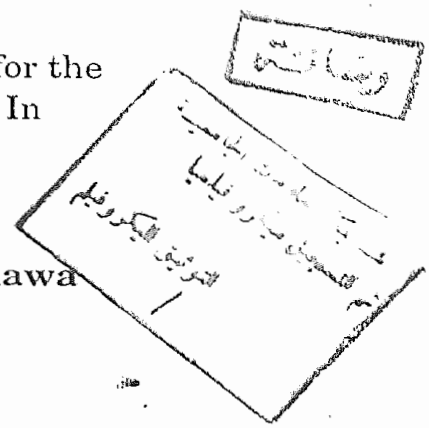
DETECTION OF BRUCELLA-AGGLUTININS IN A FARM WORKERS IN PORT-SAID CITY

THESIS

Submitted in Partial Fulfilment for the Requirments of M.Sc. Degree In **Tropical medicine**



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1992

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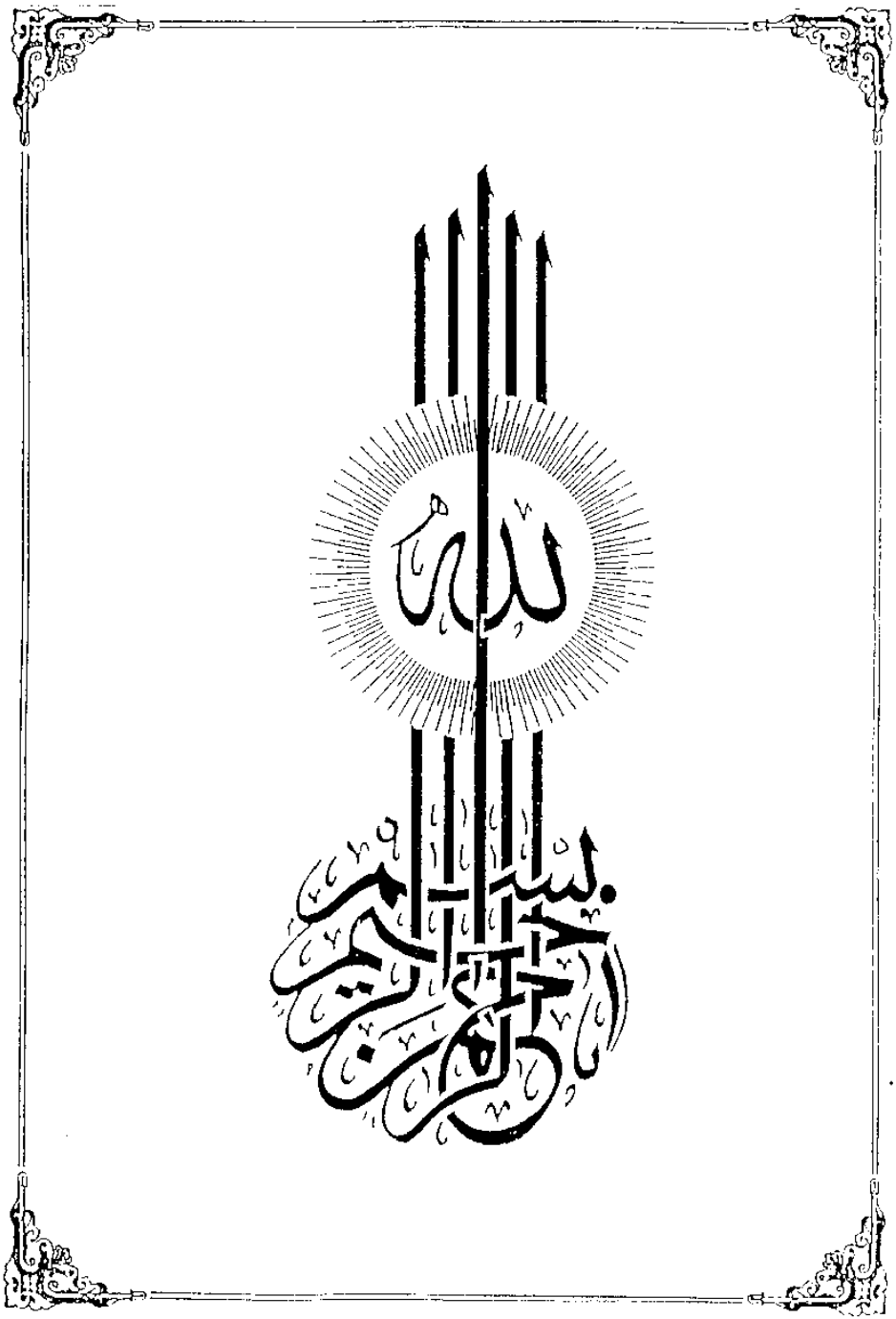

ACKNOWLEDGEMENT

"Thanks to God for enabling me to finish this work". I would like to express my deep thanks to **Dr. Mamoun Mohamed Ashour,** Assistant Professor of Tropical Medicine, Faculty of Medicine, Ain Shams University, for his helpful advice, continuous guidance and kind supervision.

I would like to express my thanks to Dr. **Mohamed Salah Mohamed Ibrahim,** "Assistant Professor of Bacteriology" Faculty of Medicine, Ain Shams University, for his help during all stages of the work. He give me much of his time experience, advice and support throughout this work.

I would like to express my thanks to Dr. **Soheir Abdel-Kader El-Sayed,** Lecturer of Tropical Medicine, Faculty of Medicine, Ain Shams University, for her helpful advice and experience.

I would like to express my thanks to Dr. **Tahany Abdel Raouf,** Lecturer of Bacteriology, Faculty of Medicine, Ain Shams University, for her help during this work. She give me much of her time.

I would like to express my thanks to Dr. **Kahiry El-NaGar,** Professor of Tropical Medicine. I would like to express my thanks to all the staff members of Tropical Medicine and Bacteriology, Ain Shams Universtiy, for their kind behaviour and continuous advice.

Introduction And Aim of The Work

INTRODUCTION

Brucellosis is also known as undulant fever, (Melitensis type), febris undulans, Malta fever, or Mediterranean fever. The genus Brucella comprises intracellular parasites that induce abortion in a variety of animal and febrile illness in human ***(Moreno et al., 1984).***

Brucellosis is an infectious disease caused by organisms of the genus brucella. It is primarily an infection of animals, man usually acquires the disease by ingesion of the infected milk or milk products and by contact with tissues or secretions of infected animals ***(Farrell, 1983).***

Brucellosis has existed for centuries in the Mediterranean Countries. In Egypt, the first case was reported in 1929.

Brucellosis comes after salmonellosis and tuberculosis as the most important systemic infection causing fever of unknown origin (FUO) in Cairo ***(Hassan and Farid, 1979).*** The cause is nearly always Brucella Melitensis ***(Pfischner et al., 1957).***

The geographical distribution of human brucellosis is closely related to the endemicity of animal infection, the

standard of hygiene and other socio-economic activities ***(Abd-El Salam and Fein, 1976).*** The acute form is a febrile illness that mimics many other diseases and is characterized by few or no localizing signs, also chronic illness occurs with or without localized findings ***(Spink, 1982).***

AIM OF THE WORK

The present study was aimed at investigating the presence of brucella, agglutinins among Farm Workers at El-Kaboti area in Port-Said City.

How much is present brucellae in our Countery.

Evalution of the tube agglutination technique in screening of brucellae .

REVIEW OF LITERATURE

GEOGRAPHICAL DISTRIBUTION OF BRUCELLOSIS

It is proved that half a million people world wide still have brucellosis each year ***(Thim, 1982).*** Brucellosis is common disease in Saudi Arabia, the incidence was 14.3% compared to 3.3% for typhoid fever as a cause of prolonged fever ***(Kambal et al., 1983).***

Hassan and Farid (1974) stated that brucelloris comes after salmonellosis and tuberculosis as the most important systemic infection causing fever of unknown origin in Cairo.

BACTERIOLOGY

Definition and classification:

Brucella are gram negative, non spore forming cocoobacilli, grow poorly on ordinary media or may require special media. They are aerobic, no growth under strict anaerobic conditions, and growth is improved by carbondioxide. They have little fermentative action on carbohydrate in usual media. Urea is hydrolysed to variable extent usually tend to produce alkali in litmus milk and brown pigmentation on potato media. ***(Wilson and Miles, 1915).***

There are three main species of brucella that differ in their choice of animal host, in certain cultural and biological characteristics. They are Brucella melitensis which infect goats and sheeps Brucella abortus which infects cattle and Brucella suis which infects pigs. The host parasite relationship is not absolutely specific and both man and animals are susceptible to all three species. Within the species strain that differ in some respects from the prototype and are considered to be biotypes there are at least nine biotypes of brucella abortus. Three of brucella melitensis and four of brucella suis ***(Mccullough, 1970).***

A number of other brucella species have been named Brucella neotomae was isolated from the desert wood rat (an animal of Western reigon of the U.S.A.).

The importance of Brucella neotomae as a pathogen is unknown ***(Stonner and Lackman, 1957).***

Brucella Ovis causes the widespread disease known as ram epididymitis (disease of sheep) which of great economic importance in most of the major sheep raising area of the world ***(Alton et al., 1975).***

Brucella Canis causes severe brucellosis infections in

dogs no infections of other species except a few cases in man ***(Alton et al., 1975).***

Morphology:

The bacilli are short, slender 0.8-1.5X0.6-0.8 micron non motile, non spore forming and arranged singly or in pairs or short chains or in small groups ***(Bruce, 1897).***

Staining:

All types stain well with ordinary dyes, thionin, basic fuchsin, methyl violet and pyronin are commonly used. Different strains of one type may vary in their sensitivity ***(Topley & Wilson, 1964).*** Modified Zeil Nelson Method, is described for pathological specimens as fetal membranes, stomach contents of aborted animals. The organism stains red against a blue background ***(Alton & Jones, 1975).***

Culture:

Media recommended by W.H.O. (1964) are serum dextrose agar, serum potato infusion agar, tryptose and trypticase soy agar, blood agar containing 5% sheep blood, optimum pH ranges from 6.2-8.8, the optimum temperature is 37 oC. ***(Zobel and Meyer, 1932).***

Brucella abortus need 5-10% CO_2 to grow while Brucella melitensis grow better with excess CO_2 but can grow without it. Brucella suis may be inhibited by it ***(Gruickshank et al., 1975).*** Growth is improved by the addition of natural animal protein to the medium. These requirements vary with different species and with different strains of the same species ***(Christie, 1987).*** On serum dextrose agar after 48 hours at 36 oC in the presence of CO_2, colonies are 1 mm. in diameter, raised, convex, with an entire edge and a moist glistening surface colonies appear greyish-white by reflected light, slightly opalescent under oblique light and transparent and honey coloured in transmitted light. Smooth colonies are soft, moist and easily emulsifiable in plysiological saline. Rough colonies are yellow, opaque, granular, and break up when touched with a needle. Mucoid colonies are glistening, greyish in colour and slimy ***(Mccullough, 1970).***

Validity:

Brucella organisms are killed at 60 oC in ten minutes hence killed by pasteurization. They are moderately sensitive to acid and die within few days in fresh cheese undergoing lactic acid fermentation. They may survive for a number of days in a butter made from infected milk. Brucella have been isolated from ice cream kept at freezing for one month.

In direct sun light they are often killed in few hours, but if unexposed may persist in dust or soil from two to three months and in dead fetal material for longer period ***(Cruichrshank et al., 1975).***

In tap water brucella may remain alive for 57 days at 8 oC for 10 days at 25 oC ***(Horning, 1935).*** In human urine they remain alive for at least one week. In animal feaces the organism have survived in the open air for 100 days and at 8 oC for over a year ***(King, 1957).***

Brucella are susceptible to sulphonamide. Streptomycin tetracycline, chloramephnicol, ampicilin ***(Cruickshank, et al., 1975)*** and rifampicin ***(Manson, Bahr and Apted, 1982).***

Metabolism:

under ordinary aerobic condition of incubation, broth culture become alkaline due to production of amonia. Litmus milk is turned weakly alkaline. On Macconkey's medium strains of brucella give rise to small non lactose fermenting colonies after 3 to 4 days. ***(Zobell and Meyer, 1932).***

Biochemical reactions:

Although carbohydrates are utilized. Brucella produce insufficient acid or gas to be demonstrable by the ordinary

method ***(Cruickshank et al., 1975).*** The methyl red and voges proskauer tests are negative, indol production is negative while oxidase and catalase are positive, ***Zobel and Meyer (1932)*** stated that all types reduce nitrates to nitrites which are rapidly reduced to amonia which is reduced also to form peptone, urea and asparagine.

Differential biological tests:

The three main species. Brucella abortus, melitensis and Brucella suis differ in certain character which form the bases of their classification these are:

1. CO2 requirement :

When cultivation is attempted directly from animal body Brucella abortus require an atmosphere containing 5-10% CO_2. Brucella melitensis grows better with excess CO_2 but can grow without it. Brucella suis may be inhibited by it ***(Cruickshank et al., 1975).***

2. Production of H_2S:

Brucella suis form H_2S more marked ***(Cruickrshank et al., 1975).***

3. Agglutination by monospecific sera:

To identify an unkown brucella strain a suspension of

the organism is prepared and diluted. The suspension is tested by monospecific sera. Brucella abortus and brucella suis are agglutinated by monospecific "A" antiserum and Brucella melitensis by "M" antiserum only ***(Cruickshank et al., 1975).***