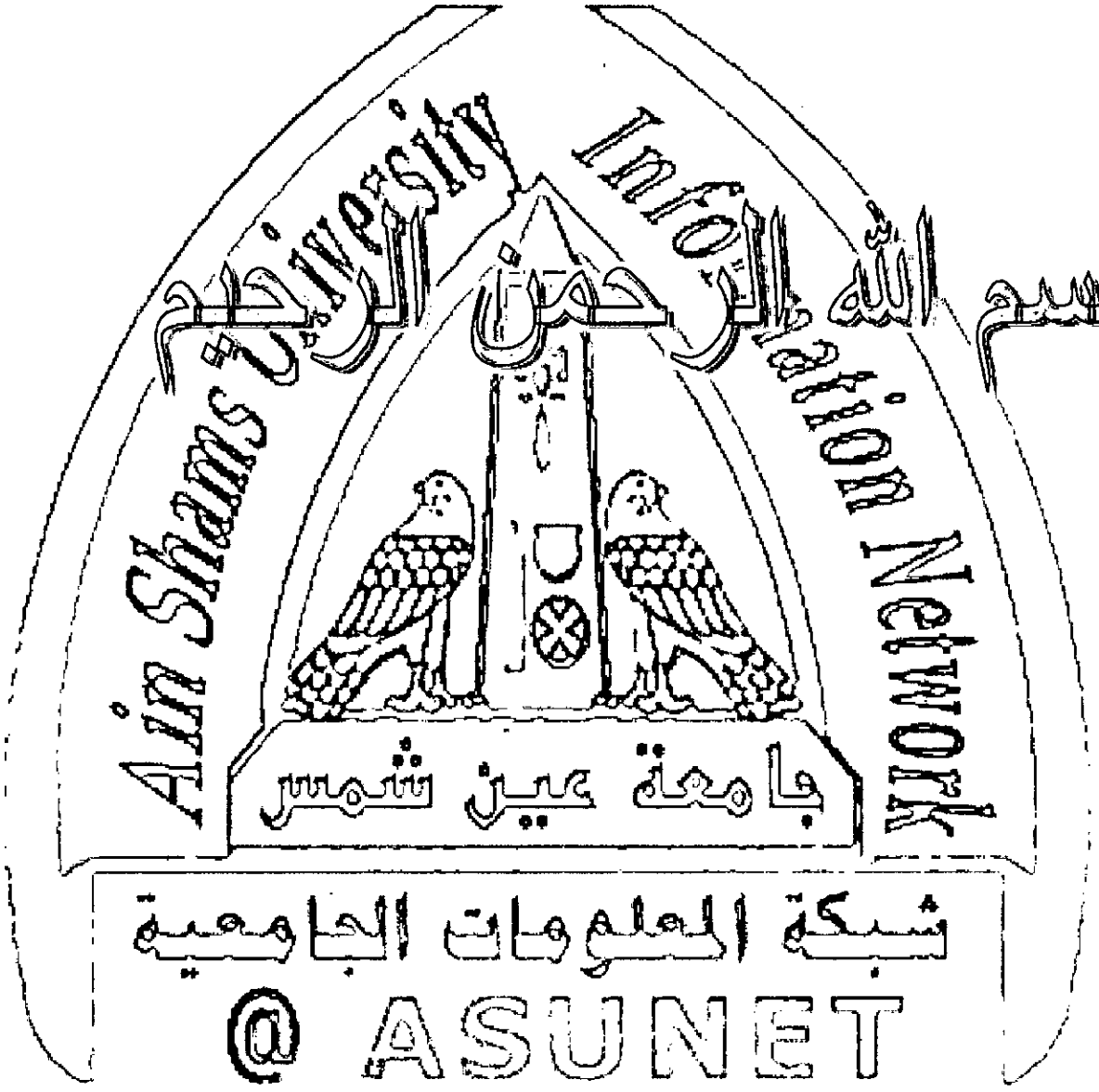
شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

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POLYMERASE CHAIN REACTION (PCR) AND ENZYME LINKED IMMUNO-SORBENT ASSAY (ELISA) TECHNIQUES IN EXPERIMENTAL TOXOPLASMOSIS

Thesis

Submitted to the Faculty of Medicine
University of Alexandria
In partial fulfilment of the
requirements of the degree of

DOCTOR OF

BASIC MEDICAL SCIENCES - PARASITOLOGY

By

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1998

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ACKNOWLEDGEMENT

I wish to acknowledge with deep gratitude the efforts of all those people who helped in the creation of this work.

Special thanks go to *Professor Dr. Helen Nashed Awadalla*, Professor of Parasitology, Faculty of Medicine, Alexandria University, for her valuable guidance, experience, support, excellent supervision and encouragement. I will remain grateful to her forever.

I would like to express my gratitude to *Professor Dr. Wagih Mohamed El-Gebali*, Professor of Parasitology, Faculty of Medicine, Alexandria University, for his expert assistance and cooperation during this study.

My grateful appreciation is extended to *Dr. Mervat Z. Omar El-Azzouni*, Assistant Professor of Parasitology, Faculty of Medicine, Alexandria University for her great effort, scientific collaboration and useful suggestion.

I wish to acknowledge with great thanks *Dr. Mona Mohamed El-Temsahi*, Assistant Professor of Parasitology, Faculty of Medicine, Alexandria University for her cooperation, valuable advice and interest in supervising this work.

I am indebted to *Dr. Ola Atef Sharaki*, Lecturer of Clinical Pathology, Faculty of Medicine, Alexandria University for her skilful excellent technical assistance, constructive discussions and generous advice.

Finally, I wish to thank all members of the Parasitology Department, who gave me helpful support to accomplish this work.

INTRODUCTION

INTRODUCTION AND REVIEW OF LITERATURE

Historical Outline

Toxoplasma gondii is one of the organisms which was discovered before it was known to cause any disease in man.[1] It was first identified in 1908 by Nicolle and Manceaux in smears of the spleen and liver of a small desert rodent called *Ctenodactylus gondi*, which was used in research on leishmaniasis and typhus fever at the Pasteur Institute in Tunisia.[2,3] However the first human case was reported in 1923 by Janku in Prague in a congenitally infected baby with retinochoroiditis.[3] Since then it has been reported to cause diseases in man which are sometimes fatal.

The organism is an obligate intracellular protozoan parasite of world wide distribution.[4] It may inhabit the nucleus of the host cell but usually lives in the cytoplasm of many kinds of tissues in different species of mammals including man, as well as in birds.[1] In heavy acute infections it can be found free in the blood and peritoneal exudate.[1]

Life Cycle and Morphology

The life cycle of the parasite involves two hosts. Man and other animals act as intermediate hosts while domestic cats and other felines serve as definitive hosts. In the cats the sexual sporogonic intestinal epithelial cycle takes place resulting in the formation of the oocysts, an asexual schizogonic extraintestinal cycle also takes place.[2,5] The **oocysts** are oval or round in shape, measuring about 9 to 11 μm in width by 11 to 14 μm in length.[6] They are surrounded by a thin cyst wall and pass unsporulated in the cat's feces.[6] Sporulation requires two to three days at 24°C or 14-21 days at 11°C. Sporulation does not occur above 37°C or below 4°C.[7] The sporulated oocyst contains two sporocysts, each of which encloses four sporozoites.[2] The oocyst can remain infectious in moist soil for more than one year. Dry heat greater than 66°C or nearly boiling water renders them non infectious.[7]

In man and other animals the extraintestinal cycle can be initiated by ingestion of sporulated oocysts or tissue cysts. The ingested oocysts release sporozoites, while the ingested tissue cysts liberate bradyzoites. Both these organisms penetrate the intestinal mucosal cells and are carried by blood and lymphatics to the mesenteric lymph nodes, where they develop into **tachyzoites**.[2] These disseminate through the blood and

lymphatics to all host tissues.[2] The organisms can invade and multiply in nucleated cells of all types of tissues but especially macrophages of reticuloendothelial system.[8] In the cell the tachyzoites divide by endodyogeny where a nest of eight to sixteen or more tachyzoites are produced within the host cell parasitophorous vacuoles forming **pseudocysts**.[9] The cell disintegrates, releasing tachyzoites, which either invade adjoining cells or disseminate widely throughout the body via the blood stream or lymphatics.[1,7,10]

After the acute stage subsides and the immunity of the host develops, which may take weeks or months,[7] the tachyzoites multiply more slowly and are transformed into the **bradyzoites**.[1] These accumulate in large numbers within a host cell, and become surrounded by a tough cyst wall of host tissue reaction, which protects them from the immune defense mechanism of the host.[1] These **true cysts** occur in a variety of host organs but principally within the central nervous system (CNS) and muscles, where they may exist for the life time of the host in a latent form.[10]

As regards the **tachyzoites**, they are the rapidly dividing forms and occur early in the infections and are the main cause of disease.[9] They are crescentric in shape, have one end more rounded than the other and

vary in length from four to eight μm and in breadth from two to three μm.[2,6] They stain well with Giemsa stain, by which they appear with a pale blue cytoplasm, and a red or purple spherical nucleus that is usually near the broad end of the parasite.[2] In tissue sections stained with haematoxylin and eosin the parasites appear in collection within the tissue cells called **pseudocysts**.[6]

As regards the **tissue cysts**, they vary in size from 10 to 100 μm in diameter and contain as many as 3000 bradyzoites.[7] Freezing to -20°C, dessication or heating above 66°C is lethal to the cysts.[7]

The **bradyzoites** are slowly dividing organisms that occur in the chronic phase of infections and are not directly related to the disease.[1,5] They are slender in shape, measure two to seven μm in diameter.[7]

The difference between tachyzoites and bradyzoites of *T.gondii* were recorded by Dubey and Frenkel in 1976,[11] who found that the bradyzoites had subterminal nuclei and contained strongly positive periodic acid- Schiff (PAS) cytoplasmic granules. The cyst wall surrounding the bradyzoites is stained weakly by PAS. In case of tachyzoites, they are slightly PAS positive and the pseudocysts are not stained by PAS.[2]

The tachyzoites are immediately destroyed by gastric juice containing pepsin-HCl while the bradyzoites that are very quickly liberated from the cyst by peptic digestion survive up to three hours in the digestion fluid so they can initiate infection.[12]

The strikingly different effects of pepsin-HCl on the two forms of the parasite suggests difference in their surface properties which can be revealed as antigenic dissimilarities.[12] Some antigens are unique to bradyzoites, while others are shared between the bradyzoites and tachyzoites. The major tachyzoites surface protein P_{30} was not observed in the bradyzoites of tissue cysts.[13]

In ultrastructural terms there are strong similarities between tachyzoites and bradyzoites of *T.gondii*.[14] The electron microscope reveals, in all stages of *T.gondii*, tachyzoites, bradyzoites and sporozoites, a complex system of penetrating organelles that clearly demonstrates the taxonomic relationship of this organism to the Apicomplexa.[2,15] These organelles facilitate entry of the parasite into the cells.[15]

Toxoplasma Strains

Several strains of *T.gondii* have been identified, and vary markedly in their virulence depending on their morbidity and mortality in *Toxoplasma*- infected mice.[16] Such differences are most marked in