live on red blood cells and will if not treated with suitable drugs (antibiotics) penetrate the wall of the intestine and produce ulcers. It can also travel into the liver and other large organs of the body. Without treatment death may ensue at any tine. Thousands of people across the world are infected with this parasite.

In 1925, a French physician suggested that there were two parasites which under the microscope looked the same. He reasoned that because not everybody who harboured the parasite become ill then only those with the parasite that could kill should receive treatment.

Nobody paid attention to what the French physician said and consequently everybody who showed this parasite in their faeces irrespective of their clinical condition was treated with drugs. Some of the drugs used produced very debilitating side effects.

More than 50 years after the suggestion that two parasites were involved, the eventual answer arrived. It has been proven irrefutably that indeed two parasites, that look the same under the microscope, are responsible for a great deal of confusion.

By looking at the proteins (enzymes) of the amoebae, clear cut differences have been shown between the two parasites.

Therefore vast amounts of drugs are no longer needed and the people to whom they have been administered are relieved of suffering.

2.0 INTRODUCTION

In 1875, Fedor Aleksandrovich Lösch described in some detail his studies on a 24 year old farmer admitted to his clinic in November 1873 in St. Petersburg. In this patient who was suffering from chronic dysentery, Lösch found in the stool amoebae in such enormous numbers that he became convinced that there was an etiologic relationship between the presence of the parasites and the disease syndrome. He then described the parasite in most careful detail with such emphasis on size, form, vacuole, and motility that there is little doubt that he was dealing with *Entamoeba histolytica*, although he named it because of its location - *Amoebae coli*. The stools of the patient contained blood and mucus associated with the presence of these amoebae. At autopsy, the patient having died of pleurisy, the colon was described as being heavily ulcerated and with submucosal lesions containing parasites.

Lösch gave two ounces of the patient's fresh, amoebae-containing stool to three dogs, orally and rectally. In one of these dogs he was able to produce a dysentery with bloody mucus. After the animal was sacrificed, an ulcerated colon was described and amoebae in great numbers were found in the ulcers.

"Koch in 1883 observed five cases of dysentery in Egypt, two of them complicated with abscess of the liver, and in the ulcers occurring in the large intestine he found numerous amoebae, and in sections these were also found deep in the tissues at the base of the ulcerations, as well as in the capillaries of the liver, close to the abscess walls" (Craig, 1944).

Koch's work stimulated Kartulis to a more intensive investigation. A series of papers published by Kartulis (1886, 1887, 1904) must be counted among the most important in the history of the infection. He reported (1886) upon 150 cases of dysentery in which he recovered amoebae from the stool and shortly thereafter found amoebae in liver abscesses which he associated with dysentery. Among the most interesting if not the most important finding was the discovery that 11 of 384 cases of liver abscess were complicated by brain abscess, and he found amoebae both in the brain and the liver in two of these.

A milestone in the history of amoebic dysentery was the discovery of the cause of bacillary dysentery by Shiga (1898); this was confirmed by Flexner in 1900. Finally, the major causes of ulcerative dysentery could be distinguished in the laboratory if not at the bedside. As late as 1914, Brown wrote, "The discovery that dysentery itself is not a single disease may be regarded as one of the most remarkable advances in modern medical science."

Levine (1973) reviews and places in perspective without prejudice, features illustrating differences within the "histolytica group, such as, "only the large forms of *Entamoeba histolytica* are generally considered pathogenic", and "different strains of *E. histolytica* differ in pathogenicity and their pathogenicity itself may vary with their environment and other factors".

Jarumilinta and Maegraith (1969) could not differentiate between pathogenic and non-pathogenic strains of *E. histolytica* when looking at their hyaluronidase and proteolytic enzymes. However, these results only reflect the fact that they were looking for the presence or absence of particular enzymes.

Deschiens (1961) recognised the intrinsic pathogenicity of a strain when he said, it has "its own pathogenic power, which varies according to the line, and is genetically transmissible".

Swellengrebel (1961) found it "difficult to reconcile certain facts with the notion of immutable pathogenicity of *E. histolytica*. However, these facts become intelligible if one considers the amoebae as a parasite, the pathogenicity of which can decrease or even disappear, in other words, if one considers "*E. histolytica* now as a commensal, now as an infective agent'. His final conclusion was "*E. dispar* described by Brumpt (1925) may be considered as a biological apathogenic subspecies or as a race of *E. histolytica*.

Sargeaunt et al. (references 1978-1988) have shown irrefutable evidence, by enzyme mobility differences, ie, basic gene coded elements, not only between strains of *E. histolytica* but differences within the whole "histolytica" group. Also new technology developed in the last 2 to 3 years, and discussed below demonstrates every antigen or gene that has been cloned has been shown to have either different antibody reactivity or differences in the restriction fragment length polymorphs between pathogenic and non-pathogenic strains.

2.2 The Parasite

Sargeaunt (1975).

There are then two stages, in the life-cycle, namely the cyst which is a host infective stage. The quadranucleate cyst is ingested and lodges in the crypts of the intestine where, if it finds a suitable microenvironment, it excysts as a trophozoite. Host tissue is then invaded. Should the organism (i.e. trophozoite) be excreted, for example, during an episode of diarrhoea, it will be seen under the microscope to contain red blood cells and to travel directionally by blunt explosive pseudopodia.

2.2.1 Trophozoite. Trophozoites measure from 9-30 μm may be round, elongated or irregular in shape. The cytoplasm consists of a clear active condition the amoebae show flowing movements of their protoplasm and rapidly protrude and retract broad pseudopodia (lobopodia), which may be composed at first mostly of ectoplasm. These movements lead to changes in shape and also to active translatory progression. The nucleus is round or oval, and in the unstained condition is not easily distinguished. It is situated in the endoplasm, usually eccentric in position. It is poor in chromatin, and the nuclear membrane is thin. The chromatin granules are small, and are collected in a ring just inside the nuclear membrane; the nucleus shows a small central karyosome.

2.1.2 Cyst. Cysts are spherical, with a thin, hyaline, refractile wall, which gives them a distinct double contour. The contents are finely granular, and the average diameter is 12.5 μm. Cysts may contain from one to four nuclei, a diffuse glycogen mass and chromatoid bodies.

The amoebae then ingest red blood corpuscles, which are observed in the endoplasm, but ingested bacteria are less frequently found. The ingested erythrocytes appear smaller than normal. Ingestion of erythrocytes by amoebae in faecal specimens is pathogonomic of *E. histolytica*. Vegetative forms after leaving the body, soon become rounded and immobile, die and disintegrate.

There is another organism thought also to be *E. histolytica*, which dwells entirely in the lumen of the gut and is a saprophytic commensal. This has a similar life-cycle, but without the ability to invade tissue, and is never found containing red blood cells although it has a similar cyst morphology. This organism was labelled by Brumpt in 1925 as *Entamoeba dispar*. He described it as similar to *E. histolytica* but not causing disease and found in temperate climates. Consequently Brumpt called *E. histolytica Entamoeba dysenteri ae*, which caused disease and was found in tropical areas of the world. Recent results demonstrated that Brumpt was correct; there are indeed two distinct species demonstrable at molecular level.

The similarity between *E. histolytica* and *E. coli*, compounded by imprecise nomenclature, led to great confusion regarding parasite identification and pathogenicity. Quincke and Roos distinguished between *E. histolytica* and *E.coli*, and described the cyst of *E. histolytica* in 1893.

They described a patient who had become ill along with 14 others after all had shared a common drinking source, thus pointing out a mode of transmission which even today is the most important means of spreading amoebiasis. Stool examination disclosed amoebae, some of which had ingested red cells and most of

which were very actively motile. Symptoms correlated with the number of amoebae. Significantly, Quincke and Roos also described cysts and noted their great resistance compared to the fragility of the motile organisms. Using cats as their experimental model, they discovered that fresh, amoebae-containing stool introduced rectally produced typical amoebic dysentery, whereas the same stool given orally produced no disease. However, when old, cyst-containing stool was given orally, cats developed amoebic dysentery. From a second patient Quincke and Roos described amoebae which were larger, less motile, more granular, and more resistant to desiccation than amoebae of their first case. Further investigation showed that these produced no disease and were often found in healthy people. This was the first distinction between *E. histolytica* and *E. coli* although the confused naming of several types at that time prevented such a clear demarcation of the species; nevertheless, the fundamental differences were described.

Huber (1903) confirmed the observations of Quincke and Roos and added the important observation that the cysts of the pathogenic amoebae contained one, two, or four nuclei but never more and thus can be distinguished from "ordinary amoebae".

Schaudinn (1903) described in accurate detail the difference between the trophozoite forms of the pathogenic and non-pathogenic amoebae and named them, to the consternation of nomenclaturologists, *E. histolytica* and *Entamoeba coli*. Schaudinn appreciated that the amoebae in East Prussia and Berlin were non-pathogenic (*E. coli*) while those he received from colleagues (Kartulis and Looss in Egypt, as well as specimens from China) were pathogenic (*E. histolytica*). His influence was so great that this differentiation and the

nomenclature were finally accepted by all.

The definitive account of the pathology of amoebiasis as well as the relationship between intestinal amoebiasis and amoebic abscess of the liver was recorded by Councilman and Lafleur (1891). These two colleagues and collaborators of Sir William Osler (1884) whom they described as "the first to find amoebas in America," published in The Johns Hopkins Hospital Reports, a massive monograph entitled "Amoebic Dysentery".

Councilman and Lafleur's meticulous description of the intestinal lesions left almost nothing to be added for the next 100 years. They recorded, in the most minute detail, the passage of amoebae through the mucosa; the localization in the submucosa; the necrosis, at first nonpurulent in character; the jeopardization of the vascular tree; and the final ulceration of the mucosa. In addition, they emphasized that bacteria were not responsible for the lesions. They described amoebic abscess of the liver, in addition they also described abscess of the lung secondary to abscess on the upper surface of the liver.

Considering the clinical picture as the patient presents, would leave no doubt as to treatment if the subject has a liver abscess, or infection at some other extraintestinal site. Intestinal invasion by the organism may reveal ulceration on sigmoidoscopy. We could then, reasonably expect the laboratory to report the presence of haematophagous trophozoites present in extravasated material from those sites. Thus a pathogenic condition is diagnosed. The question now arises, can the laboratory identify those organisms, present in either cyst or trophozoite form, producing a life threatening infection from similar morphologically indistinguishable organisms found as gut commensals in asymptomatic subjects? The answer is an unquestionable yes! It follows that recognition of the two states

of infection would lead to treatment only of the organisms capable of causing disease. Those infections with the commensal form would be left untreated. Evidence to support this proposal is substantiated by the research of **Sargeaunt** et al. (1978 -1993).

Also by the reports of Allason Jones *et al.* (1986), Goldmeier *et al.* (1986) and particularly that of Weber *et al.* (1990) comes from research on male homosexuals. Of many separate faecal samples, examined by culture, from those subjects, approximately 12.6 per cent were found to be excreting *E. histolytica.* None of those subjects had related clinical symptoms, and none of them were treated for *E. histolytica* infection. To have such a high incidence of infection in any group of subjects may at first appear to be alarming. But, there has never been anything but an exceedingly rare incidence of a pathogenic infection with *E. histolytica* in this group of people. The last statement is supported albeit obliquely, by the knowledge that there are no reported cases of overwhelming infections with *E. histolytica* in male homosexuals with Acquired Immune Deficiency Syndrome. Thus indicating that the commensal form of *E. histolytica* shows very little response to an immunologically compromised system. Conversely, there are reports of occult infections with the invasive form of *E. histolytica* in patients treated with corticosteroids (Stamm, 1975) where the outcome of such treatment has resulted in death due to a fulminating infection with the parasite. This last circumstance does not occur in the homosexual situation outlined above.

Massive support for the need to reclassify *Entamoeba histolytica* into the two species according to Brumpt (1925): *E.dysenteriae* and *E.dispar* came not only from a decade of zymodeme classification (Sargeaunt; 1978 - 1983) but also from

original research by various "Amoebic groups" around the world

By mixing pathogenic strains each with a different zymodeme profile, **Sargeaunt** *et al.* (1985,1988b) demonstrated genetic exchange. However, repeating this process many times over a long period with mixtures of pathogenic and non-pathogenic strains has resulted in complete failure.

Results from further research to confirm that *E. histolytica* is two species has led to a rash of new publications. Garfinkel *et al.* (1989) developed DNA probes which hybridized selectively to DNA of either pathogenic or non-pathogenic *E. histolytica* as classified by zymodemes. Of the two non-pathogenic strains used in this work, the report says "a probe derived from", a pathogenic strain, "did not hybridize to DNA from *E. histolytica* SAW 1734R c1AR and WI: 1285: 1 which have non-pathogenic zymodemes".

Tannich *et al.* (1989) reported genomic DNA differences between pathogenic and non-pathogenic *E. histolytica*, one cDNA library being made from SAW 1734.

Bracha *et al.* (1990) constructed DNA probes which recognised respectively pathogenic or non-pathogenic *E. histolytica* SAW 1734R c1AR was used for the non-pathogenic probe which in turn recognised many other non-pathogenic strains, many of which were from the SAW bank.

(SAW = Sargeaunt and Williams followed by a number represents a particular isolate).

Petri *et al.* (1990) differentiated between pathogenic and non-pathogenic *E. histolytica* by monoclonal antibodies to the galactose specific adherence lectin

"No significant difference in lectin content was detected" when a pathogenic strain (HM1 :-MSS) was grown, either with or without bacterial concomitants. Of the 18 pathogenic and 32 non-pathogenic strains as determined by zymodeme profile which were tested, lectin was detected in only the pathogenic strains.

Edman et al. (1990) characterized an immuno-dominant variable surface antigen from pathogenic and non-pathogenic *E. histolytica*. The non-pathogenic strain used in this work, REF 91 (supplied by Dr. S.Reed, San Diego) together with a pathogenic strain were compared, at the same amino acid sequence level with the work of Tannich *et al.* (1989), described above. There is remarkably good correlation not only between the pathogenic strains used but also between the non-pathogenic strains REF 91 and SAW 1734.

By extending their genomic DNA analysis into restriction fragment analysis of a single gene amplified *in vitro*, Tannich *et al.* (1991) conclude that their results support the concept that pathogenic and non-pathogenic strains of *E. histolytica* constitute distinct species. 48 isolates were used in their latest work and each corresponded to the clinical status of the subjects. Among the non-pathogenic strains isolated 13 were from male homosexuals, "all of these isolates exhibited a non-pathogenic isoenzyme pattern and a non-pathogenic DNA restriction fragment pattern".

Que and Reed (1991) used a nucleotide sequence of a small subunit ribosomal RNA gene from *E. histolytica* and differentiated pathogenic from non-pathogenic isolates. Their illustration of an autoradiograph of southern blot shows a glaringly obvious difference between the types of amoeba. Included with the non-pathogenic strains was SAW 1734.

Tannich *et al.* (1991a) produced a cDNA clone derived from the gene encoding a cysteine proteinase of pathogenic *E. histolytica* isolated using antiserum to the purified enzyme. Part of their conclusion from use of this material is "the difference in genomic organization and its consistency within the groups of pathogenic and non-pathogenic strains", again support the notion that the two groups are genetically distinct. Interestingly in their discussion of the clone they draw no conclusion as to whether it may be lysosomal/vacuolar, cell membranes associated or secreted. But "electron microscope studies indicated that the enzyme is located inside the vacuoles/lysosomes of the amoebae".

Reed *et al.* (1991) used molecular and cellular characterization of the 29 kDa peripheral membrane protein *of E. histolytica* to differentiate between pathogenic and non-pathogenic isolates. This is an extension of their previous work (Blakely *et al.* 1990). In this present paper they describe analysis of the complete nucleotide sequence and compared the immunoreactivity of this antigen in pathogenic and non-pathogenic strains. They characterised the 29kDa antigen with monoclonal antibodies which distinguish between pathogenic and non-pathogenic zymodeme classified strains. These workers make the important point that this 29 kDa antigen was extremely labile when after 2 weeks storage at -70°C, the antigen was not found intact by immunoblotting. Their results show quite clearly well defined differences between pathogenic and non-pathogenic clinical isolates of *E. histolytica* immuno-blotted with nine monoclonal antibodies specific for the 29kDa.

Recently Clark and Diamond (1991) have shown in a most neatly executed piece of work, irrefutable evidence that ribosomal RNA genes of pathogenic and non-

pathogenic (including SAW 1734) *E. histolytica* are distinct. These workers like ourselves in London attempted to replicate the published results of "conversion" (Mirelman, 1986). "Using the same strains and following the published methodologies, but despite considerable effort we have not met with success (unpublished results)". The final sentence of their discussion is: "All the available data including those presented above support the notion that *E. histolytica*, as currently defined, is in fact two distinct species."

Gathiram and Jackson (1987) demonstrated in a very precise experiment that "pathogenic zymodemes" of *E. histolytica* remain unchanged throughout the life cycle of the organism. With their permission it is reported here that they also endeavoured, over a period of a year, to repeat the experiments of **Sargeaunt's** work in an endeavour to breed pathogenic and non-pathogenic strains together (cf. page 11). Sargeaunt (1992). They also met with no success.

2.3 Morphology in Tissues

In the intestinal lumen, *E. histolytica* may be seen as both cysts and trophozoites; however, only trophozoites are found in tissue in this situation (Stamm, 1970; Griffin and Juniper, 1971). The trophozoite is a 15-40 μm diameter protozoan containing numerous vacuoles and a characteristic nucleus with a central karyosome surrounded by delicate chromatin along the nuclear membrane (Adams and Macleod, 1977; Brandt and Perez-Tamayo, 1970; El-Hashimi and Pittman, 1970; Griffin and Juniper, 1971).

The trophozoite may contain ingested red blood cells and by electron microscopy lacks mitochondria and Golgi complexes (Griffin Juniper, 1971; El-Hashimi and Pittman, 1970; Patterson and Schoppe, 1982). On histological

examination the trophozoites often appear to be surrounded by a clear halo (Brandt and Perez-Tamayo, 1970) that may be due to shrinkage during fixation. In all tissues, *E. histolytica* trophozoites are found within an amorphous eosinophilic debris, presumably the result of tissue lysis (Messersmith and Chase, 1984).

2.4 Intestinal Infection

Amoebic infection of the human intestine ranges in spectrum from luminal colonization to mucosal invasion. The colonic mucosa may be entirely normal or may have non-specific changes, even with the clinical syndrome of "symptomatic cyst passers" (Nanda *et al.*, 1984). Ulceration of the colonic mucosa is the hallmark of invasive disease and is found in acute colitis, toxic megacolon, and chronic nondysenteric syndromes (Haider and Rasul, 1975). Ulcerations develop more frequently in the caecum and ascending colon (Adams and MacLeod, 1977a; Brandt and Perez-Tamayo, 1970; Castro, 1974; Perez-Tamayo and Brandt, 1971; Aikat *et al.*, 1979), followed in frequency by the sigmoid, appendix, and descending colon (Craig, 1944; Clark, 1925; Reddy and Thangavelu, 1948; Kean *et al.*, 1956). The transverse colon is relatively spared, with ulceration occurring in only up to 10% of cases. Seventy percent of patients with amoebic colitis have ulcerations in segmental areas of the colon, but in 35% the entire colon is involved (Castro, 1974). Ulceration may progress to intestinal perforation, which occurs most frequently in the cecum (Jimenez, 1981) but can originate from other areas of the colon. Peritonitis may ensue, and formation of a fistular communication with the skin, retroperitoneal space, other hollow viscera or thorax can occur. Severe invasive diseases may result in necrosis of the entire colon and a clinical picture of "toxic megacolon" (Luvano *et al.*, 1982; Ballkian *et al.*, 1977; Prathap and Gilman, 1970; Friedrich *et al.*, 1980; Elhence *et al.*, 1979).

Pathological findings have contributed to understanding of the pathogenesis of intestinal amoebiasis. Studies of experimental human amoebic infection have been few in number and limited to establishment of intestinal infection after oral doses of *E. histolytica* cysts (Walker and Sellards, 1913; Beaver *et al.*, 1956). Early investigators believed that the amoebic trophozoite would penetrate the colonic mucosa and go directly to the submucosa. By setting up a nidus of infection the overlying mucosa would undergo necrosis, resulting in ulcer formation (Councilman and LaFleur, 1891; Paterson, 1935; Harris, 1898; Rogers, 1913). However, other observers felt that tissue necrosis began on the mucosal surface, resulting from direct invasion by the trophozoites or release of toxic products (Ratcliffe, 1931; Craig, 1927; Martin, 1953; Dopter, 1905; Boeck, 1933).

Initially, amoebic trophozoites are found in the lumen and within mucous, the underlying mucosa is normal in appearance (Brandt and Perez-Tamayo, 1970; Juniper *et al.*, 1958). Following attachment to the interglandular epithelium, the trophozoites have been found associated with microulcerations of the mucosa (Faust, 1941, 1954).

Following attachment of amoebae, the epithelium apparently undergoes lysis and the submucosa is invaded. Amoebic invasion of rectal crypts has also been reported (Juniper *et al.*, 1958). The human inflammatory response to amoebic invasion has been noted for its paucity. (Castro, 1974; Marcial and Marcial-Rojas, 1985; Robbins, 1984).

This may be due to the ability of *E. histolytica* to lyse host inflammatory cells (Guerrant *et al.*, 1981; Salata *et al.*, 1985). The ulcer is covered by a

necrotic layer containing eosinophilic granular debris, amoebic trophozoites, and few inflammatory cells (Pittman *et al.*, 1973). The ulcer extends into the lamina propria with the edges of normal tissue becoming edematous and hyperemic (Brandt and Perez-Tamayo, 1970; Pittman, *et al.*, 1973; Prathap and Gilman, 1970; Juniper, 1962). Ulceration proceeds through the lamina propria to the depth of the muscularis mucosa, where the progress usually stops prior to perforation. A plug of necrotic debris accumulates in the ulcer's center as the submucosa becomes undermined with amoebic trophozoites found in the leading edges of the ulcer base (Brandt and Perez-Tamayo, 1970; Prathap and Gilman, 1970; Juniper, 1978). The undermined edge of necrosis under viable-appearing mucosa results in the "flask" or "button-hole" ulcer classically described in amoebic colitis (Brandt and Perez-Tamayo, 1970; Castro, 1974; Marcial and Marcial-Rojas, 1985; Robbins, 1984; Pittman *et al.* 1973; Juniper, 1958, 1962; Musgrave, 1931).

2.5 Pathology Of Liver Infections

Amoebic infection of extracolonic sites results from prior intestinal infection, whether initially symptomatic or not. Mechanisms of spread involve direct extension of the hematogenous route. Direct extension can occur by intestinal perforation or involvement of perianal skin. Amoebae have been shown to travel to the liver by means of the portal venules and to lodge in the portal radicals (Brandt and Perez-Tamayo, 1970; Aikat *et al.*, 1979; Gulati *et al.*, 1967; Carrera, 1950; Tsutsumi *et al.*, 1984; Rasaretnam and Wijetilaka, T976).

Amoebic liver abscess remains the most important nonintestinal infection, due to the high frequency of occurrence and serious clinical consequences (Kean *et al.*, 1956; Ochsner and DeBakey, 1943a & 1943b; Katzenstein, 1982). Frequency of liver abscess varies greatly with geography and has been reported to

occur in up to 95% of fatal cases of amoebiasis (Brandt and Perez-Tamayo, 1970). The location is in the right liver lobe in up to 82% of liver abscess cases (Chatgidakis, 1953). Multiple abscesses are more common in cases with an acute presentation (Katzenstein, 1982). In Brandt and Perez-Tamayo's series from Mexico, multiple abscesses occurred in 85% of cases (Brandt and Perez-Tamayo, 1970; Perez-Tamayo and Brandt, 1971). Scragg (1960) in South Africa reported single abscess in 75%, and Kapoor *et al.* (1972) in Bombay reported 80% of the abscesses to be single.

2.6 Epidemiology

"*Entamoeba histolytica* is dispersed widely and produces an enormous burden of illness and death from colitis and liver abscess. Approximately 10% of the world's population carry the parasite, although a smaller percentage suffer disease. In some countries, such as Mexico, it is among the top 10 causes of death; worldwide, only the parasites malaria and schistosomiasis cause a greater number of deaths. A wide array of imperfect tests for diagnosis of amoebiasis severely limits our understanding of the magnitude and epidemiology of this infection. A greater hindrance is the varied and inconsistent application of existing diagnostic methods for surveys in different areas of the world. Yet our best estimate suggests that, in 1984, probably 500 million people were infected with *E. histolytica* and 40 million developed disabling colitis or extraintestinal abscesses. At least 40,000 deaths that year were attributable to amoebiasis. Much remains to be learned of its epidemiology but awaits the development of improved diagnostic tools." (Walsh, 1988).

3.0 DISCUSSION OF PUBLISHED WORKS SUBMITTED

In 1978 using modified techniques described by Wraxall and Culliford (1968), **Sargeaunt** and Williams began to show differences in the enzyme patterns of *E. histolytica* and *E. coli.* Subsequently they described (**Sargeaunt** and Williams, 1979) electrophoretic iso-enzyme patterns of the pathogenic and non-pathogenic intestinal amoebae of man. In 1978, **Sargeaunt**, Williams and Grene demonstrated four groups (later labelled as zymodemes, **Sargeaunt** and Williams, 1982b) of *E. histolytica* among which was one group originating exclusively from cases of clinical amoebiasis. A total of 85 isolations of the amoebae were made from subjects in New Delhi, India, where amoebiasis is recognised as being endemic. 17 of the subjects had clinical amoebiasis and all of these showed an exclusive pattern of iso-enzymes on electrophoresis. The remaining subjects showed non-pathogenic zymodemes and were clinically well.

Subsequent testing of further subjects in New Delhi and other areas in India (**Sargeaunt** *et al.*, 1984) showed a remarkable feature in that all the "pathogenic isolates" were of one single zymodeme. The inference drawn from this feature was that geographical factors may influence the distribution of "pathogenic zymodemes" of E.histolytica Also again these were only limited numbers of isolates showing a pattern consistent with clinical amoebiasis in the host.

A clinical follow-up to the above research by Nanda *et al.*(1984) showed a remarkable correlation to **Sargeaunt**'s results.

Mexico has been recognised as a country where amoebiasis caused by *Entamoeba histolytica* is among the top 10 causes of death (Walsh, 1988). Numerous surveys have shown varying degrees of infection throughout the

population and among these, Gutierrez (1976) conducted a survey of the entire country using (serum') counterelectrophoresis. His results showed a 6 per cent rate of positivity. However, it must be recognised that circulating antibodies remain for some time and consequently Gutierrez's results should be viewed with this in mind.

Two surveys (**Sargeaunt** *et al.*, 1980, 1982c) and a review (**Sargeaunt** *et al.*, 1982d), have shown a great diversity of zymodeme profiles of *E. histolytica* and the other amoebae found in man. Of *E. histolytica* patterns thirteen different profiles were shown among 85 isolations, with only 17 of these indicating a pathogenic infection in the host. In the first survey (**Sargeaunt** *et al.*, 1980) it was shown that 11.2 per cent of the total cases tested gave a pattern consistent with a pathogenic condition in the host.

Many laboratories around the world retain from as early as 1948 stock cultures of *Entamoeba histolytica* and related organisms. **Sargeaunt** *et al.* (1988a) have studied these cultures using isoenzyme electrophoresis in an effort to rationalize these isolates into easily recognisable forms and groups. All the isolates have reliable histories including clinical details. Some of the isolates, were made from higher primates other than man, and these became a useful marker for subsequent work (**Sargeaunt** *et al.*, 1982b, 1982f).

In Durban, South Africa, which is an endemic area for *E. histolytica* a survey was undertaken (**Sargeaunt** *et al.*, 1982) using both hospital patients, each with a clear clinical history, and also out-patients with no history of amoebic disease, but attending hospital for physical disabilities. Among the hospital patients, clinical disease was firmly established and many cases of amoebic liver abscess and amoebic dysentery showed, by isolation of the amoebae in culture, a zymodeme pattern consistent with pathogenicity in the host.

Three subjects, i.e., 4.5 per cent of 67 symptom free cy ' passers, among 344 individuals from the out-patient group were shown to have amoebae with a pathogenic pattern.

A random zymodeme study of amoebae in a group of South African school children (**Sargeaunt** *et al.*, 1982e), showed the presence of *E. histolytica* in 31 children in a total of 94 tested. Only one of the children provided a zymodeme consistent with a pathogenic condition. The results of the two surveys In South Africa mentioned above, prompted by the zymodeme research, fit together with the report (Editorial, Lancet, 1985') by Jackson *et al.* (1985) on serological testing of subjects with and without clinical amoebic disease.

When isolating amoebae in culture (Robinson, 1968), prior to harvesting and application to thin layer starch gel electrophoresis, it became necessary to examine the morphology of the organisms themselves (**Sargeaunt** and Williams, 1982a). All the amoebae colonising the lower intestinal tract have been described since the morphology is not always the same as in fresh material.

The presence of circulating infections of *E. histolytica* within closed communities (Mental homes) has been constantly reported (Faust and Russell, 1964, Thacker *et al.*, 1979) **Sargeaunt** and Williams, (1982b) reported a 7.0 per cent infection rate with *E. histolytica* in patients in a group of Mental Hospitals. 174 samples were examined and apart from the *E. histolytica* infections, other species of amoebae were demonstrated. As expected none of the *E. histolytica* isolates gave a zymodeme pattern consistent with a pathogenic condition in the host. These results added together with those of Thacker *et al.* (1979) show a very low incidence of *E. histolytica* in a closed community.

In North West Italy a mental home, in which are housed a particularly debilitated group of subjects has produced an interesting feature. Three patients among 80 have shown *E. histolytica* in their faeces which on isoenzyme electrophoresis demonstrate a pattern consistent with pathogenicity in the host (Scaglia *et al.* and **Sargeaunt**, 1991). The salient point to make about these isolations is that they come from a very unusual group of subjects.

Over time it became clear that a pattern rarely shown of isoenzyme profiles on electrophoresis was not that usually seen from human isolates. Since the higher primates other than man also carry amoebae, a survey of zoological stock was undertaken (**Sargeaunt** *et al.*, 1982f). The results showed that not only was *E. histolytica* being passed by apes and monkeys, it was also being passed to man (zoo keepers) with an *Entamoeba* species (i.e. *E. chattoni*) commonly found only in those animals. This fact was confirmed by a further isolate (**Sargeaunt** *et al.*, 1992b) from a subject who had travelled in India.

In 1983 **Sargeaunt** summarised the electrophoretic patterns so far established for *E. histolytica* and linked these with the ability of the parasite to resist lysis by human serum (Reed, **Sargeaunt** and Braude, 1983).

Epidemiological studies during the late 1970's reported the transmission of amoebiasis among homosexual men (Felman *et al.*, 1979, Pomerantz *et al.*, 1980, Schmerin *et al.*, 1977). These subjects presented with varying symptoms which were not necessarily related to amoebic disease. Druckman and Quinn (1988) refer to these infections. "As a sexually acquired pathogen, *E. histolytica* and the other pathogens of the gay bowel syndrome frequently produce syndromes that differ from the clinical illnesses observed in heterosexuals, where sexual

transmission is believed to be less frequent (Quinn, 1984, Quinn *et al.*, 1983; Williams, 1983). Subtle differences in clinical presentation, differential diagnosis, and often the presence of multiple pathogens in a single host all contribute to the unique nature of amoebic infections in homosexual males". All these authors were dealing with an unknown quantity since they did not know the specific identity of the potential pathogen with which they were dealing and if *E. histolytica* was present they concluded that that parasite was the cause of symptoms.

In 1983, **Sargeaunt** *et al.* reviewed the distribution of faecal protozoa in 470 male homosexuals. 52 of the total number produced *E. histolytica* but none of these infections proved on isoenzyme electrophoresis to be capable of producing a pattern consistent with clinical amoebiasis in man. Following this survey three other in-depth studies on homosexual males, Goldmeir, **Sargeaunt**, Price, Munday *et al* (1986), Allason-Jones, Mindel, **Sargeaunt** *et al.* (1986). Allason-Jones, Mindel, **Sargeaunt** *et al.* (1988) were carried out. Of the very large numbers of subjects examined many produced *E. histolytica* in their stools but not a single subject showed an isoenzyme electrophoresis pattern other than that which would be expected from a commensal infection in the host. This fact was also confirmed by intestinal biopsies in AIDS/HIV infections (**Sargeaunt** *et al.* (1988a).

A very important survey was undertaken (Weber, Mohr, Fleischer and **Sargeaunt**, 1990) on a cohort of travellers whose clinical condition and parasitological events were monitored over a period of two and a half years. 99 subjects from a total of *6856* people examined were carrying *E. histolytica*, but only 4 subjects produced amoebae which identified with a pathogenic strain by isoenzyme electrophoresis. The probable areas where these travellers became

infected was identified. One of the subjects, despite continuing treatments, continued to excrete his pathogenic amoeba.

A small review of isolates made from homosexuals in Japan (Nozaki et al. and **Sargeaunt**, 1989) suggests that *E. histolytica* isoenzyme patterns consistent with pathogenicity in the host may be circulating in that community.

Because it became clear, as the various surveys had demonstrated, that *E. histolytica* was in fact composed of two species, *E. histolytica* and *E. dispar*, as Brumpt (1925) had suggested, an attempt was made to interbreed "pathogenic strains" in an *in-vitro*, and then in an *in-vivo* system (**Sargeaunt**, 1985; **Sargeaunt** *et al.*,1988; Blanc, Nicholls and **Sargeaunt,** 1989). Success was achieved and three new zymodemes were 'made". Each of these was found to be stable and gave reproducible results. These experiments then obviously allowed an attempt which was the whole objective of interbreeding the pathogenic strains, to try and interbreed together pathogenic (*E. histolytica*) and non-*pathogenic (E. dispar*) strains. Numerous attempts were made with varying zymodemes but no success was achieved. This last experiment, although negative, fortifies acceptance of two species.

The Seychelles survey (**Sargeaunt,** 1992a), illustrates a very clear picture of what is happening across the world, particularly in an *E. histolytica* endemic area. Because of the continuing presence of *E. histolytica* infections in the community, there is a tendency for over diagnosis of the parasite, due to the presence also of *E. dispar*, which has a similar morphology at the cyst stage of its life cycle The

consequence is over treatment. This survey also throws light on epidemiology of *E. histolytica* since only two of a family of seventeen were infected *with E. histolytica* and both of these were children of school age.

During the course of the enzyme classification research described above, a suggestion was made by Mirelman *et al.* (1986) that concomitant growths of bacteria in culture with amoebae could influence the zymodeme pattern. To answer this suggestion, **Sargeaunt** *et al.* (1987) carried out an in-depth study which showed that no such possibility could occur. The fact that zymodemes were immutable was reinforced in a publication by **Sargeaunt** in 1993. Added to the above facts was an editorial in the **Lancet** (1986) which succinctly reviews the subject.

4.0 SUMMARY

To put the whole picture of *Entamoeba histolytica* infections into perspective, the work on homosexuals, apart from any of the other research described in the text above, illustrates quite clearly in irrefutable fashion, one particular point. Brumpt (1925) was absolutely correct - there were two species being labelled *E. histolytica (Sargeaunt, 1989)*

A) One should be known as *Entamoeba histolytica* causing disease in the host.

B) Another should be known as *Entamoeba dispar* (Brumpt, 1925) acting as a commensal in the intestine of man.

To this end, the World Health Organization (1997) now formerly accepts *E. dispar* as a separate species.

Therefore the status of *Entamoeba histolytica* - causing clinical amoebiasis is established.

The incidence of amoebic disease quoted by Walsh (1984) and the World Health Organization (1981) needs to be reduced after attention to *E. dispar* infections.

Now that *E. dispar* is recognised as a separate species, it clearly answers the question posed in an Editorial in The Lancet (1979), "Is the way going to be cleared for treating throughout the world only patients with pathogenic amoeba? That would be revolutionary indeed".

The answer, of course, is an emphatic yes.'

Sargeaunt's work has now revolutionised and rationalised drug treatment of amoebic infections so that vast amounts of drugs are no longer given indiscriminately to all subjects passing amoebic cysts.

Further questions posed again in another Editorial in The Lancet (1985), (regarding **Sargeaunt's** work) have also been answered by isoenzyme characterization.

5.0 REFERENCES

Adams, E.D., and MacLeod (1977). Invasive amoebiasis. 1. Amoebic dysentery and its complications. *Medicine* **56,** 315-320

Aikat, B.K., Bhusnurmath, S.R., Pai, A.K. *et al.* (1979). The pathology and pathogenesis of fatal hepatic amoebiasis -study based on 79 autopsy cases. *Trans. R. Soc. Trop. Med. Hyg.* **73,** 188.

Allason-Jones, E., Mindel, A., Sargeaunt, P.G., and Williams, P. (1986). *Entamoeba histolytica* as a commensal Intestinal parasite in, homosexual men. *N. Engl. J. Med.* 315, 353-356.

Allason-Jones, E., Mindel, A., Sargeaunt, P.G., and Katz, D. (1988). Outcome of untreated infection with *Entamoeba histolytica* in homosexual men with and without HIV antibody. *Br. Med. J.* 297, 654-657.

Balikian, J.P., Bitar, J.G., Rishani, K.K., Rashini R.T., and Bachti, G.R. (1977). Fulminant necrotizing amebic colitis in children. *Am. J. Proct.* **28,** 69-73

Beaver, P.C., Jung, R.C., Sherman, H.J., Bennett P. and Correr R. (1955). Experimental *Entamoeba histolytica* infections in man. *J. Trop. Med. Hyg.* 4, 1000-1005.

Blakely, P., Sargeaunt, PG., and Reed, S.L. (1990). An immunogenic 30-kda surface antigen of pathogenic clinical isolates of *Entamoeba histolytica. J. Infect. Dis.* 162, 949-954

Blanc, U., Nicholls, R., and Sargeaunt, P.G. (1989). Experimental production of new zymodemes of *Entamoeba histolytica* supports the hypothesis of genetic exchange. *Trans. R. Soc. Trop. Med. Hyg.* 83, 787-790.

Boeck, W.C., (1933). Amebic invasion of lymphoid tissue and its probable clinical significance. *Am. J. Intern. Med.*, **6,** 1549-1552.

Bracha, R., Diamond, L.S., Ackers, J.P, Burchard, G.D., Mirelman, D. (1990). Differentiation of clinical isolates of *Entamoeba histolytica* using specific DNA probes. *J. Clin. Microbiol.* **28,** 680-684.

Brandt, H. and Perez-Tamayo, R. (1970) Pathology of human amebiasis. *Human pathology, Prenza Medica Mexicana Mexico City* **1,** 351-389.

Brown, W.C. (1911). Human Infection by *Entamoeba histolytica*. In Ravdin J.I. (ed), *Amebiasis*. John Wiley & Sons, Oxford.

Please note; publications submitted for this Doctorate are marked in bold.

Brumpt, E. (1925). Élude sommaire de l'"*Entamoeba dispar*" n.sp. amide à kystis quadrinuclees, parasite de l'hemme. Bull. Acad. Med., Paris, 94, 942-952.

Brumpt, E. (1928). Differentiation of human intestinal amoeba with four nucleated cysts. *Trans. R. Soc. Trop. Med. Hyg.* **XXII,** 101-124.

Carrera, G.M. (1950). Pathology of early amebic hepatitis. *Arch. Pathology.* **50,** 440.

Castro, H.R. (1974). Anatomic and Pathological findings in Amoebiasis report of 320 cases. In Padilla, Y., Padilla, C.A. and Padilla, G.M. (eds), *Amoebiasis in Man.* C.C.Thomas, Springfield, Illinois, 44-73.

Councilman, W.R. and La Fleur, H.A. (1891). Amebic dysentery. *Johns Hopkins Hosp. Reports* **2,** 395-548.

Chatgidakis, C.B.(1953). The pathology of hepatic amoebiasis as seen on the Witwatersrand. *S. Afr. J. Clin. Science* **4,** 230-241.

Clark, C. (1925). The distribution and complications of amoebic lesions found in 186 post-mortem examinations. *Am. J. Trop. Med. Hyg.* **5,** 157-162.

Clark, G.C, and Diamond, L.S. (1991). Ribosomal RNA genes of "pathogenic and "non pathogenic" *Entamoeba histolytica*, are distinct. *Mol. Biochem. Parasitol* **49,** *297-302.*

Craig, C.F. (1927). Observation upon the hemolytic, cytolytic and complement-binding properties of extracts of *Entamoeba histolytica. Am. J. Trop. Med. Hyg.* **7,** 225-227.

Craig, C.F. (1944). *The Etiology, Diagnosis and Treatment of amoebiasis.* Williams and Wilkins, Baltimore,

Descheins, R. (1961). L'Ecologie D'*Entamoeba histolytica* et L'Epidemiologie De L'Amibiase. *Bulletin de la Societe de Pathologie. Exotique et des ses Filiales* **54,** 482-488.

Dopter, M, (1905). Sur quelques points relatifs a laction pathogene de l'amibe dysenterique. *Annales de l'Institut Pasteur* **19,** 415-417.

Druckman, D.A., and Quinn, T.C. (1988). In: . Ravdin, J.I. (ed), *Amebiasis Human Infection by Entamoeba histolytica.* Wiley, New York. and Chichester.

Please note; publications submitted for this Doctorate are marked in bold.

Edman, V., Meraz, M.A., Rausser, S., Agabian, N., and Meza, I. (1990). Characterization of an immuno-dominant variable surface antigen from pathogenic and non pathogenic *Entamoeba histolytica*. *J. Exp. Med.* **172,** 879-888.

El-Hashimi, W., Pittman, F. (1970). Ultrastructure of *Entamoeba histolytica* trophozoites obtained from the colon and from *in vitro* cultures. *Am. J. Trop. Med. Hyg.* **19,** 215-226.

Elhence, I.P., Agerwal, B.M., and Sharma, B.D. (1979). Amebic necrosis of bowel. *Int. Surg.* **64,** 57-63.

Faust, E.C. (1941). Amebiasis in New Orleans population as revealed by autopsy examination of accident cases. *Am. J. Trop. Med. Hyg.* **21,** 35-48.

Faust, E.C. (1954). *Amebiasis.* Charles C. Thomas, Springfield, Illinois. USA.

Faust, EC and Russell, P.F. (1964). *Clinical Parasitology.* 7th Edition. Lea and Febiger, Philadelphia, U.S.A.

Felman, Y.M., and Nikitas, J.A. (1979). Sexual transmission of enteric parasites in New York City. *NY State J. Med.* **79,** 412-414.

Flexner, S. (1900). On etiology of tropical dysentery. *Bull. Johns I* **11,** 231-233.

Friedrich, I.A., Korsten, M.A., and Gottfried, E.B. (1980). Necrotizing amebic colitis with pseudo-membrane formation. *Am. J. Gastroeterol.* **74,** 529-531

Garfinkel, L.I., Giladi, M., Huber, M., Gitler, C., Mirelman, D., Revel, M., and Rozenblatts (1989). DNA probes specific for *Entamoeba histolytica* possessing pathogenic and non-pathogenic zymodemes. *Infect. Immun.* **57,** 926-931.

Gathiram, V., and Jackson, T.H.F.G. (1987). A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. *S. Afr. Med. J.* **72,** 669-672.

Goldmeier, D., Sargeaunt, P.G., Price, AB., Munday, P.E., Billington, 0., Dixon, I., Borriello, P., Carder, J.M., Shaw, A., Hilton, J. and Jeffries, D.J. (1986). Is *Entamoeba histolytica* in homosexual men a pathogen? *Lancet* i, 641-644.

Griffin, J.L., Juniper, K. (1971). Ultrastructure of *Entamoeba histolytica* from human amebic dysentery. *Arch. Pathol.* **21,** 271-280.

Please note; publications submitted for this Doctorate are marked in bold.

Guerrant, R.L., Brush, J., and Ravdin, J. *et al.* (1981). The interaction between *Entamoeba histolytica* and human polymorphonuclear leukocytes. *J. Infect. Dis.* **143,** 83-85.

Gulati, P.D., Gupta, D.M., and Chuttani, H.K. (1967). Amoebic liver abscess and disturbances of portal circulation. *Am. J. Med.* **42,** 852-854.

Gutierrez, G., Ludlow, A., and Espinosa, G. *et al. (1976).* National National serologic survey II. Search for antibodies against *Entamoeba histolytica.* In Sepulveda, B. and Diamond, L.S. (eds.), *Amebiasis Mexico Institute Mexicano de Seguro Social,* 609-638.

Haider, Z., and Rasul, A. (1975). Chronic non-dysenteric intestinal amoebiasis - a review of 159 cases. *J.P.M.A.* **25,** 75-82.

Harris, H.F. (1898). Amoebic dysentery. *Am. J. Med. Sci.* **115,** 384-385.

Huber, J. (1903). Dysenterie Amoben. *Deutsch Med. Sochenschr.* **29,** 267-271

Jackson, T.F.H.G., Gathiram, V., and Simjee, A.E. (1985). Seroepidemiological study of antibody response to the zymodemes of *Entamoeba histolytica. Lancet* **i,** 716-718.

Jarumilinta, R., Maegraith, B.G. (1969). *Bull. WHO.* **41,** 269-272.

Jimenez, F. (1981). Pathology of amoebiasis. *Bull. N. W. Acad. Med.* **57,** 217-218.

Juniper, K., Steele, V.W., and Chester, C.L. (1958). Rectal biopsy in the diagnosis of amebic colitis. *South Med. J.* **51,** 545-546.

Juniper, K. (1962). Acute amebic colitis. *Am. J. Med.* **33,** 377-379e

Juniper, K. (1979. Amoebiasis. *Clin. Gastroenterol.* **7,** 3-5.

Kapoor, O.P., Joshi, V.R., and Sabnio, AM. (1972). Amoebic appendicitis. *J. Trop. Med. Hyg.* **75,** 1.

Kartulis, S. (1886). Sur Aetiologie der Dysentle Aegypten. *Arch. fur. Path. Anat.* **105,** 521-531

Kartulis, S. (1887). Zur Aetiologie der Leberabscesse. *Centralbl. Bakt.* **2,** 745-748.

Kartulis, S. (1904). Gehirnabscesse nach Dysenterischen Lebanabscesse. *Centralbl. Bakt.*, **37,** 527-532.

Please note; publications submitted for this Doctorate are marked in bold.

Katzenstein, D., Rickerson, V., and Braude, A. (1982). New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis and hepatic enzymes in 67 consecutive cases in San Diego. *Medicine* **61,** 237-246.

Kean, B.H., Gilmore, H.R., and Van Stone, W.W., (1956). Fatal amoebiasis. Report of 148 fatal cases from the armed forces institutes of pathology. *Am. Intern. Med.* **44,** 831-849.

Lancet; Editorial (1979). Pathogenic *Entamoeba histolytica*. *Lancet* i, 303

Lancet; Editorial (1985). Is that amoeba harmful or not. *Lancet.* i, 732-734

Lancet; Editorial (1986). On three stages of amoebic research. ***Lancet i,1133-1134***

Levine, N.D. (1973). *Protozoan parasites of domestic animals and of man.* Burgess Publishing Company, Minnesota, U.S.A.

Lösch, *F.A.* (1875). Massive development of Amoeba in the large intestine. *Archiv. f. Pathologische Anatomie und Phiysiologie* **65,** 196-211.

Luvano, F.M., Mtshali, Z., and Baker, L. (1982). Toxic dilatation complicating fulminant amoebic colitis. *Br. J. Surg.* **69,** 56-57.

Marcial, M.A., and Marcial-Rojas, R.A. (1985). Protozoal and helminthic diseases. In Kissane, J.M.(ed.). *Anderson's Pathology,* Eighth edition. C.V. Mosby, Co., St.Louis. 401-482.

Martin, GA., Garfinkal, B.T., Brooke, M.M., *et al.* (1953). Comparative efficacy of amebacides.and antibiotics in acute amebic dysentery. *JAMA* **151,** 1055-1057.

Messersmith, RN., and Chase, G.J. (1984). Amoebiasis presenting as multiple apple core lesions. *Am. J. Gastroenterol.* **79,** 238-241.

Mirelman, D., Brach, R., Chayen, A., Aust.Kettis, and Diamond, L.S. (1986). *Entamoeba histolytica* effects on growth conditions and bacterial associates on isoenzyme patterns and virulence. *Exp. Parasitol.* **62,** 142-148.

Musgrave, W.E. (1931). Amebiasis. A clinical study. *Am. J. Trop. Med. Hyg.* **11,** 469-472.

Nanda, R., Baveja, U., and Anand, B.S. (1984). *Entamoeba histolytica* cyst passers. Clinical features and outcome in untreated subjects. *Lancet* **2,** 301-304.

Please note; publications submitted for this Doctorate are marked in bold.

Nozaki, T., Motta, S.R.N., Takeuchi, T., Kobayashi, S., and Sargeaunt, P.G. (1989). Pathogenic zymodemes of *Entamoeba histolytica* in Japanese male homosexual population. *Trans. Roy. Soc. Trop. Med.* 83, 525.

Ochsner, A., and DeBakey, M. (1943a). Amebic hepatitis abscess. An analysis of 181 cases with review of the literature. Part 1. *Surgery* **13,** 460-468.

Ochsner, A., and DeBakey, M. (1943b). Amebic hepatitis abscess. An analysis of 181 cases with review of the literature. Part 2. *Surgery* **13,** 612-634.

Osler, W. (1984). Cases of dysentery under care of Dr. Osler. 1. Sloughs in the colon and rectum of unusual extend. 11.Abscess of liver pointing externally – death. *Can. Med. Surg. J.* **12,** 330-333

Paterson, J.C. (1935). The diagnosis and treatment of amoebiasis. *Can. Med. Assoc. J.* **66,** 399-402.

Patterson, M., and Schoppe, L.E. (1982). The presentation of amoebiasis.. *Med. Clin. North. Am.* **66,** 689-692.

Perez-Tamayo, R., and Brandt, H.(1971). Amebiasis. In Marcial-Rojas, R.A. (ed.), *Pathology of Protozoal and Helminthic Diseases.* Williams and Wilkins Baltimore. 145-173.

Petri, W.A.Jr., Jackson, T.F.H.G., Gathiram, V. et al. (1990). Pathogenic and non-pathogenic strains of *Entamoeba histolytica* can be differentiated by monoclonal antibodies to the galactose-specific adherence lectin. *Infect. Immun.* **58,** 1802-1806.

Pittman, F.E., El-Hashimi, W.K. and Pittman, J.C. (1973). Studies of human amebiasis. II. Light and electron microscopic observations of colonic mucosa and exudate in acute amoebic colitis. *Gastroenterology* **65,** 588-590.

Pomerantz, B.M., Marr, J.S. and Geldman, W.D. (1980). Identification of the male homosexual high risk population. *Bull. N. Y. Acad. Med.* **56,** 232-233.

Prathap, K., and Gilman, R. (1970). The histopathology of acute intestinal amebiasis. A rectal biopsy study. *Am. J. Pathol.* **60,** 229-239

Que, X., and Reed, S.L. (1991). Nucleotide sequence of a small subunit ribosomal RNA (165-like rRNA) gene from *Entamoeba histolytica*: differentiation of pathogenic from non-pathogenic isolates. *Nucleic Acids Res. 19,* 5438-5440.

Quincke, H.I., and Roos, E. (1893). On amoebic enteritis. *Berliner Klinische Wochenschift* **30,** 1089-1095.

Please note: publications submitted for this Doctorate are marked in bold.

Quinn, T.C., Stamm, W.E., and Goodell, S.E. et al. (1983). The polymicrobial origin of intestinal infection in homosexual men. *N. Engl. J. Med.* **309,** 576-582.

Quinn, T.C.(1984). Parasitic infections in homosexual men. *Microbiol.* 192-195.

Rasaretnam, R., and Wijetilaka, S.E. (1976). Left lobe amoebic liver abscess. *Postgrad. Med. J.* **52,** 269-272.

Ratcliffe, H.L. (1931). A comparative study of amoebiasis in man, monkeys and cats with special reference to the formation of early lesions. *Am. J. Hyg.* **14,** 337-339.

Reddy, UG., and Thangavelu, M. (1948). Some aspects of amoebiasis in Madras. *India Med. Gaz.* **83,** 557-559.

Reed, S.L., Sargeaunt, P.G. and Braude, A.I.(1983). Resistance to lysis by human serum of pathogenic *Entamoeba histolytica. Trans. R. Soc. Trop. Med. Hyg.* 772, 248-253.

Reed., S.L., Flores, B.M., Batzer, MA., Stein, MA., Carlton, J.E., Diedrich, D.L., and Torian, B.E. (1991). Molecular and cellular characterization of the 79kDa peripheral membrane protein of *Entamoeba histolytica*: Differentiation between pathogenic and non pathogenic isolates. *J. Infect. Dis.* **162,** 949-954.

Robbins, S.L., Cotran, R.S., and Kumar, V. (1984). *Pathologic Basis of Disease.* Third edition. WB Saunders Co., Philadelphia. 362-365.

Robinson, G.L. (1968). The laboratory diagnosis of human parasitic amoebae. *Trans. R. Soc. Trop. Med. Hyg.* **62,** 285-294.

Rogers, L. (1913). *Dysenteries. Their Differentiation and Treatment.* Oxford University Press, London.

Salata, R.A., John, J.E., Ahmed, P., Randin, J.I., and Pearson, R.D. (1985). The interaction *of Entamoeba histolytica* with human neutrophils. Chemoattraction, decreased chemoluminence, and enhanced amebic cytopathogenicity for liver cells. *Clin. Res.* **33,** 418-425.

Sargeaunt, P.G. (1975). Protozoa in Medical Microbiology. In Cruickshank, R., Duguid, B.P., Marmion, B.P., and Swain, R.H.A. (eds.). *The practice of medical microbiology,* Twelth Edition. Churchill Livingstone, Edinburgh. Volume 2, 544-558

Please note; publications submitted for this Doctorate are marked in bold.

Sargeaunt, P.G., Williams, J.E., and Grene, J.D. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. Trans. R. Soc. Trop. Med. Hyg. 72, 519-521.

Sargeaunt, P.G., and Williams, J.E. (1979). Electrophoretic isoenzyme patterns of the pathogenic and non-pathogenic intestinal amoebae of man. *Trans. R. Soc. Trop. Med. Hyg.* 73, 225-227.

Sargeaunt, P.G., Williams, J.E., Kumate, J., and Jimenez, E. (1980). The epidemiology of *Entamoeba histolytica* in Mexico City. A pilot survey I. *Trans. R. Soc. Trop. Med. Hyg.* 74, 653-656.

Sargeaunt, P.G., Williams, J.E., and Neal, R.A. (1980a). A comparative study of *Entamoeba histolytica* (NIH:200, HK9, etc.) "*E. histolytica* like" and other morphologically identical amoebae using isoenzyme electrophoresis. *Trans. R. Soc. Trop. Med. Hyg.* 74, 469-474.[9]

Sargeaunt. P.G., Jackson, T.F.H.G., and Sinijee, A. (1982). Biochemical homogeneity of *Entamoeba histolytica* isolates, especially those from liver abscess. *Lancet* i, 1386-1388.

Sargeaunt, P.G., and Williams, J.E. (1982a). The morphology in culture of the intestinal amoebae of man. *Trans. Roy. Soc. Trop. Med. Hyg.* 76, 465-472.

Sargeaunt, P.G., and Williams, J.E. (1982b). A Study of intestinal protozoa including non-pathogenic *Entamoeba histolytica* from patients in a group of mental Hospitals. *Amer. J. Public Health.* 72, 178-180.

Sargeaunt, P.G., Williams, J.E., Bhojnani, R., Campos, J. and Gomez, A. (1982c). The epidemiology of Entamoeba histolytica in a rural and an urban area of Mexico. A pilot survey II. *Trans. R. Soc. Trop. Med. Hyg.* 76, 208 - 210.

Sargeaunt, P.G., Williams, J.E., Bhojnani, R., Kumate, J. and Jimenez, E. (1982d). A review of isoenzyme characterization of *Entamoeba histolytica* with particular reference to pathogenic and non-pathogenic stocks isolated in Mexico. *Arch. Invest. Med. (Mex.)*, 13, 89-94.

Sargeaunt, P.G. (1989). Chapter 15; *Entamoeba histolytica* - pathogen or commensal. In Jewell, D.P and Lowe, J.R. (eds). *Topics in gastroenterology*. Blackwell Scientific Publications, Oxford.

Sargeaunt, P.G. (1990). The epidemiological aspects of *Entamoeba histolytica* zymodemes. *Mitt. Österr. Ges. Tropenmed. Parasitol.* 12, 1-4

Please note: publications submitted for this Doctorate are marked in bold.

Sargeaunt, P.G. (1992). *Entamoeba histolytica* is a complex of two species. *Aust. Microbiol.* 13, 359-361.

Sargeaunt, P.G. (1992a). A Survey of *Entamoeba histolytica* and *Entamoeba dispar* (Brumpt) infections on Mahe, The Seychelles. *Arch. Med. Res.* 23, 265-267.

Sargeaunt, P.G., Patrick, S., and O'Keeffe, S. (1992b). Human infections *of*, *Entamoeba chattoni* masquerade as *Entamoeba histolytica. Trans. R. Soc. Trop. Med. Hyg.* 86, 633- 634.

Sargeaunt, P.G. (1993). *Entamoeba histolytica:* a question answered. *Trop. Dis. Bull.* 90, R1-R3.

Sargeaunt, P.G., Williams, J.E., Jackson, T.H.F.G., and Simjee, A.E. (1982e). A zymodeme study of *Entamoeba histolytica* in a group of South African school children. *Trans. R. Soc. Trop. Med. Hyg.* 76, 401-405.

Sargeaunt, P.G., Williams, J.E., and Jones, D. (1982f). Electrophoretic Isoenzyme patterns of *Entamoeba histolytica* and *Entamoeba chattoni* in a primate survey. *J. Protozool.* 29, 36-139.

Sargeaunt, P.G. (1983). Identification of stocks of non-pathogenic *Entamoeba histolytica. Bull. Soc. Path. Exot. D.S. Fil.* 76, 588-591.

Sargeaunt, P.G., Oates, J.K., MacLennan, I., Oriel, J.D., and Goidmejer, D. (1,983a). *Entamoeba histolytica* in male homosexuals. *Brit. J. Ven. Dis.* 59, 193-195.

Sargeaunt, P.G., Baveja, U.K., Nanda, R. and Anand, B.S. (1984). Influence of geographical factors in the distribution of pathogenic zymodemes *of Entamoeba histolytica*: identification of zymodeme XIV in India. *Trans. R. Soc. Trop. Med. Hyg.* 78, 96-101.

Sargeaunt, P.G. (1985). Zymodemes expressing possible genetic exchange in *Entamoeba histolytica. Trans. R. Soc. Trop. Med. Hyg.* 79, 86-89.

Sargeaunt, P.G., Jackson, T.F.H.G., Wiffen, S., Bhojnani, R., Williams, J.E., Felmingham, D. , Goldmeier, U., Allason-Jones, E., Mindel, A., and Phillips, E. (1987). The reliability of *Entamoeba histolytica* zymodemes in clinical laboratory diagnosis. *Arch. Invest. Med.* (Mex)., 18, 69-75.

Sargeaunt, P.G. (1988). Zymodemes of *Entamoeba histolytica.* Chapter 25. In Ravdin, J.I. (ed.), *Amebiasis. Human infection by Entamoeba histolytica*."John Wiley & sons, Oxford. 370– 414.

Please note; publications submitted for this Doctorate are marked in bold.

Sargeaunt, P.G., Goldmeier, D., and Allason-Jones, E. (1988a). *Entamoeba histolytica* and AIDS/HIV. *Trans. R. Soc. Trop. Med. Hyg.* **82,** 63.

Sargeaunt, P.G., Jackson, T.F.H.G., Wiffen, S.R. and Bhojnani, R.(1988b). Biological evidence of genetic exchange in *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* 82, **862-867.**

Scaglia, M., Gatti, S., Bruno, A., Cevini, C., Marchi, L., and Sargeaunt, P.G. (1991). Autochthonous amoebiasis in institutionalized mentally - retarded patients: preliminary evaluation of isoenzyme patterns in three isolates. *Ann. Trop. Med. and Para.* 85, **509-513**

Schaudinn, F. (1903). Anbeiten aus dem kaiserlichen Gesundheitsante **19,** 547-561.

Schmerin, M.J., Gelston, A., and Jones, T.C. (1977). Amoebiasis. An increasing problem among homosexuals in New York City. *JAMA* **238,** 1387.

Scragg, J. (1960). Amoebic liver abscess in African children. *Arch. Dis. Child.* **35,** 176.

Shiga, K. (1898). Uber den Erneger der Dysenteries in Japan. *Centralblatt f. Bkt.* **23,** 599.

Stamm, W.P. (1970). Amoebic aphorisms. *Lancet* **ii,** 1355.

Stamm, W.P. (1975). Epidemiology of Amoebiasis in England and Wales. Brit. Med. J., **284,** 452-453.

Swellengrebel, N.H. (1961). LEcologie D'*Entamoeba histolytica* et L'Epidemiologie De L'Amibiase. *Bull. Soc. Path. Exot. et des Filial.*, **54,** 459-466.

Tannich, E., Horstmann RD., Knobloch, J. and Arnold, H.H. (1989). Genomic DNA differences between pathogenic and non-pathogenic *Entamoeba histolytica*. *Proc. Natl. Acad. Sci. USA* **86,** 5118-5122.

Tannich, E., and Burchard, G.D. (1991). Differentiation of pathogenic from non-pathogenic *Entamoeba histolytica* by restriction fragment analysis of a single gene amplified in vitro. *J. Microbiol.* **29,** 250-255.

Tannich, E., Scholze, H., Nickel, R. and Horstmann R.D. (1991a). Homologous cysteine proteinase of pathogenic and non-pathogenic *Entamoeba histolytica* differences in structure and expression. *J. Biol. Chem.* **266,** 4798-4803.

Please note; publications submitted for this Doctorate are marked in bold.

Thacker, S.B., Simpson, S., Gordon, T.J., Wolfe, M., and Kimball, AM. (1979). Parasitic disease control in a residential facility for the mentally retarded. *Am. J. Public Health* **69,** 1274-1281.

Tsutsumi, V., Mena-Lopez,R., Anaya-Velzquez, F., and Martinez-Paloma, A. (1984). A cellular basis of experimental amebic liver abscess formation. *Am. J. Pathol.* **117,** 81-83.

Walker, E.L., and Sellards, A.N. (1913). Experimental entamoebic dysentery. *Phil. J. Sci.* **8,** 253-331.

Walsh, J.A.(1984). The burden of illness. In Warren K.S., Mahmoud MF (eds) *Tropical and Geographic Medicine*. McGraw-Hill Book Co., New York. 1073-1092.

Walsh, J.A. (1988). Prevalence of *Entamoeba histolytica* infection. In Ravdin J.I. (Ed.) *Amebiasis*. J. Wiley and Sons. New York and Chichester. 93-105

Weber, G., Mohr, W., Fleisc her, K. and Sargeaunt, P.G. (1990). *Entamoeba histolytica* infections in flight personnel of an international airline. *Trans. R. Soc. Trop. Med. Hyg.* 84, 803-805.

William, D.C. (1983). Amebiasis. In Ostrow, D.B., Sandholtzer, T.A., Felman (Eds.), *Sexually Transmitted Diseases in homosexual men*. Plenum. New York. 234-280

World Health Organization (1981). Intestinal Protozoan and Helminthic Infections. *Technical Report Series*. No. 666.

World Health Organization (1981). Epidemiological bulletin. Pan American Health Organisation (1997). WHO/PAHO/UESCO Report. A consultation with experts on *Amoebiasis*. Mexiico City. Mexico. Vol. 18 No. 1.

Wraxall, B.C.D,, and Culliford, B.J. (1968). A thin-layer starch gel method for enzyine typing of blood stains. *J. Forensic Sci. Soc.* **8,** 81-82.

Please note; publications submitted for this Doctorate are marked in bold.

PUBLISHED WORK

SUBMITTED FOR

DOCTORATE

LIST OF PUBLISHED WORK SUBMITTED FOR DOCTORATE

1. Allason-Jones, E., Mindel, A., **Sargeaunt**, P.G., and Williams, P. (1986) *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. *N. Engl. J. Med.* **315,** 353-356.

2. Allason-Jones, E., Mindel, A., **Sargeaunt, P.G.,** and Katz, U. (1988). Outcome of untreated infection with *Entamoeba histolytica* in homosexual men with and without HIV antibody
Br. Med. J. **297,** 654-657.

3. Blakely, P., **Sargeaunt, P.G.,** and Reed, S.L. (1990). An immunogenic 30-kda surface antigen of pathogenic clinical isolates of *Entamoeba histolytica.*
J. Infect. Dis. **162,** 949-954.

4. Blanc, U., Nicholls, R., and **Sargeaunt, P.G.** (1989). Experimental production of new zymodemes of *Entamoeba histolytica* supports the hypothesis of genetic exchange.
Trans. R. Soc. Trop. Med. Hyg. **83,** 787-790.

5. Goldmeier, D., **Sargeaunt, P.G.,** Price, A.B., Munday, P.E., Billington, Dixon, I., Borriello, P., Carder, J.M., Shaw, A., Hilton, J. and Jeffries, D.J. (1986). Is *Entamoeba histolytica* in homosexual men a pathogen?
Lancet **i,** 641-644.

6. Lancet; Editorial (1979). Pathogenic *Entamoeba histolytica.*
Lancet **i,** 303.

7. Lancet; Editorial (1985). Is that amoeba harmful or not?
Lancet **i,** 732-734.

8. Lancet; Editorial (1986). On Three Stages of Amoebic Research.
Lancet **i,** 1133-1134.

9. Nozaki, T., Motta, S.R.N., Takeuchi, T., Kobayashi, S., and **Sargeaunt, P.G.** (1989). Pathogenic zymodemes of *Entamoeba histolytica* in Japanese male homosexual population. *Trans. R. Soc. Trop. Med. Hyg.* **83,** 525.

10. Reed, S.L., **Sargeaunt, P.G.,** and Braude, A.I. (1983). Resistance to lysis by human serum of pathogenic *Entamoeba histolytica.*
Trans. R. Soc. Trop. Med. Hyg. **772,** 248-253

11. **Sargeaunt, P.G.** (1975). Protozoa in Medical Microbiology. In Cruickshank, R., Duguid, B.P., Marmion, B.P., and Swain, R.H.A. (eds.). *The practice of medical microbiology,* Twelfth Edition. Churchill Livingstone, Edinburgh. Volume 2, 544-558

12. **Sargeaunt, P.G.,** Williams, J.E., and Grene, J.U. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis.
Trans. R. Soc. Trop. Med. Hyg. **72,** 519-521.

13. **Sargeaunt, P.G.** and Williams, J.E. (1979). Electrophoretic isoenzyme patterns of the pathogenic and non-pathogenic intestinal amoebae of man.
Trans. R. Soc. Trop. Med. Hyg. **73,** 225-227.

14. **Sargeaunt, P.G.,** Williams, J.E., Kumate, J., and Jimenez, E. (1980). The epidemiology of *Entamoeba histolytica* in Mexico City. A pilot survey I.
Trans. R. Soc. Trop. Med. Hyg. **74,** 653-656.

15. **Sargeaunt, P.G.,** Williams, J.E., and Neal, R.A. (1980a). A comparative study of *Entamoeba histolytica* (NIH:200, Hk9, etc.) " *E. histolytica* like" and other morphologically identical amoebae using isoenzyme electrophoresis. *Trans. R. Soc. Trop. Med. Hyg.* **74,** 469-474.

16. **Sargeaunt, P.G.,** Jackson, T.F.H.G., and Simjee, A. (1982). Biochemical homogeneity of *Entamoeba histolytica* Isolates, especially those from liver abscess. *Lancet* **i,** pp. 1386-1388.

17. **Sargeaunt, P. G.,** and Williams, J . E. (1982a). The morphology in culture of the intestinal amoebae of man. *Trans. R. Soc. Trop. Med. Hyg.* **76,** 465-472.

18. **Sargeaunt, P.G.,** and Williams, J.E. (1982b). A Study of Intestinal Protozoa Including non-pathogenic *Entamoeba histolytica* from patients in a group of mental hospitals. *Am. J. Public Health* **72,** 78-180.

19. **Sargeaunt, P.G.,** Williams, J.E., Bhojnani, R., Campos, J., and Gomez, A. (1982c). The epidemiology of *Entamoeba histolytica* in a rural and an urban area of Mexico. A pilot survey II. *Trans. R. Soc. Trop. Med. Hyg.* **76,** 208-210.

20. **Sargeaunt, P.G.,** Williams, J.E., Bhojnani, P., Kumate, J. and Jinienez, E. (1982d). A review of isoenzyme characterization of *Entamoeba histolytica* with particular reference to pathogenic and non-pathogenic stocks isolated in Mexico. *Arch. Invest. Med. (Mex.)* **13** (*Suppl. 3*), 89-94.

21. **Sargeaunt, P.G.,** Williams, J.E., Jackson, T.H.F.G., and Simjee, A. E. (1982e). A zymodeme study of *Entamoeba histolytica* in a group of South African School Children *Trans. R. Soc. Trop. Med. Hyg.* **76,** 401-405.

22. **Sargeaunt, P.G.,** Williams, J.E., and Jones, D.M.. (1982f). Electrophoretic isoenzyme patterns of *Entamoeba histolytica* and *Entamoeba chattoni* in a primate survey. *J. Protozool.* **29,** 136-139.

23. **Sargeaunt, P.G.,** (1983). Identification of stocks of non-pathogenic *Entamoeba histolytica*. *Bull. Soc. Path. Ex.* **76,** 588-591

24. **Sargeaunt, P.G.,** Oates, J.K., MacLenran, I., Oriel, J.D., and Goldmeier, D. (1983a). Entamoeba histolytica in male homosexuals. *Br. J Vener. Dis.* **59,** 193-195.

25. **Sargeaunt, P.G.,** Baveja, U.K., Nanda, R. and Anand, B.S. (1984). Influence of geographical factors in the distribution of pathogenic zymodemes of *Entamoeba histolytica*: identification of zymodeme XIV in India. *Trans. R. Soc. Trop. Med. Hyg.* **78,** 96-101.

26. **Sargeaunt, P.G.** (1985). Zymodemes expressing possible genetic exchange in *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* **79,** 86-89.

27. **Sargeaunt, P.G.,** Jackson, T.F.H.G., Wiffen, S., Bhojnani, R., Williams, J.E. , Felmingham, D, Goldmeir, U., Allason-Jones, E., Mindel, A., and Phillips, E. (1987). The reliability of *Entamoeba histolytica* zymodemes in clinical laboratory diagnosis. *Arch. invest.Med.(Mex.)* **18,** 69-75.

28. **Sargeaunt, P.G.** (1988). Zymodemes of *Entamoeba histolytica*. Chapter 25. In Ravdin, J.I. (ed.), *Amebiasis. Human infection by Entamoeba histolytica*. John Wiley & sons, Oxford. 370 - 414

29. **Sargeaunt, PG.,** Goldmeier, D., and Allason-Jones, E. (1988a). *Entamoeba histolytica* and AIDS/HIV. *Trans. R. Soc. Trop. Med. Hyg.* **82,** 653-

30. **Sargeaunt, P.G.,** Jackson, T.F.H.G., Wiffen, S.R. and Bhojnani, R.(1988b). Biological evidence of genetic exchange in *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* ***82,*** 862-867.

31. **Sargeaunt, P.G.** (1989). Chapter 15; *Entamoeba histolytica* - pathogen or commensal. In Jewell, D.P and Lowe, J.R. (eds). *Topics in gastroenterology*. Blackwell Scientific Publications, Oxford.

32. **Sargeaunt, P.G.** (1990). The epidemiological aspects of *Entamoeba histolytica* zymodemes. *Mitt. Osterr. Ges. Tropenmed. Parasitol.* **12,** 1-4.

33. **Sargeaunt, P.G.** (1992). *Entamoeba histolytica* is a complex of two species. *Aust. Microbiologist* **13,** 359-361.

34. **Sargeaunt, P.G.** (1992a). A Survey of *Entamoeba histolytica* and *Entamoeba dispar* (Brumpt) infections on Mahé, the Seychelles. *Arch. Med. Res.* **3,** 265-267.

35. **Sargeaunt, P.G.,** Patrick, S., and OuKeeffe, S. (1992b). Human Infections *of Entamoeba chattoni* masquerade as *Entamoeba histolytica. Trans. R. Soc. Trop. Med. Hyg.* **34,** 633-634.

36. **Sargeaunt, P.G.** (1993). *Entamoeba histolytica*: a question answered. *Trop. Dis. Bull.* **90.**

37. Scaglia, M., Gatti, S., Bruno, A., Cevini, C., Marchi, L. and **Sargeaunt, P.G.** (1991). Autochthonous amoebiasis in institutionalized mentally - retarded patients: preliminary evaluation of isoenzyme patterns in three isolates. *Anna Trop. Med. Parasitol.* **85,** 509 -513.

38. Weber, G., Mohr, W., Fleischer, K., and **Sargeaunt, P.G.** (1990). *Entamoeba histolytica* infections in flight personnel of an international airline *Trans. R. Soc. Trop. Med. Hyg.* **84,** 803-805.

39. **Sargeaunt, P.G.** (1997). Intestinal protozoan and helminthic infections. Epidemiological Bulletin Pan American Health Organisation vol 18 No.1 WHO/PAHO/UNESCO Report. Bulletin of the World Health Organization 75.

LIST OF PUBLISHED WORKS SPECIFYING THE PERCENTAGE OF WORK CARRIED OUT BY THE CANDIDATE

Number of reprint	Percentage of work by the candidate
1	50
2	50
3	40
4	60
5	40
6	*not applicable*
7	*not applicable*
8	*not applicable*
9	50
10	75
11	95
12	80
13	60
14	83
15	70
16	80
17	80
18	75
19	75
20	80

Number of reprint	Percentage of work by the candidate
21	75
22	80
23	100
24	85
25	75
26	100
27	75
28	100
29	90
30	70
31	100
32	100
33	100
34	95
35	95
36	100
37	70
38	57
39	*not applicable*

LIST OF LABORATORIES WHERE THE RESEARCH WAS CONDUCTED

Please Note:

- Most work was undertaken at The London School of Hygiene and Tropical Medicine.

- Dates of visits to other laboratories is given together with dates of publications resulting from those visits

LONDON

SITE	PUBLICATIONS
Department of Pathology, London School of Hygiene & Tropical Medicine, Keppel Street, London.	*1978, 1979, 1980a,b,c,* *1982a,b,c,d,e,1983,a,b,* *1985, 1985b, 1986, 1986b,1986c,* *1987, 1987b,* *1987c, 1988, 1988b, 1989, 1990,* *1992, 1.993*

OTHER LABORATORIES, VISITS AND PUBLICATIONS

SITE	PUBLICATIONS
Department of Pathology, Medical Centre, University of California, San Diego, U.S.A.	*1983*
Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria, 3052, Australia.	*1987*

SITE	PUBLICATIONS
Microbiology Department, Royal Hobart hospital, Hobart, Tasmania	*1987*
Bacteriology Department, Maulana Azad Medical College, New Delhi, India	*1978, 1984*
Department of Parasitology, School of Medicine, Keio University, Shinjuku-Ku, Tokyo, Japan.	*1989*
Department of Immuno Chemico, Hospital Infantil, Mexico City.	*1980, 1982*
Microbiology Department, Instituto Mexicano Del, Seguro Social, Mexico City.	*1986, 1987*
Research Institute for Disease in a Tropical Environment South African M.R.C. Durban, South Africa.	*1982a,9b, 1988, 1989*

SITE	PUBLICATIONS
Laboratory of Clinical Parasitology Institute of Infection Diseases, University - IRCCS S.Matteo, Pavia, Italy.	*1989, 1990, 1991, 1992*
Laboratory of The hospital, Botanical Gardens, Victoria, Mahe, The Seychelles	*1992*