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Seroprevalence of Toxoplasmosis amongst Healthy Adults in the Al Ain Area

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Seroprevalence of Toxoplasmosis Amongst Healthy Adults in the Al Ain Area

(A Thesis Submitted in Partial Fulfillment for the Degree of
M.Sc. in Environmental Science)

by
Hamad Noor Mohamed Ahmad (B.Sc.)

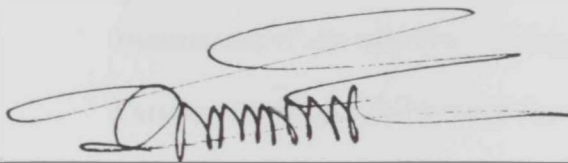
**Faculty of Science
United Arab Emirates University**

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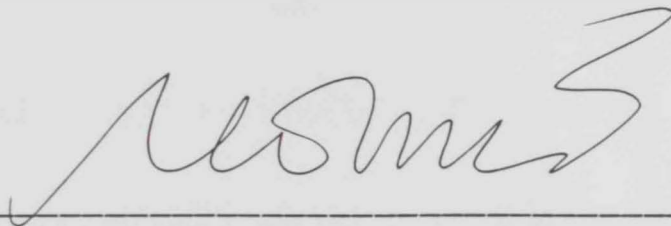
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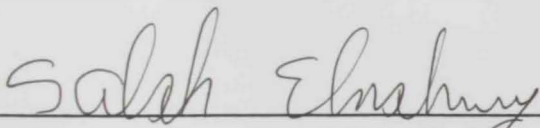
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ABSTRACT

There being no published data on the seroprevalence of toxoplasmosis in the UAE, a base -line study was carried out on a randomly selected population of healthy adults drawn from subjects presenting themselves for a health certificate at the Department of Preventive Medicine, Al Ain, or presenting for blood donation at the two main hospitals in Al Ain, the Tawam Hospital and Al Ain Hospital. Commercially available VIDAS IgG and IgM kits were used to test for seropositivity. A validated questionnaire was used to record personal data and blood was drawn from each donor after informed consent. All raw data and test results were fed into a computer for a statistical analysis of results.

The overall IgG seropositivity rate was 33.6% and the IgM seropositivity rate 2.7 %, the former on a sample size of 941 and the latter on a smaller sample of 75 owing to limitation of research funds.

No significant difference of IgG seropositivity was found between samples drawn from Preventive Medicine department and two Blood Banks, or between the sexes, and although rural dwellers showed lower seropositivity (25%) compared to urban dwellers (35.1%), the difference was not significant.

IgG seropositivity increased with every successive decade of life, with 19% for <20 years old to 42.2% for >50 year old.

Statistically significant association was found between IgG seropositivity and both nationality groups (Higher amongst the Jordanians, Palestinians, Lebanese and Syrians at 56.4% and lowest amongst Far East Asians at 21.9%) and occupation (surprisingly highest amongst professional groups at 48.3% and lowest amongst farm labourers at 22.4%).

These findings are clearly related to the different environmental and behavioural factors such as different culinary habits and varying risk factors associated with occupation.

An important finding was the seropositivity of IgM antibodies against *Toxoplasma gondii* amongst blood donors. Even at the low rate of 2.7% a transmission risk has been identified.

DEDICATION

*This work is dedicated to
My Loving & Caring Family Members especially my Mother & my brother Humaid
who has given me all the encouragement and support throughout my studies
and has been the backbone of all my success*

HAMAD

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*Seroprevalance of Toxoplasmosis
Amongst
Healthy Adults in the Al Ain Area*

AIMS

To establish base-line data on the seroprevalence of toxoplasmosis and assess some of the associated risk factors in the Al Ain area.

OBJECTIVES

1. Use of a simple questionnaire to obtain basic epidemiological data from two healthy adult sub-populations:
 - (a) of persons presenting for normal health screening
 - (b) of blood donors, in the Al Ain area.
2. Obtain blood samples and prepare sera from consenting participants.
3. Use the Enzyme Linked Fluorescent Assay (ELFA) for specific anti-*Toxoplasma* IgG, to measure prevalence rates; then to assess to any correlation between seropositivity and such variables as age, sex, domicile, nationality and occupation of each population sub groups.
4. Use specific anti-*Toxoplasma* IgM in human serum which is IgG positive, with ELFA technique (Enzyme Linked Fluorescent Assay) to assess transmission risk amongst potential and actual blood donors .
5. Assess inherent and environmental risk factors that may be associated with toxoplasmosis.

CHAPTER I

INTRODUCTION

I. INTRODUCTION

Toxoplasmosis is a zoonotic infection caused by an intracellular protozoan parasite *Toxoplasma gondii*. Nicolle and Manceaux (1908) first discovered it in a colony of North African rodents, the 'gondii' (*Ctenodactylus gondii*), kept at the Institute Pasteur in Tunis and named it *Leishmania gondii*. The following year (Nicolle and Manceaux, 1909), they renamed it *Toxoplasma gondii*, derived from the Greek "toxon", an arc or bow, for the shape of the organism.

There are a number of reviews of the history of *Toxoplasma* and toxoplasmosis which have contributed to our understanding of the organism and its many forms of disease transmission and presentation (Garnham, 1971; Frenkel, 1973; Jacobs, 1973; Jackson and Hutchinson, 1989).

1.1 HISTORICAL

Soon after the description of the organism by Nicolle and Manceaux, Mello (1910) described the disease in domestic animals. The human disease with eye involvement was first recognized by Janku in 1923 and the congenital infection in human neonates confirmed by Wolf *et al.*, in 1939. Sabin (1941) first described the tetrad of symptoms: choroidoretinitis, cerebral calcification, hydrocephalus and mental subnormality. The recognition of acquired toxoplasmosis, or glandular form of the disease in adults, came in 1952

(Siim, 1952) and two years later the suggestion was that undercooked meat might be the source of human infections (Weinman and Chandler, 1954). Desmonts *et al*, (1965) provided good epidemiological evidence for this when they observed seroconversion in children fed on raw meats in a TB hospital in Paris.

Little was known of the life cycle and hence the taxonomic status of the organism, despite numerous experimental transmissions in animals. A report (Hutchinson, 1965) on faecal transmission from the cat stimulated a great deal of research. Initially, Hutchinson had held that *Toxoplasma* was transmitted only in association with the nematode *Toxocara cati*; however further work by Hutchinson *et al* (1969, 1970, 1971), *n et al* (1970), Sheffield and Melton (1970), Overdulve (1970), Weiland and Kühn (1970), Witte and Piekarski (1970), and Zaman and Colley (1970) led to the identification of the oocyst as the infective stage in cat faeces and the description of the coccidian nature of *Toxoplasma*.

1.2 CLASSIFICATION

The following modified scheme of classification is that adopted by the Society of Protozoologists in 1980 (Schmidt and Roberts, 1989):

Phylum	Apicomplexa		:		
Class	:	Sporozoea	Subclass	:	Coccidia
Order	:	Eucoccidiida	Suborder	:	Eimeriina
Family	:	Sarcocystidae	Subfamily	:	<i>Toxoplasmatinae</i>
Genus	:	<i>Toxoplasma</i>	Species	:	<i>gondii</i>

1.3. MORPHOLOGY AND LIFE CYCLE

The defin

Sexual reproduction occurs in the intestinal epithelium resulting in the formation of oocysts (Figure. 1).

Oocysts are shed in cat faeces for 7-20 days and as many as 10×10^6 oocysts may be shed in a single day, thus heavily contaminating the environment. In the soil, oocysts sporulate in 1-21 days, depending on humidity and temperature (22°C optimum) and may remain infective for a year in a humid environment. Desiccation and high temperatures destroy the oocysts.

The intermediate hosts are all warm blooded animals including man; and infection occurs through ingestion of mature oocysts through direct contamination of food, water, hands; and mechanical vectors such as flies and cockroaches.

The ingested oocysts hatch in the intestine releasing the sporozoites which invade the intestinal epithelium to multiply intracellularly, disrupting the host cells to infect neighbouring cells. Tachyzoites also enter leukocytes, including macrophages in the lymph drainage, and proliferate rapidly disseminating to every organ and tissue in the body (Figure 2).

Parasite proliferation and host tissue destruction in immunocompetent hosts is contained by specific antibody production and a cell-mediated immunity. Not all parasites are eliminated. Surviving organisms now enter a dormant stage in the form of intracellular cysts or pseudocysts composed of a mass of cystozoites now called bradyzoites enclosed by a resistant membrane (Figures 3a-d).

Tissue cysts are found in virtually every organ but the brain, the eye and striated muscles are the most common sites of latent infection, probably remaining viable for the rest of the life of the host.

Toxoplasma gondii

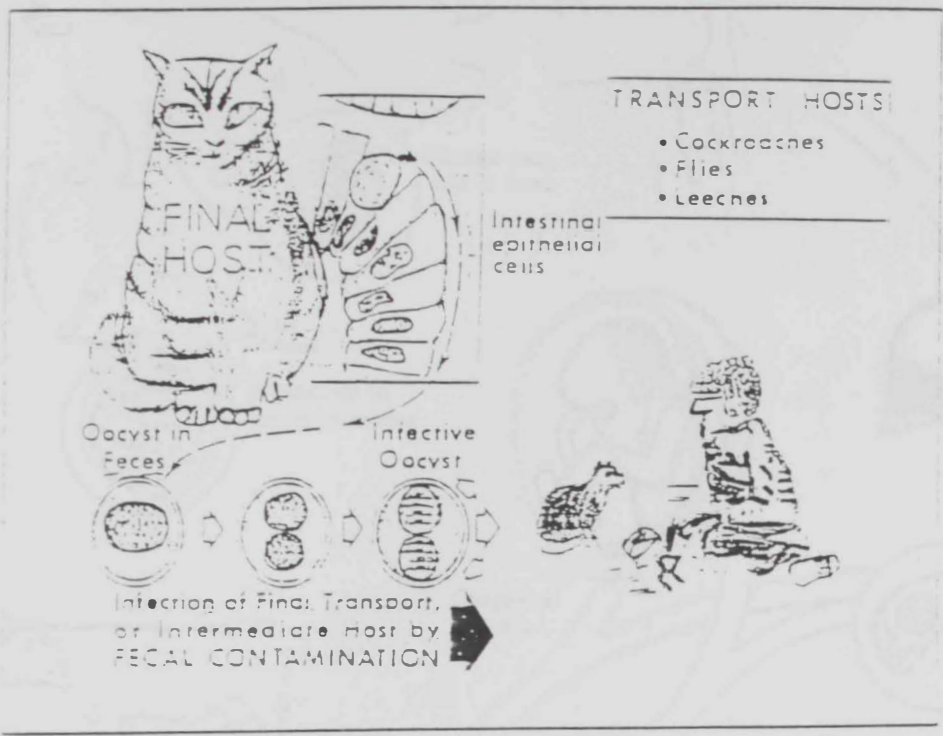


Figure 1: Enteroepithelial cycle of sexual reproduction of *Toxoplasma gondii* in cat intestine.

Toxoplasma gondii

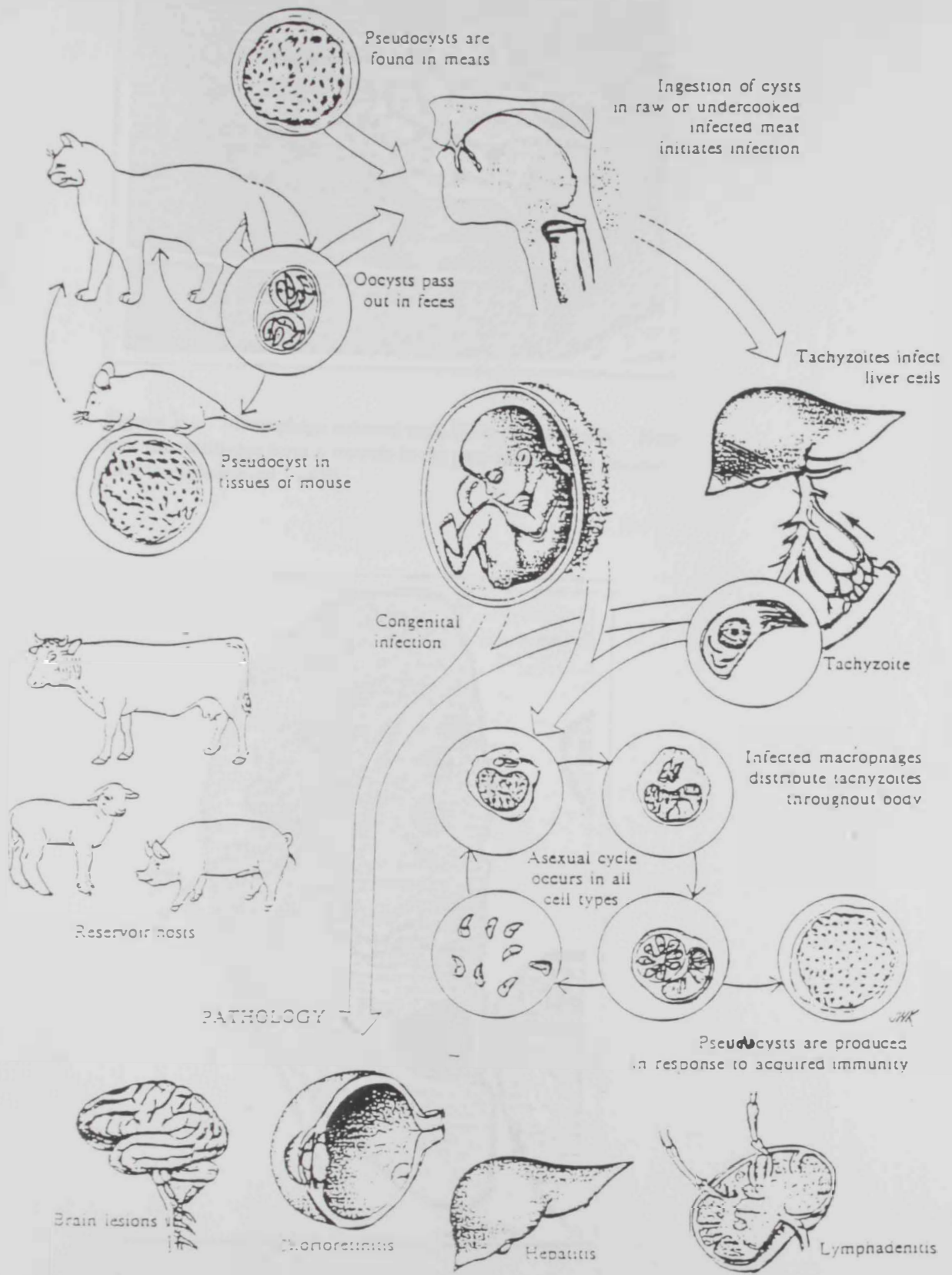


Figure 2: Infection and spread of Toxoplasma to other tissues and organs.

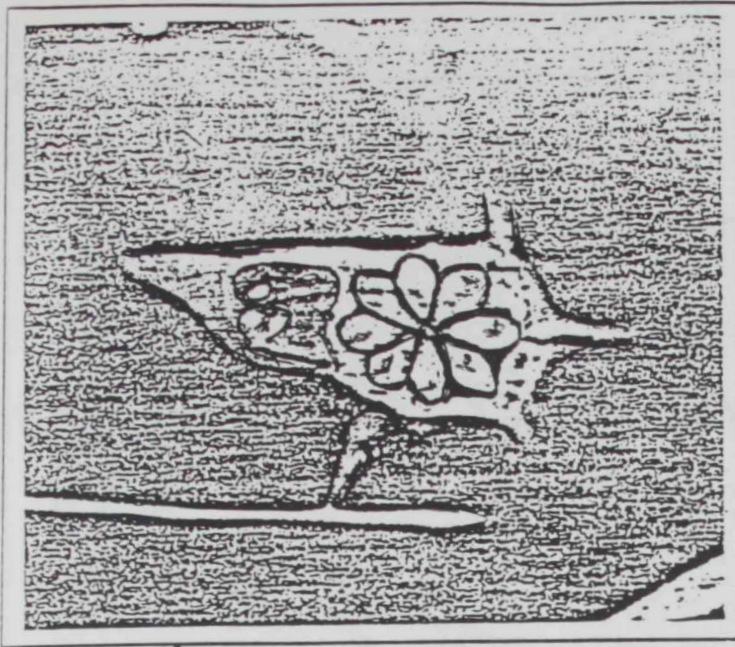


Figure 3a: Macrophage infected with *Toxoplasma gondii*. Note how the tachyzoites form a rosette in the parasitophorous vacuole with the infected cell. x450



Figure 3b: Transmission electron micrograph of a tachyzoite of *Toxoplasma gondii*. Various subcellular organelles are visible in this view. Nucleus (N), rhoptries (R), Golgi apparatus (G), conoid (C) x26,000 (Courtesy of Dr T Jones).



Figure 3c: Transmission electron micrograph of a portion of macrophage infected with the tachyzoites of *Toxoplasma gondii* (T). Although the lysosomes (Iy) have taken up ironcontrast, there is no evidence of fusion with the parasitocercous vacuole (PV). x 31,000 (Courtesy of Dr T Jones)

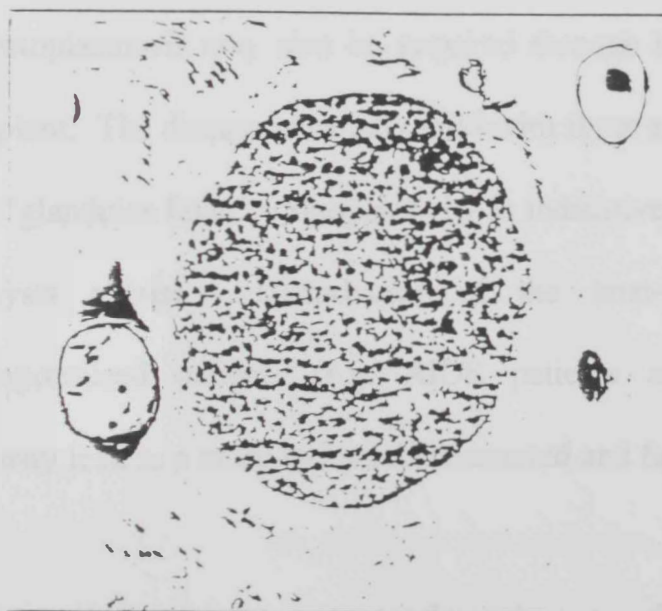


Figure 3d: Pseudocyst of *Toxoplasma gondii* in mouse brain, x1000

Figure 3d: Pseudocyst of *Toxoplasma gondii* in mouse brain, x1000

Presence of these tissue cysts in rodents and other meat animals (sheep, cattle, pigs, chicken, etc) may account for the enteroepithelial cycle in the cat and other feline hosts which feed on raw meat.(Jackson and Hutchinson, 1989)

1.4. TRANSMISSION AND DISEASE

Acquired toxoplasmosis in humans thus has two sources of infection: ingestion of oocysts and eating undercooked meats of various animals with tissue cysts of *Toxoplasma gondii*. Bradyzoites released from the cysts in the intestine quickly gain access to the lymphatics and behave like trophozoites, proliferating intracellularly in non-immune hosts; and forming cysts in various tissues and organs.

Toxoplasmosis may also be acquired through blood transfusion and organ transplant. The disease is usually mild with fever and lymphadenopathy, resembling "glandular fever". Seropositivity is indicative of immunity, though dormant cysts persist. Disturbance of the host-parasite balance in immunocompromised patients (e.g. AIDS patients and organ transplant recipients) may lead to a more serious disseminated and fatal disease.

Congenital toxoplasmosis is dependent on the unique ability of tachyzoites to cross the placental barrier. Depending on the time of maternal

infection during pregnancy, foetal infection may result in abortion, stillbirth, mild to severe CNS, eye, heart damage to infant, or the apparently normal child developing blindness later in life (Schmidt and Roberts, 1989).

1.5. HOST PARASITE INTERACTIONS

Toxoplasma is able to infect cells and tissues of all organs in the body. The usually mild nature of this acquired disease is therefore surprising. However, there is no evidence of toxin production by the parasite, and its intracellular location partially protects the parasite from the host immune responses. Cystozoites presumably prime the immune responses to provide long-term immunity.

Immunity in toxoplasmosis has been reviewed by Frenkel (1973). It appears that immunity does not eradicate the infection. Tissue cysts with viable cystozoites may be recovered from animals and humans many years after the primary infection. These tissue cysts, moreover, may persist even though antibody levels may decline or disappear.

Reactivation of chronic latent infection can occur, as seen in AIDS patients, leading to formation of new tissue cysts. Thus, depending on the immune status of the host reactivation and proliferation of the parasites, may lead to local pathology, for example retino-choroiditis and blindness or death

due to dissemination and proliferation in vital organs such as the brain or the heart. (Schmidt and Roberts,1989).

1.6 DIAGNOSIS

Diagnosis of toxoplasmosis may be made by clinical, histopathological, or serological methods, or by isolation by direct smear preparation, or passage of tissues from suspected infected hosts into cell cultures or laboratory mice.

Clinical signs and symptoms are invariably non-specific. Demonstration of *Toxoplasma* either in smears or in tissue sections is difficult because of the limited amount of biopsy material; though tissue cysts in autopsy samples are more readily seen. Isolation in cultures or in laboratory mice is time consuming and although failure to demonstrate the presence of the organisms by this method may be suggestive of lack of infection in the suspected case, it does not exclude it.

Serological tests are used both in diagnosis and in surveillance. The test antigen is either whole, unbroken organisms or extracts from lysed parasites. Tests using whole organisms whether live or killed, detect antibodies against parasite membrane antigens. These are the dye test (Sabin-Feldman Dye test), the agglutination test, and the immunofluorescent antibody tests.

Methods using extracts of lysed organisms include the Complement Fixation Test (CFT), the Indirect Haemagglutination Test (IHAT), the Latex

Agglutination Test (LT), the Enzyme Linked Immuno Sorbent Assay (ELISA) and Enzyme Linked Fluorescent Assay (ELFA). Antigenic extracts, being a mixture of many membrane and cytoplasmic antigens, have to be standardized in order to interpret test results. For this reason, the tests vary in their specificity and sensitivity.

Although the Sabin Feldman Dye Test still remains a diagnostic standard, technological advancements in automation and sensitivity of detection of enzyme reaction products have made ELISA a test of choice for both individual patient diagnosis and mass population surveys. A number of commercial ELISA systems offer kits that measure specific anti-IgG and anti-IgM antibodies in test sera.

Specific anti-*Toxoplasma* IgM and IgG antibodies develop as a result of infection. IgM antibodies reach their peak within a few weeks of infection and then decline to insignificant levels by the fourth month. A sign of acute infection, these antibodies may persist for longer in some patients. IgG antibodies take 2-3 months to reach their peak and remain elevated, or may persist for several months or years.

1.7. EPIDEMIOLOGY AND ENVIRONMENTAL RISK FACTORS

A cat may shed upto 10 million oocysts in a day and in the soil oocysts sporulate to become infective in 1-21 days depending on humidity,

available oxygen and temperature of 4-37°C, with 22°C being optimal. In a humid environment they may remain viable for over one year. They are resistant to common disinfectants and only killed by desiccation or heat. This provides a ready explanation for the wide spread seropositivity of herbivorous animals like sheep, cattle, goats, camels and even chickens, which all provide meat for man. That oocysts are the prime cause of toxoplasmosis of man is more difficult to establish. There is considerable evidence of direct oocyst contamination (Stagno et al., 1980), fly and cockroach borne oocyst infection (Wallace, 1972), and seroprevalence related to presence or absence of cats (Wallace, 1981).

Acquisition of infection from tissue cysts in meats first suggested by Weinman and Chandler (1954), and Desmonts et al (1965) provided clear evidence of importance of undercooked meat as a source of infection in children fed on 'rare' meat in a TB hospital in Paris. Serological surveys of meat animals world-wide show mean positive rates of 25% (0-99%) in cattle, 31% (0-96%) in sheep and 29% (1-98%) in pigs (Fayer, 1981). Tissue cysts are able to survive for over 2 months at 4°C but are killed at +60°C or by freezing at -9 to -20°C for 3-4 hours. It is therefore unlikely that well-cooked meat is a source of infection.

The practice of eating undercooked or raw meat is now a well established risk factor and there is also evidence of transmission through

ingestion of unpasteurised goats milk (Rieman et al., 1975; Sacks et al., 1982). The local practice of drinking raw camel milk may be another risk factor. Other risk factors may include degree of association with different animals (veterinarians, farm workers, slaughter-house workers etc).

Since *Toxoplasma* may be found in body tissues or fluids, transmission through blood transfusion, bone-marrow or organ transplant, remains a possibility, even though the donor may be asymptomatic. Recipients of blood or grafts who are under chemotherapy are at particular risk of developing serious disease.

Seroprevalence amongst selected groups is summarised in Table I

Population seroprevalence in different geographical areas is subject to variations in climate, sanitary conditions, food habits and presence of cats. These are summarised in Table II.

There is only one study about *Toxoplasma* seroprevalence among pregnant women in the U.A.E. (Dhaiban , 1994). She sampled 400 pregnant women and found the IgG positive rate of 29.75% .

Table I: Prevalence of human toxoplasmosis in various groups based on serological evidence

<i>Group</i>	<i>Region</i>	<i>Prevalence (%)</i>
Women of childbearing age	Mali	34
Pregnant women	Paris	84
	Glasgow	13.4
	Brussels	53
	England	14.9
Blood donors	Kenya	42
	Thailand	1.2-4.6
	Scotland	7.6-7.8
Veterinarians	California	43.7
	New York	8.3
Abattoir workers	Brazil	60-92
Military recruits	USA	3-20
Cat owners	Washington County, USA	20.9
	England	35.8
Without dogs	Iceland	18.3
Isolated island populations:		
With cats	Pacific (tropics)	43 and 56
Without cats	Pacific (tropics)	7
Isolated jungle populations	Brazil (tropics)	39-77
Remote populations	Alaska (arctic)	28
Medical outpatients	England	35.7
Travelling people	Scotland	28

NB: Comparison between results is complicated because of different tests used to determine prevalences. In addition, prevalences in control groups or the rest of the population were not usually reported for comparison.

(Adapted from Table 3: Jackson and Hutchinson, 1989)

Table II : *World-wide prevalence of antibodies to Toxoplasma gondii*

<i>Region</i>	<i>Prevalence (%)</i>
Africa	
Algeria	52
Egypt	18-31
Ethiopia	48
Ghana	10-70
Kenya	42-59
Mali	48-58
Mauritania	4-32
Morocco	27-38
Nigeria	21-64
Senegal	18-23
Somalia	10-53
Sudan	61
South Africa	7-37
Tanzania	40-72
Uganda	11-33
Rest of the world	
Brazil	74
Iran	13
Jordan	26
Hong Kong	10
Taiwan	4
Tahiti	71
Solomon Islands	80-89
Caroline Islands	56-87
Malaysia	4.5-27
Indonesia	2.3-51
United States	38
England	22
France	87
Norway	12-13
El Salvador	75

(Adapted from Table 7.1: Couvreur and Desmonts, 1988)

CHAPTER II

MATERIALS AND METHODS

II MATERIALS AND METHODS

2.1. GEOGRAPHY AND DEMOGRAPHY

The United Arab Emirates lies on the Arabian Gulf with a coast line of about 650 km . It covers an area about 77,000 km square, most of which is flat desert except for the eastern side of the Peninsula, which is mountainous. It has hot, humid summers and the annual rainfall is less than 10 cm. which occurs during the winter when the temperature is milder. Sand storms blow in the spring from the North and the North-West.

UAE is a federation of seven emirates stretching from the borders of Qatar and Saudi Arabia to the Musandam peninsula, just south of the strait of Hormuz.. The largest of which is Abu Dhabi, consisting of 87% of the total area, while Ajman is the smallest with 259 km square, or 0.33% of the total area. Before the discovery of oil in 1958, the economy of the country was limited primarily to oasis agriculture, fishing, pearling and trade with neighbouring countries. Oil now represents 95% of total exports and 70% of the GDP.

The population of the UAE was estimated to be approximately 2,011,400 in 1992 according to the estimate of the Ministry of Planning. The economic and social development witnessed by the UAE over the past two

decades has led to significant demographic changes, mainly characterized by declining mortality, changing age structure and increasing fertility in the indigenous population.

A significant characteristic is male:female ratio which stands currently at 2.2:1. Of the population 15 years and over 75% are males (Bener et al 1994), a large proportion being single male expatriates which affects the male:female population ratio. In addition, the UAE has become highly urbanized with more than 70% of the population living either in cities or in semi-urban townships.. Only 32% are below 15 years of age due to the population pyramid caused by the expatriates, particularly males, who mainly fall into the age group of 15-44 years (Bener et al., 1994).

2.2. STUDY AREA AND POPULATION:

Al Ain City is situated in the eastern part of Abu Dhabi Emirate near the Omani border. Al Ain is considered the "Garden City" and "Cultural capital" of the UAE. In Al Ain, there is an abundance of natural springs and fertile land. Long before the oil age, the dates and palm trees were one of the basic sources of income, at the same time providing food and shelter. The city and its surrounding area has a population of about 250,000 and again most of this number are expatriates, reflecting the national demographic make-up.

The study was designed to be in Al Ain Medical District, where the department of Preventive Medicine screens all imported manpower coming to

work in Al Ain to ensure they are free of infectious diseases. Though these expatriates belong to different nationalities, the majority are from Arabic countries, India, Pakistan, Bangladesh, Sri Lanka and the Phillipines.

2.3. SAMPLING DESIGN

A prospective study was carried out during the period between January 1994 to May 1995. All subjects were randomly selected from Al Ain and Tawam Hospitals which are Ministry of Health administered teaching hospitals. As these are the main hospitals in a city catering to a population of around 250,000, patients would be representative of the Al Ain population. The two hospitals were included in the study also to ensure that all blood donors presenting at both teaching hospitals during a period of one year were included.

The methods used included a simple questionnaire (Fig 4) following the multi-stage sampling technique used to select subjects . Stratification allowed both urban and rural areas to be proportionally represented. The sample size was obtained by assuming that the prevalence rate would be around 30%, similar to neighbouring Gulf countries of Bahrain (Yousif et al., 1991) and Saudi Arabia (Abbas et al., 1986). Therefore, on computing for 99% confidence

TOXOPLASMA SEROPREVALANCE IN HEALTHY ADULTS

SERUM SAMPLE NO: _____ HOSPITAL NO: _____

DATE: _____

NAME: _____

AGE: _____

SEX: _____

NATIONALITY: _____

DOMICILE: _____

CITY: _____ AREA: _____

LENGTH OF DOMICILE IN AREA: _____

PROFESSION: _____

Doctor: _____

Field Worker: _____

Teacher: _____

Farm Labourer: _____

Engineer: _____

Housewife: _____

Office Worker: _____

Others: _____

REMARKS: _____

RESULTS

IgG: _____

IgM: _____

Figure 4 : The questionnaire

limits and with 5% error bound it yielded the required sample size of 1200. The questionnaire was completed by nurses with the help of a senior medical officer and under the supervision of the investigator. The questionnaires and administration team were the same in urban and rural areas. Urban areas were those areas situated within the periphery of the city of Al Ain. Rural areas were defined as those villages located immediately outside the Al-Ain city limits.

Prior to the commencement of this study, the repeatability of the questionnaire was determined and the same questionnaire a second time, five weeks later was administered. No alteration was made to the questionnaire prior to its use in the main study sample. The assessment of repeatability of the questionnaire was performed through the use of Kappa test.

2.3.1. Sampling

From June 1993 to the end of March 1995, 1200 samples were collected randomly from healthy adults who came for health checks or to donate blood. A qualified technician drew 5-7 ml blood by venepuncture into a serum separator bottle (vacutainer). After clot formation in a plain tube, serum was separated by centrifugation, dispensed into vials and stored in a deep freezer at -30°C until used. The time from collecting the sample to storing the serum was not more than 48 hours.

Personal data for each individual was recorded on the questionnaire.

Collections were made at the following sites:

1. Preventive Medicine Department, Al Ain
2. Blood Bank Departments in both the Al Ain and the Tawam Hospitals

Each donor was invited to give blood for this survey after the purpose of the study was explained.

All samples were numbered serially according to the date of collection.

2.3.2. Serological Tests.

Commercially available test kits used were:

- (a) VIDAS toxo IgG (TXG)
- (b) VIDAS toxo IgM(TXM)

This is an enzyme immunoassay system for the determination of anti *Toxoplasma* IgG and-IgM in human serum using ELFA technique (Enzyme Linked Fluorescent Assay) supplied by Biomeriux - France.

2.3.3. Principle

The vidas toxo IgG/IgM is an automated assay system which enables anti *Toxoplasma* IgG and-IgM. in human serum to be quantitatively measured.

The assay principle combines the enzyme immunoassay method with a final fluorescence detection system. The disposable solid phase receptacle serves both as a solid phase and pipetting device during the assay.

2.3.4. Procedure:

For each test run, computer based VIDAS System was standardized according to a set procedure.

1. Programming the machine.

Because the machine can run different tests, we have to choose the test we require, i.e. TXG or TXM

2. Loading of reagents.

100 μ l of samples were loaded with calibration and control reagents in the strips and the work was followed. The detailed methodology is described in the system instruction (Fig 5 and Fig 6).

2. Calibration of the machine.

The machine should be calibrated for each kind of test by entering the calibration card from the kit and to read it. The machine should be calibrated every 14 days.

3. Work list creation

To create a work list regarding to the number of tests for either TXG or TXM

4. Running Control and Standards

Positive control 1 and negative control 2 and system standards 1 and standard 2 were run using 100 μ l amounts.

5. Loading of samples SPR

100 μ l of samples were loaded with calibration and control on to the strips and the work list followed. The detailed methodology is described in the suppliers instruction (Fig 5 and Fig 6).

Data computation and Analysis of the results was based on the following values

Titer (iu/ml)	Interpretation
<8	Negative
from 8 to 10	Equivocal
≥ 10	Positive

In the running of the samples the following precautions were followed to avoid by contamination or introduction of any variation in the results.

1. To avoid mixing of reagents from different lots
2. All reagents were brought to room temperature at least 30 minutes before using.
3. To handle the strips and samples carefully to treat them as potential infectious.
4. Using disposable latex gloves in handling samples.
5. Shaking all reagents well to obtain a better result reproducibility.

VIDAS TOXO IgG (TXG)

For in vitro diagnostic use

Enzyme immunoassay for the determination of anti-Toxoplasma IgG in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay)

PRINCIPLE

The VIDAS TOXO IgG is an automated assay for the VIDAS system, which enables anti-Toxoplasma IgG in human serum to be quantitatively measured. The assay principle combines the enzyme immunoassay method with a final fluorescent detection (ELFA).

All reaction steps are performed by the VIDAS instrument. The disposable Solid Phase Receptacle serves both as a solid phase and pipetting device during the assay. Reagents for the assay are all contained in the sealed Reagent Strips.

REAGENTS

Store at 2-8°C. Do not freeze the reagents.

The expiration date is indicated on the kit labeling.

Kit composition (60 tests) :

60 TXG strips	Ready-to-use.
60 TXG SPRs 2 x 30	Ready-to-use. SPRs coated with membrane and cytoplasmic Toxoplasma antigen. Each SPR is clearly marked. Only remove the required number of SPRs. Make sure the pouch is well closed after opening.
Positive TXG control* 1 x 2 ml (liquid)	Human serum containing anti-Toxoplasma IgG + protein stabilizer + 0.1 % of sodium azide. Titer in IU/ml : range given on vial label.
Negative TXG control* 1 x 3 ml (liquid)	Human serum negative for anti-Toxoplasma IgG + protein stabilizer + 0.1 % of sodium azide.
TXG calibrator* 1 x 1 ml (liquid)	Human serum containing anti-Toxoplasma IgG and calibrated against the WHO standard + protein stabilizer + 0.1 % of sodium azide. Titer in IU/ml given on vial label.

Description of the TXG Reagent Strip

Wells	Reagents
1	Sample well
2	Serum diluent : TRIS buffer (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 0.1 % of sodium azide (600 µl)
3	Pre-washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 0.1 % of sodium azide (600 µl)
4 - 5 - 7 - 8	Washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 0.1 % of sodium azide (600 µl)
6	Conjugate : Alkaline phosphatase labelled monoclonal anti-human IgG antibodies (mouse) + 0.1 % of sodium azide (400 µl)
9	Empty well
10	Cuvette with substrate : 4 methyl-umbelliferyl-phosphate + 0.1 % of sodium azide (300 µl)

* This product has been tested and shown to be negative for HBs surface antigen and antibodies to HIV and HCV. However, since no existing test can totally guarantee their absence, this product must be treated as potentially infectious, and handled with care. Furthermore, no known method can offer complete assurance that this human blood product does not contain any infectious agent.

Caution : Product of human origin for in vitro diagnostic use only, not intended for therapeutic use

The test identification, lot number and expiration date are printed on the strip bar code label.

The test identification, lot number, calibration parameters and calibrator titer are both clearly indicated in the kit's specifications sheet and printed on the bar code label.

Figure 5: Protocols for IgG determination

Materials required but not provided

Pipette with disposable tip calibrated to dispense 100 µl.

Warnings and precautions

1. Do not mix reagents from different lots.
2. Bring reagents to room temperature at least 30 minutes before inserting them into VIDAS.
3. It is recommended to handle the calibrator, controls and human samples carefully, while observing routine laboratory procedures. They should be treated as being potentially infectious.
4. The indicator on the desiccant in the SPR storage pouch should be blue. If the indicator has turned pink, do not use the remaining SPRs in that pouch.
5. Mix calibrator, controls, samples well with a vortex to obtain a better result reproducibility.
6. Use powderless, disposable latex gloves.

SAMPLES

Non-hemolyzed, uncontaminated sera or plasmas (EDTA, heparin, citrate). They can be stored for five days at 2-8°C but must then be frozen at -20°C. Freeze once only.

Heat inactivated (56°C for 30 minutes) sera may be used with VIDAS Toxo IgG.

PROCEDURE

1. Enter patient and assay data on the VIDAS terminal to create a Work List (see VIDAS User's Manual). The assay code for VIDAS TXG is TXG.
2. Mix samples, calibrator, and/or controls with a vortex.
3. Pipette 100 µl of sample, calibrator and/or controls into the strip's first sample well, if this has been directed in the Work List.

Note : After receipt of a new lot of reagents and every 14 days, calibrate using the TXG calibrator assayed in duplicate (see Manual, Chapter on "Calibration Menu").

4. Place the TXG SPR and TXG Reagent Strip into the positions indicated by the VIDAS Work List.
5. Follow procedure as directed in the VIDAS User's Manual. The assay will be completed in approximately 40 minutes.

QUALITY CONTROL

Periodically check the results' validity using the negative and positive controls.

The expected positive control value will be included in the range given on the vial label.

RESULTS

When the assay is completed, the results are analyzed automatically and the anti-toxoplasma IgG sample concentrations are calculated by VIDAS. The results are expressed in IU/ml, in relation to a calibration curve stored in VIDAS's memory.

Thresholds and Interpretation of results

Titer : IU/ml	Interpretation
< 8	Negative
from 8 to 10	Equivocal
≥ 10	Positive

Equivocal samples (titers between 8 and 10 IU/ml) should be retested. If the interpretation remains equivocal, then a new sample must be collected.

Samples containing antitoxoplasma IgG concentrations above 300 IU/ml can be diluted 1:4 in the negative TXG control. Multiply the result by the dilution factor to obtain the samples' titer.

PERFORMANCES

Accuracy

The presence of IgG antibody to *T. gondii* was determined using another commercially available EIA. All specimens which had discrepant results were tested using the Dye-Test and the hemagglutination method.

642 sera were tested.

The sera found to be equivocal with VIDAS Toxo IgG have not been used to calculate the performances.

Specificity

Of the 331 specimens found to be negative, 330 were negative by the VIDAS Toxo IgG assay, corresponding to a specificity of 99.7 % (99 % using the other EIA assay).

Sensitivity

Of the 305 specimens found to be positive, 300 were positive by VIDAS Toxo IgG assay, corresponding to a sensitivity of 98.4 % (91.8 % using the other EIA assay).

Within-run reproducibility of VIDAS TOXO IgG

		Number of assays	Average	Standard deviation	Within-run CV %
Serum 1	Signal	30	1373	43	3.12
	IU/ml	30	25	1	4.52
Serum 2	Signal	30	2238	63	2.81
	IU/ml	30	55	3	4.85
Serum 3	Signal	30	4174	88	2.11
	IU/ml	30	240	19	7.72

Between-run reproducibility of VIDAS TOXO IgG

		Number of assays	Average	Standard deviation	Between-run CV %
Serum 1	Signal	10	1403	34	2.43
	IU/ml	10	27	1	4.34
Serum 2	Signal	10	2252	81	3.62
	IU/ml	10	55	3	5.59
Serum 3	Signal	10	4157	86	2.06
	IU/ml	10	236	20	8.65
Positive Control	Signal	10	1773	53	2.98
	IU/ml	10	38	1	3.86

Complete information, specifications and references can be found in the VIDAS Package Insert Compendium.

VIDAS TOXO IgM (TXM)

For in vitro diagnostic use

Enzyme immunoassay for the detection of anti-Toxoplasma IgM in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay)

PRINCIPLE

The VIDAS TOXO IgM is an automated assay for the VIDAS system, which enables anti-Toxoplasma IgM in human serum to be detected. The assay principle combines the enzyme immunoassay method with a final fluorescent detection (ELFA). After IgM serum immunocapture, the anti-toxoplasma IgM are specifically detected with alkaline phosphatase labelled immune complex.

All reaction steps are performed by the VIDAS instrument. The disposable Solid Phase Receptacle serves both as a solid phase and pipetting device during the assay. Reagents for the assay are all contained in the sealed reagent strips.

REAGENTS

Store at 2-8°C. Do not freeze the reagents.

The expiration date is indicated on the kit labeling.

Kit composition (60 tests) :

60 TXM strips	Ready-to-use.
60 TXM SPRs 2 x 30	Ready-to-use. SPRs sensitized with anti-human μ chain antibodies (goat) purified by affinity chromatography. Each SPR is clearly marked. Only remove the required number of SPRs. Make sure the pouch is well closed after opening.
Positive TXM control 1 x 2 ml (liquid)	Human serum* containing anti-Toxoplasma IgM + protein stabilizer + 1 g/l of sodium azide. Index : range indicated on vial label.
Negative TXM control 1 x 2 ml (liquid)	Human serum* negative for anti-Toxoplasma IgM + protein stabilizer + 1 g/l of sodium azide.
TXM standard 1 x 1 ml (liquid)	Human serum* containing anti-Toxoplasma IgM + protein stabilizer + 1 g/l of sodium azide.

Description of the TXM Reagent Strip

Wells	Reagents
1	Sample well
2	Serum diluent : TRIS buffer (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (300 μ l)
3	Pre-washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (600 μ l)
4 - 5 - 7 - 8	Washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (600 μ l)
6	Conjugate : alkaline phosphatase labelled immune complex (toxoplasma antigen - mouse monoclonal anti P-30 antibodies) + 1 g/l of sodium azide + 0.02 % Gentamicin (400 μ l)
9	Empty well
10	Cuvette with substrate : 4 methyl-umbelliferyl-phosphate + 1 g/l of sodium azide (300 μ l)

* This product has been tested and shown to be negative for HBs surface antigen and antibodies to HIV and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

Caution : Product of human origin for in vitro diagnostic use only, not intended for therapeutic use.

The test identification, lot number, and expiration date are printed on the strip bar code label.

The test identification, lot number and calibration parameters are both clearly indicated in the kit's specifications sheet and printed on the bar code.

Figure 6: Protocols for IgM determination

Materials required but not provided

Pipette with disposable tip calibrated to dispense 100 µl.

Warnings and precautions

1. Do not mix reagents from different lots.
2. Bring reagents to room temperature at least 30 minutes before inserting them into VIDAS.
3. This kit contains human blood products. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions.
4. The indicator on the desiccant in the SPR storage pouch should be blue. If the indicator has turned pink, do not use the remaining SPRs in that pouch.
5. Mix standard, controls, and samples well with a vortex to obtain better result reproducibility.
6. Use powderless, disposable latex gloves.

SAMPLES

Non-hemolyzed, uncontaminated sera or plasmas (EDTA, heparin, citrate). They can be stored for five days at 2-8°C but must then be frozen at -20°C. Freeze once only.

Heat inactivated (56°C for 30 minutes) sera may be used with VIDAS Toxo IgM.

PROCEDURE

1. Enter patient and assay data on the VIDAS terminal to create a Work List (see VIDAS User's Manual). The assay code for VIDAS TXM is TXM.
2. Mix samples, standard, and/or controls with a vortex.
3. Place 100 µl of sample, standard and/or controls into the strip's first sample well, if this has been directed in the Work List.

Note : After receipt of a new lot of reagents and every 14 days, calibrate using the TXM standard assayed in duplicate (see Manual, Chapter on "Calibration Menu").

4. Place the TXM SPR and TXM Reagent Strip into the positions indicated by the VIDAS Work List.
5. Follow procedure as directed in the VIDAS User's Manual. The assay will be completed in approximately 40 minutes.

QUALITY CONTROL

Periodically check the results' validity using the negative and positive controls.

The expected positive control value will be included in the range given on the vial label.

RESULTS

When the assay is completed, the results are analyzed automatically and the index i (ratio of serum to be tested's fluorescent signal to the memorized standard signal) is calculated by VIDAS.

Thresholds and Interpretation of Results

Index	Interpretation
$i < 0.55$	Negative
$0.55 \leq i < 0.65$	Equivocal
$i \geq 0.65$	Positive

Equivocal samples (indices between 0.55 and 0.65) should be retested. If the interpretation remains equivocal, then a new sample must be collected.

PERFORMANCES**Accuracy****Comparison of VIDAS TOXO IgM method with ISAGA method**

N = 1116		VIDAS TOXO IgM		
		+	Equivocal	-
ISAGA	+	72	0	5*
	Equivocal	4	0	3
	-	7	5	1020

Sensitivity : 93.5 %

Specificity : 99.3 %

Agreement : 98.9 %

* for the 5 samples found to be negative with the VIDAS TOXO IgM and positive with ISAGA, three of them were found to contain residual IgM.

Note : The sera found to be equivocal with ISAGA and/or with VIDAS TOXO IgM have not been used to calculate the sensitivity, specificity and agreement.

Within-run reproducibility of VIDAS TOXO IgM

Sera	Number of assays	Signal Average	Standard deviation	Within-run CV %
S1	30	82	11.04	13.4
S2	30	574	30.04	5.2
S3	30	1443	43.09	3.0

Between-run reproducibility of VIDAS TOXO IgM

Sera	Number of assays	Index Average	Standard deviation	Between-run CV %
S4	7	2.09	0.10	4.8
S5	7	0.76	0.04	5.3
S6	7	0.11	0.01	8.2

Complete information, specifications and references can be found in the VIDAS Package Insert Compendium.

2.3.5. Data Analysis

The questionnaires were coded, entered and processed in the Department of Community Medicine on the IBM computer of Faculty of Medicine and Health Sciences at the UAE University.

The statistical package program (SPSS) [Norusuis (1992)] was used to calculate Chi-square to ascertain the association between two or more categorical variables in 2x2 tables instead of Chi-square, in particular when the sample size was small.

The relative risk (RR) and their confidence intervals (CI) were obtained by using Mantel Haenszel test Ep15 package. Student's-t (two-tailed) test was used to determine the significance of difference between two continuous variables.

The level $p < 0.05$ was considered as cut-off value for significance.

CHAPTER III

RESULTS

III RESULTS

A total of 1200 blood samples were obtained from willing donors. However, as the number of kits needed do the tests was limited by the availability of research funds only 941 of the samples were actually tested. These were chosen randomly from the two main sources of collection, i.e. the Preventive Medicine Department (49.9%) and Blood Banks at the Tawam and Al-Ain Hospitals (50.1%). (Fig 7)

Table III summarises the socio-demographic characteristics of the population surveyed. Of 941 serum samples tested, 317 (33.6%) were found positive for anti-*Toxoplasma* IgG antibodies, 616 (65.5%) samples were seronegative and 8 (0.8%) gave equivocal results (Figure 8).

Age was divided into five separate age groups: less than 20 years (4.5%), 21-30 years (27.4%), 31-40 years (51.3%), 41-50 years (13.1%) and more than 50 years (3.7%) (Figure 9).

Of 941 serum samples tested for IgG positivity 857 (91.7%) were males and 78(8.3%) were females (Figure 10).

Fig.7: Distribution of samples according to the source in Al Ain.



Fig.8: Seropositivity rate of the sample population in Al Ain.

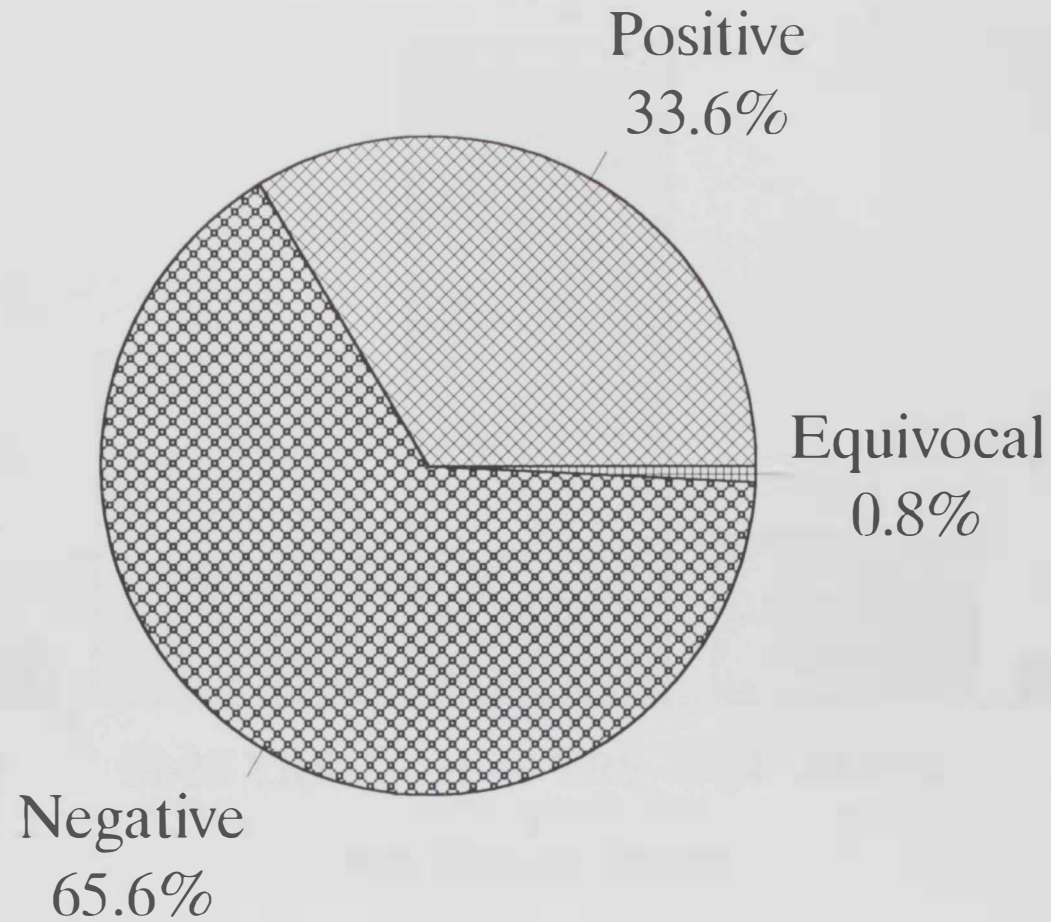


Fig.9: Distribution of samples according to the age groups

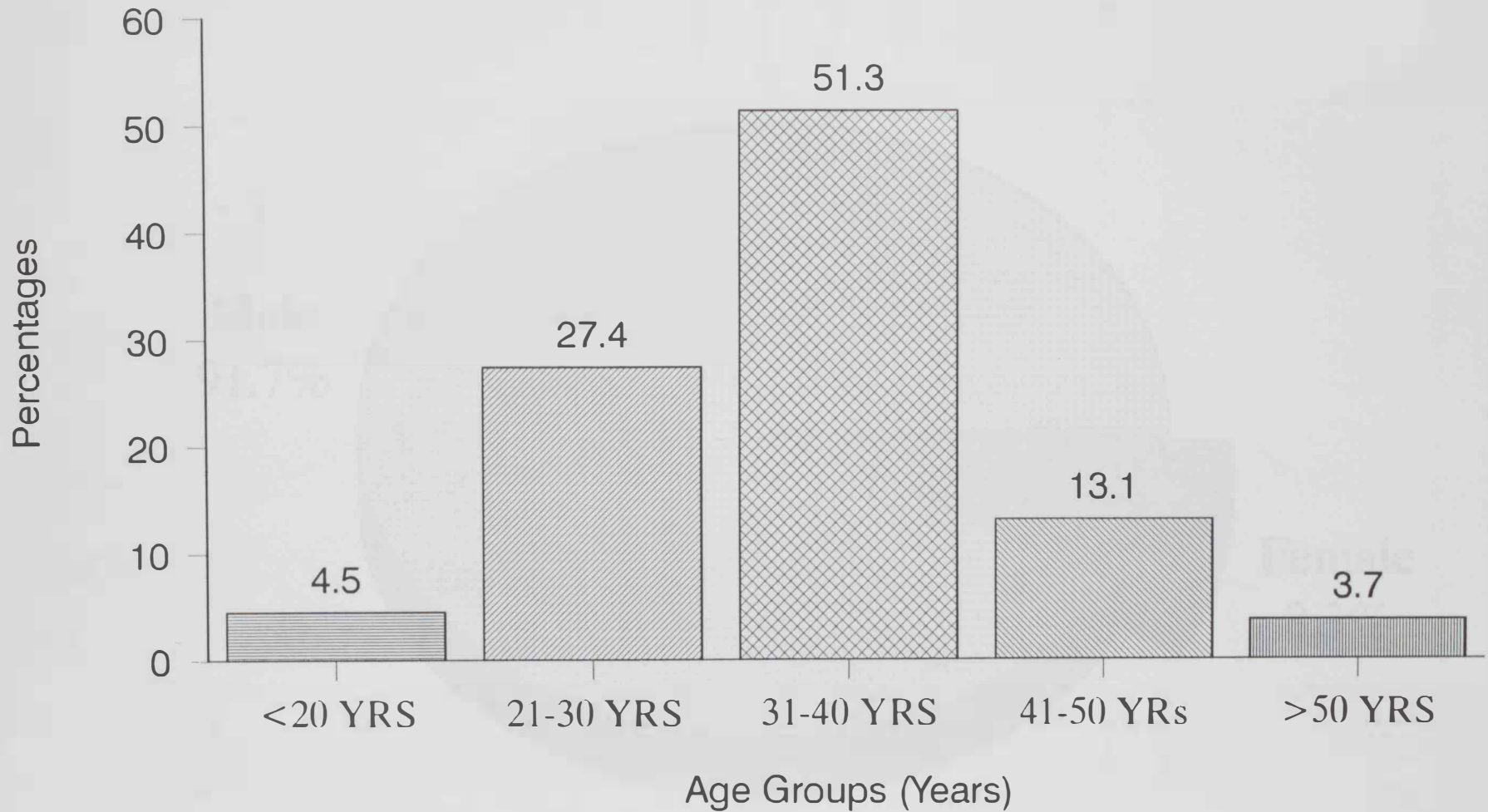
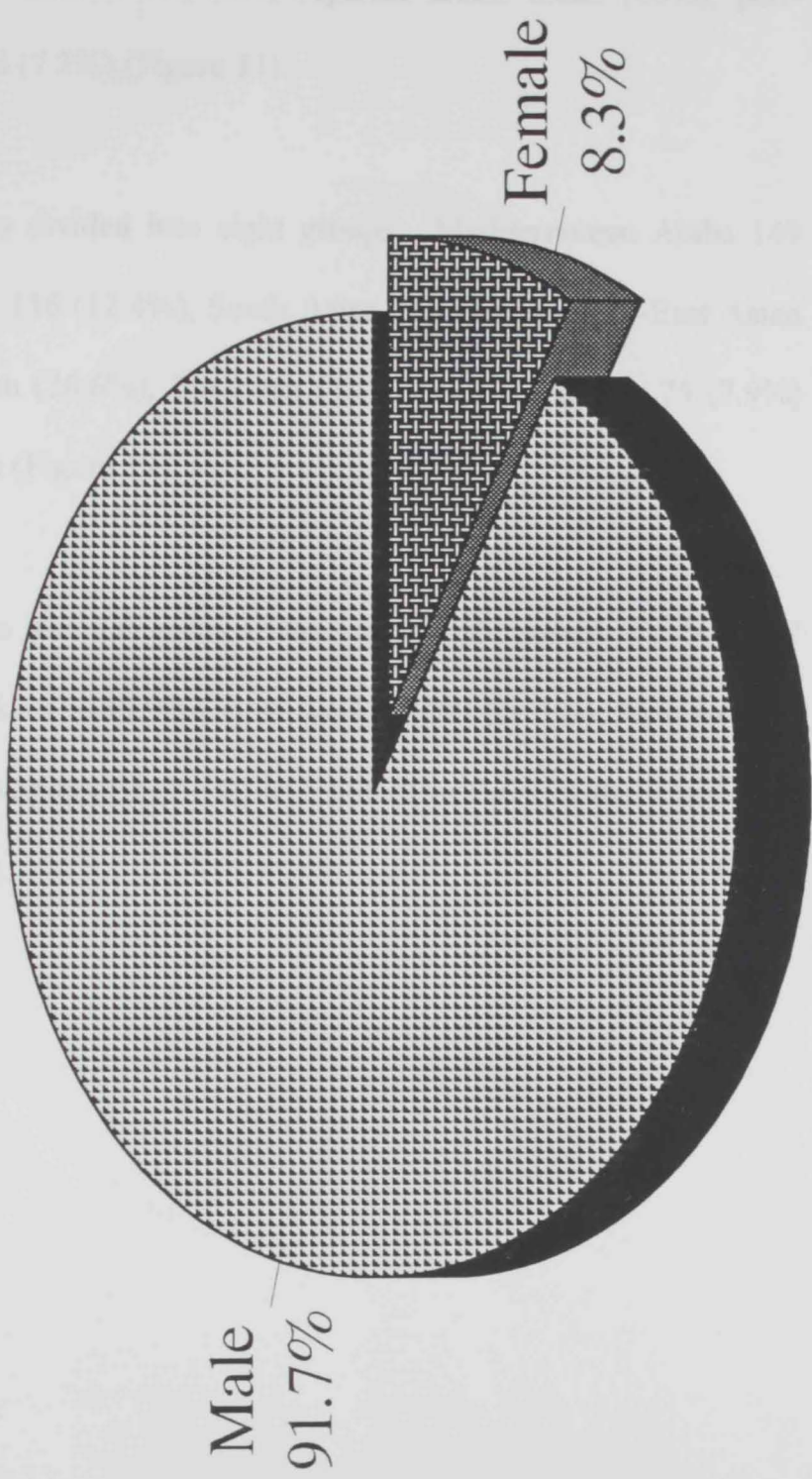


Fig.10: Distribution of samples according to the sex groups



Residence was divided into three separate areas: urban (86%), peri-urban (6.6%) and rural (7.3%),(Figure 11).

Nationality was divided into eight groups: Mediterranean Arabs 149 (16%), African Arabs 116 (12.4%), South Asian 178 (19.1%), Far-East Asian 32 (3.4%), West Asian (26.6%), European (3.4%) ,Gulf Countries 75 (7.9%) and UAE 103 (10.9%) (Figure 12).

Occupation was also categorized into six separate groups: Professional 62 (6.6%), office workers 93 (9.9%), field workers 189 (20.1%), farm workers 86 (9.1%), domestic workers 22 (2.3%) and skilled workers or daily labourers 489 (52%) (Figure 13).

Fig.11: Distribution of samples according to residential status in the Al Ain area.

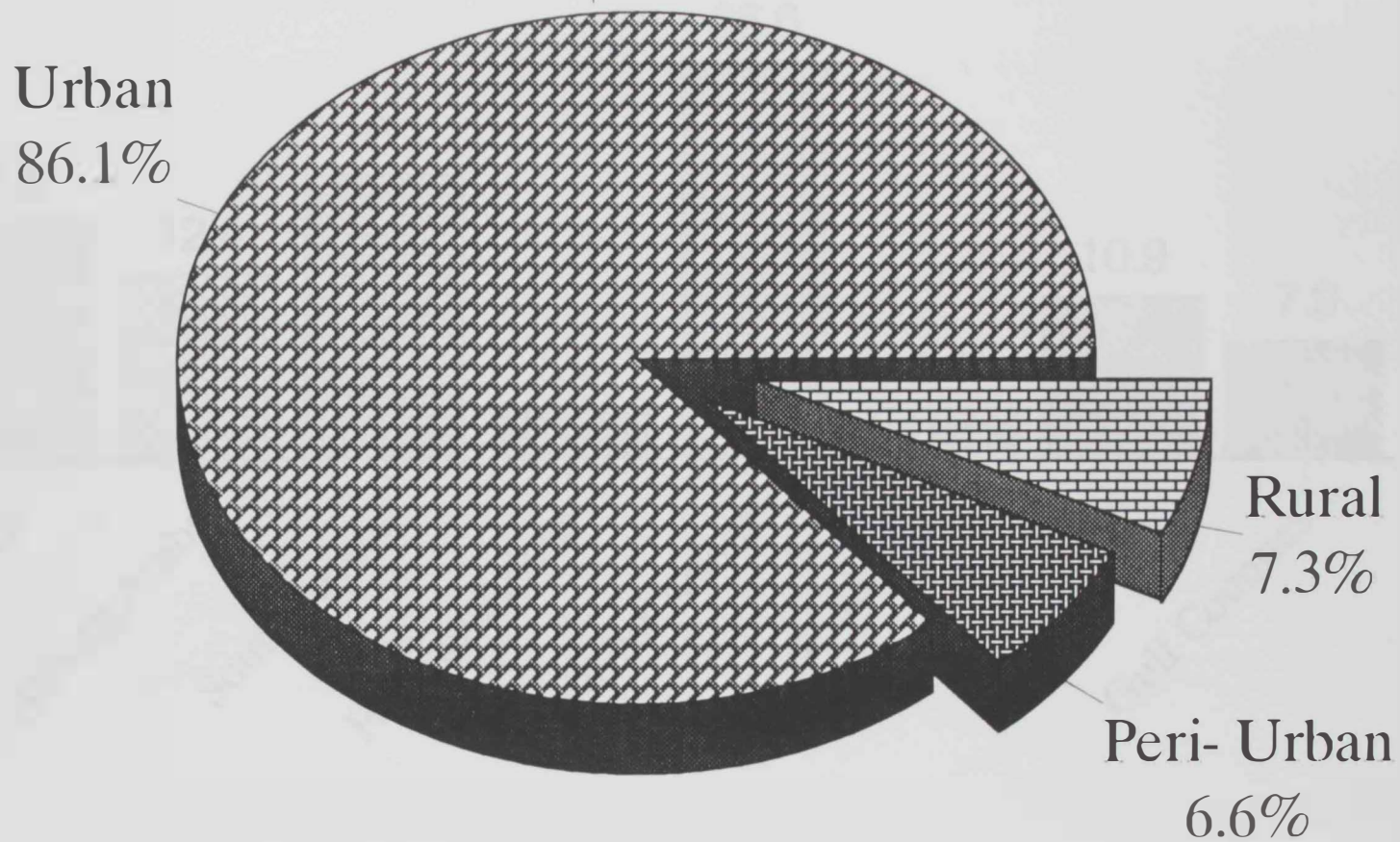


Fig.12: Distribution of sample size according to and geographical groups

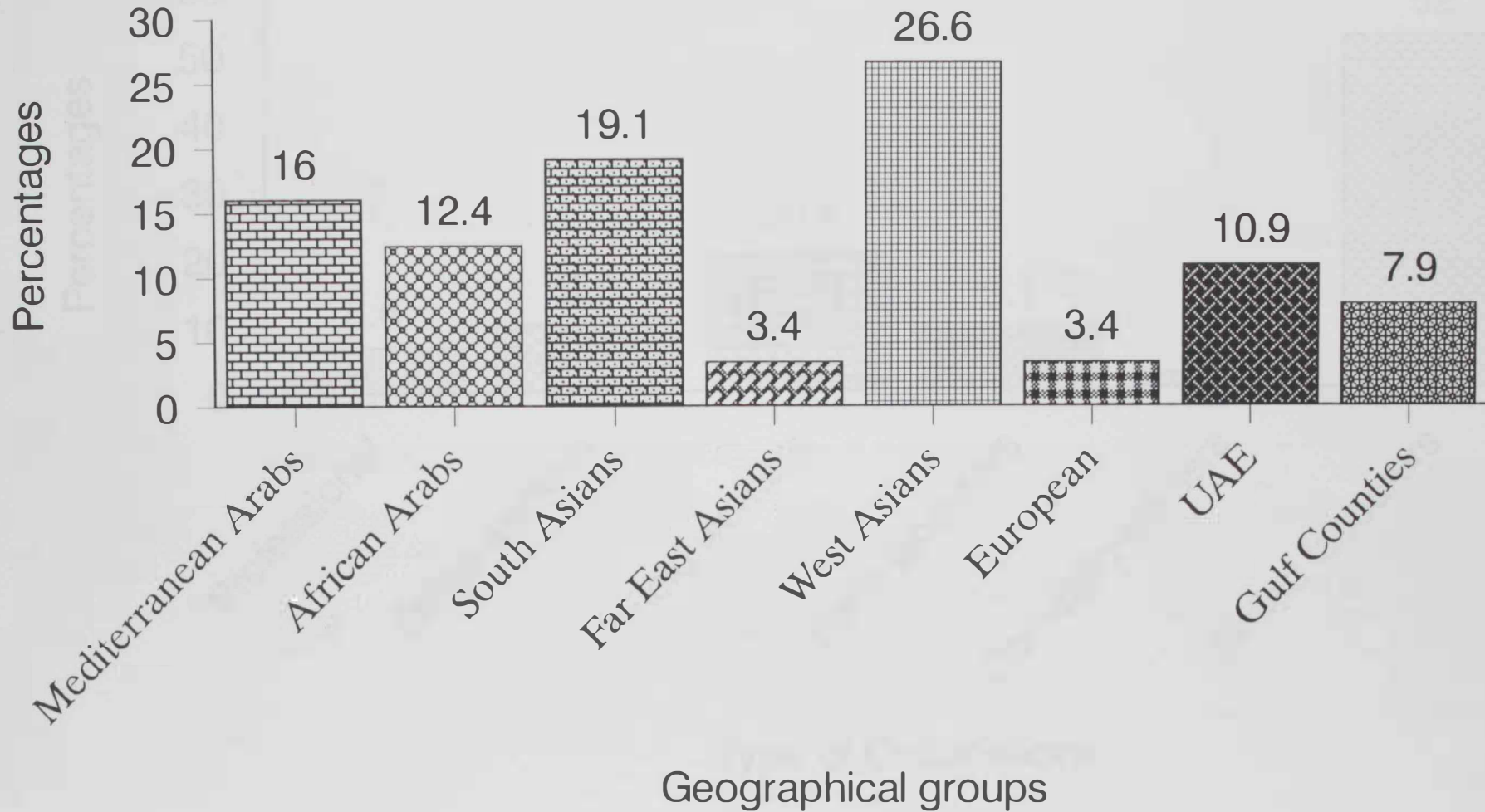


Fig.13 : Distribution of Sample size according to the type of occupation

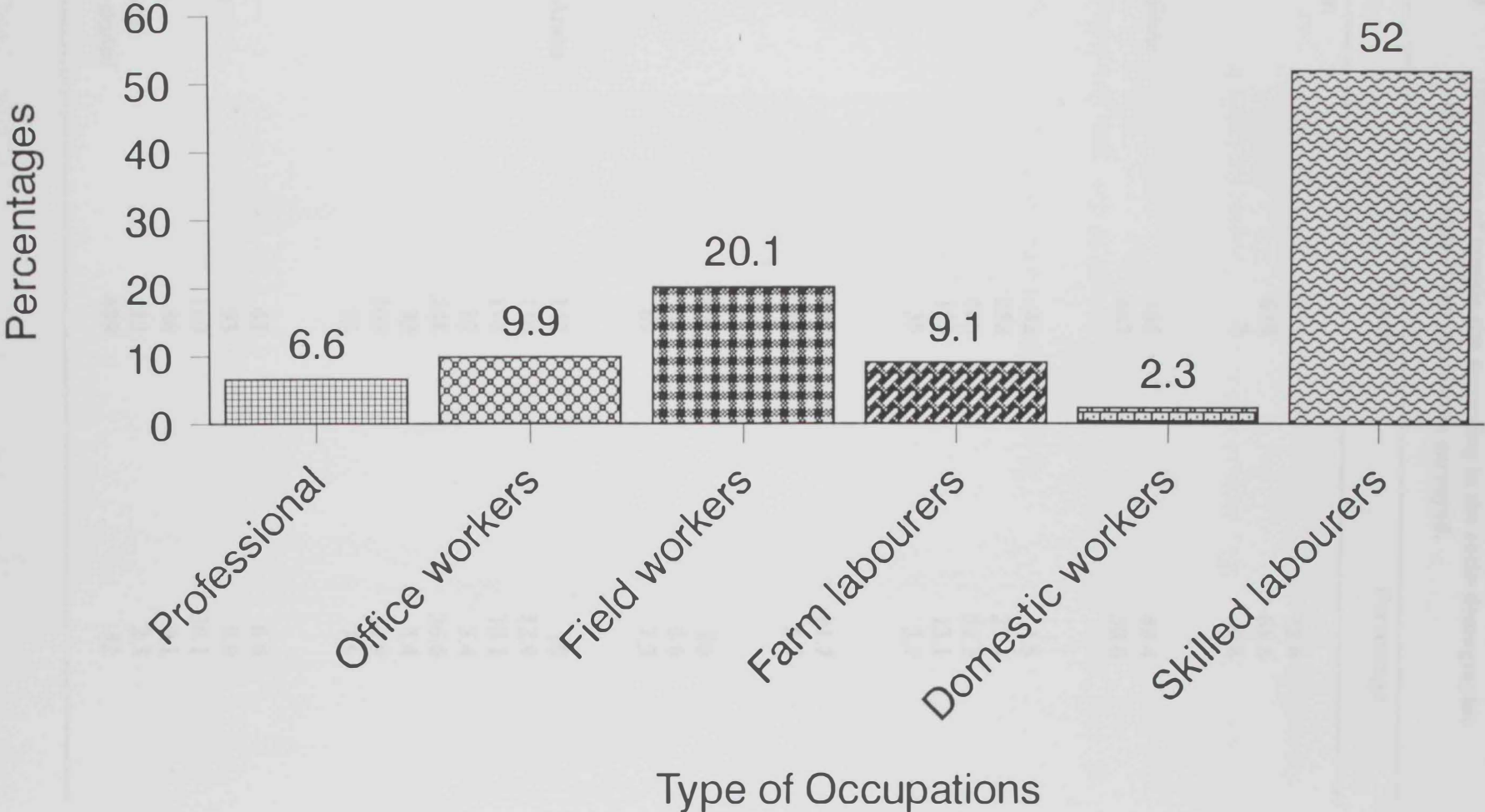


Table III Distribution of sample size according to the socio-demographic characteristics of the population surveyed.

	Number	Percentage
Toxoplasmosis:		
Positive IgG	317	33.6
Negative	618	65.6
Equivalence	8	0.8
Locations:		
Preventive Medicine	466	49.4
Blood Bank	467	50.6
Age in Years:		
<20 years	42	4.5
21-30 years	258	27.4
31-40 years	483	51.3
41-50 years	123	13.1
>50 years	35	3.7
Sex:		
Males	863	91.7
Females	78	8.3
Area:		
Urban	808	86
Peri-Urban	62	6.6
Rural	69	7.3
Nationality:		
Mediterranean Arabs	149	16
African Arabs	116	12.4
South Asian	178	19.1
Far East Asian	32	3.4
West Asian	248	26.6
European	32	3.4
UAE	103	10.9
Gulf Countries	75	7.9
Occupation:		
Professional	62	6.6
Office Workers	93	9.9
Field Workers	189	20.1
Farm labourers	86	9.1
Domestic workers	22	2.3
Skilled or semi-skilled	489	52

Table IV summarises the socio-demographic characteristics of the population according to the IgG seropositivity to *Toxoplasma gondii*. As can be seen from this table, there was no significant differences between Preventive Medicine Department and Blood Bank concerning *Toxoplasma gondii* seropositivity. There was a trend of increasing seropositivity with age. There was a significant association between nationality and seropositivity ($p < 0.0001$) and between occupation and seropositivity ($p < 0.03$). However, there was no significant effect of sex or residence on seropositivity.

Characteristic	n	%	OR	95% CI	p-value
Sex					
Male	72	42.1	0.9	0.5 - 1.7	NS
Female	100	57.9	1.0		
Age					
18-29	200	35.1	1.0		
30-39	40	22.6	1.1	0.6 - 1.9	NS
40-49	11	6.2	1.2	0.4 - 3.5	NS
50-59	34	19.4	1.3	0.7 - 2.3	NS
60-69	14	7.8	1.5	0.7 - 3.0	NS
70-79	41	23.1	1.6	0.9 - 2.7	NS
80-89	7	3.9	1.8	0.6 - 5.0	NS
90-99	20	11.3	1.9	1.0 - 3.5	NS
European	11	6.2	1.0		
Other countries	24	13.5	2.2	1.1 - 4.1	$p < 0.0001$
USA	17	9.6	1.5	0.8 - 2.6	NS
City residence					
Professional	29	16.2	1.0		
Office workers	40	22.6	1.4	0.8 - 2.3	NS
Field workers	30	16.9	1.1	0.6 - 1.9	NS
Home help	17	9.6	0.7	0.4 - 1.3	NS
Domestic workers	7	3.9	0.5	0.2 - 1.1	$p < 0.03$
Unemployed	150	84.0	1.0		

Table IV -Association of *Toxoplasma* IgG seropositivity with the socio-demographic characteristics of the population surveyed

	POSITIVE		NEGATIVE		p-value
	Number	Percentage	Number	Percentage	
Locations:					
Preventive Medicine	158	33.9	308	66.1	NS
Blood Bank	159	34	308	66	
Age in Years:					
< 20 years	8	19	34	81	<0.05
21-30 years	82	31.8	176	68.2	
31-40 years	162	33.8	317	66.2	
41-50 years	49	41.2	70	58.8	
>50 years	16	45.7	19	54.3	
Sex:					
Male	285	33.3	572	66.7	NS
Female	32	42.1	44	57.9	
Area:					
Urban	281	35.1	520	64.9	NS
Peri-urban	19	30.6	43	69.4	
Rural	17	25	51	75	
Nationality:					
Mediterranean Arabs	84	56.4	65	43.6	p<0.0001
African Arab	34	29.3	82	70.7	
South Asian	67	37.6	111	62.4	
Far-East Asian	7	21.9	25	78.9	
West Asian	59	23.8	189	76.2	
European	11	34.4	21	65.6	
Gulf countries	24	32	51	68	
UAE	31	30.1	72	69.9	
Occupation:					
Professional	29	48.2	31	51.7	p<0.03
Office workers	40	43.5	52	56.5	
Field workers	63	33.3	126	66.7	
Farm labourers	19	22.4	66	77.6	
Domestic workers	7	31.8	15	68.2	
Skilled or semi-skilled	159	32.8	326	67.2	

Seropositivity results related to the different sample variables are as follows:

1. Sample sources:

Approximately equal numbers of randomly selected serum samples from the Preventive Medicine Department and the Blood Banks were tested for IgG seropositivity. The results showed no significant difference between the two sample populations (Table V , Fig. 7, Fig.14).

2. Sex:

Of 941 serum samples tested for IgG positivity, 857 were males and 76 were females. The seropositivity rate among males was 33.3% and 42.1% in females (Table VI, Fig.10, Fig.15). Relative risk (RR) and their 95% confidence interval (CI) of toxoplasmosis with sexes are also presented and estimates were found for males [RR: 0.79 ; 95% CI: 0.60-1.05 , p=0.118], and females [RR: 1.27 ; 95% CI: 0.96-1.68 , p=0.118]. However, there was statistically no significant differences between males and females concerning seropositivity.

3. Age:

The age distribution of this population sample according to the seropositivity is given in (Table VII and Fig. 9, Fig.16). The sample represents a normal distribution of groups ranging from 15-60 years old. More frequent IgG seropositivity rates being in the 21-30 years and 31-40 years among the five age groups. A Chi-square test for trends showed a significant progressive

rate increase from 19% to 45.7% (Table VII). Relative risk (RR) and their 95% confidence interval (CI) of toxoplasmosis with age groups are also presented and estimates were found for each age group.

4. Domicile:

The seropositivity distribution of this sample population according to domicile is shown in (Table VIII , Fig. 11, Fig.17). Most of the sample population 801 (86%) was resident in the city of Al Ain. Results of IgG seropositivity rates amongst inner city (urban) outer city (peri-urban) or isolated (rural) dwellers showed no statistically significant differences in their rates. Relative risk (RR) and their 95% confidence interval (CI) of toxoplasmosis with domicile are also presented. There was statistically no significant difference between urban, peri-urban and rural dwellers concerning seropositivity .

5. Nationality:

The sample represented some 22 nationalities. Those were grouped into 8 geographical areas representing broad socio-cultural sub-groupings (Table IX , Fig. 12, Fig. 18).

a. Subgroup I: Designated as Mediterranean Arabs consisted of 149 subjects or 16% of sample population and included Jordanians, Syrians, Palestinians, and Lebanese. This sub-group showed the highest seropositivity rate of 56.4%.

- b. Subgroup II:** Designated as African Arabs consisted of 116 subjects or 28.2% of sample population, included Egyptian, Moroccan, Mauritanian, Sudanese and Somali nationals. This sub-group showed a seropositivity rate of 29.3%.
- c. Subgroup III:** Designated as South Asians consisted of 178 subjects or 19.1% of sample population and included Indians, Sri-Lankans, and Bangladeshis. This sub-group showed the second highest seropositivity rate of 37.6%.
- d. Subgroup VI:** Designated as Far East Asians consisted of 32 subjects or 49.4% of sample population and included, Filipino and Japanese nationals . This sub-group showed a seropositivity rate of 21.9%.
- e. Subgroup V:** Designated as West Asians consisted of 248 subjects or 26.6% of sample population and included Pakistani and Iranian nationals. The seropositivity rate of this sub-group was 23.8%.
- f. Subgroup VI:** Designated as Europeans consisted of 32 subjects or 3.4% of sample population and included Britons, Americans and Canadians. Seropositivity rate in this sub-group was 34.4%.

g. Subgroup VII: Designated as Peninsula Arabs or Gulf Countries, consisted of 75 subjects or 8% of sample population and included Saudi, Omani, Yemeni, Bahraini and Kuwaiti nationals. This sub-group showed a seropositivity rate of 32%.

h. Subgroup VIII: Designated as UAE nationals, consisted of 103 subjects or 11% of the sample. All were citizens of United Arab Emirates. This sub-group had the lowest seropositivity rate of 18.2%.

We then compared UAE nationals with other nationalities with regard to toxoplasmosis seropositivity (Table X). We obtained Relative Risk, 95% Confidence Interval and p-value for significance differences between the UAE and other nationalities. Statistically significant differences were found between seropositivity in UAE nationals and others (Table X).

6. Occupation:

The seropositivity distribution of this sample population according to occupation is shown in (Table XI , Fig.13, Fig.19). The sample distribution of the various occupations were classified in six categories: professionals (doctors, engineers, teachers), Office workers (secretarial and clerical jobs), field workers, farm workers (agricultural workers or workers looking after animals) domestic workers (mostly house-maids or servants, or house wives) and skilled or semi-skilled jobs.

The results of the IgG seropositivity rates amongst the different occupation groups was surprising. The highest was among the professional groups (48.3%) [RR: 1.47 ; 95% CI: 1.11-1.93 , p=0.015]; followed by office workers (43.5%) [RR: 1.32 ; 95% CI: 1.03-1.70 , p=0.042]; field workers (33.3%) [RR: 0.98 ; 95% CI: 0.78-1.22 , p=0.834].; skilled or semi-skilled(32.8%), [RR: 0.93 ; 95% CI: 0.78-1.11 , p=0.423]; domestic workers (31,8%) [RR: 0.94 ; 95% CI: 0.51-1.75 , p=0.845].; and the lowest amongst farm labourers (22.4%) [RR: 0.64 ; 95% CI: 0.42-0.96 , p=0.017].

IgM seropostivity amongst Blood Donors

60 of the IgG positive and 15 of the IgG negative sera were randomly selected and tested for presence of *Toxoplasma* specific IgM antibodies, using the VIDAS IgM Kit.

Table XII shows the results of test for *anti-Toxoplasma* IgM antibodies amongst a sample of Blood Donors. Two of the 60 tested (3.3%) were positive, one with a significant titre and the other with a very high titre. The overall IgM seropositivity amongst blood donors was 2/75 or 2.7%.

Table V Seropositivity for toxoplasmosis according to the sample source*

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
	Locations :					
Preventive Medicine	158	(33.9)	308	(66.1)	1.00 [0.83-1.19]	0.963
Blood Banks	159	(34)	308	(66)	1.00 [0.84-1.20]	0.963
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table VI Seropositivity for toxoplasmosis according to sex distributions

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
Sex:						
Male	285	(33.3)	572	(66.7)	0.79 [0.60-1.05]	0.118
Female	32	(42.14)	44	(57.9)	1.27 [0.96-1.68]	0.118
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table VII Seropositivity for toxoplasmosis according to the age distribution

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
	Age in years:					
<20 years	8	(19)	34	(81)	0.55 [0.29-1.03]	0.366
21-30 years	82	(31.8)	176	(68.2)	0.91 [0.74-1.20]	0.382
31-40 years	162	(33.8)	317	(66.2)	0.99 [0.83-1.18]	0.917
41-50 years	49	(41.2)	70	(58.8)	1.25 [0.99-1.58]	0.75
> 50 years	16	(45.7)	19	(54.3)	1.33 [0.92-1.94]	0.162
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table VIII Seropositivity for Toxoplasmosis according to the domicile*

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
Living area:						
Urban	281	(35.1)	520	(64.9)	1.29 [0.96-1.73]	0.079
Peri-urban	19	(30.6)	43	(69.4)	0.90 [0.61-1.32]	0.566
Rural	17	(25)	51	(75)	0.72 [0.47-1.70]	0.104
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table IX. Seropositivity for toxoplasmosis according to the nationality distributions*

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
Nationality:						
Mediterranean Arabs	84	(56.4)	65	(43.6)	1.90 [1.59-2.27]	0.0001
African Arabs	34	(29.3)	82	(70.7)	0.85 [0.63-1.14]	0.257
South Asian	67	(37.6)	111	(62.4)	1.14 [0.92-1.41]	0.251
Far-East Asian	7	(21.9)	25	(78.1)	0.64 [0.33-1.23]	0.140
West Asian	59	(23.8)	189	(76.2)	0.63 [0.50-0.81]	0.0001
European	11	(34.4)	21	(65.6)	1.01 [0.62-1.65]	0.961
Gulf countries	24	(32)	51	(68)	0.94 [0.67-1.32]	0.706
UAE	31	(18.2)	72	(6.1)	0.87 [0.64-1.19]	0.378
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table X Comparison of toxoplasmosis seropositivity between UAE and other nationalities*

	Relative Risk (RR)	95% Confidence Interval (CI)	p-value Significance
Nationalities :			
UAE - Mediterranean Arabs	0.49	[0.36-0.67]	p<0.0001
UAE - African Arabs	0.95	[0.73-1.24]	p=0.694
UAE - South Asian	0.59	[0.44-0.80]	p<0.0001
UAE - Far East Asian	1.70	[1.43-2.02]	p<0.0003
UAE - West Asian	0.66	[0.49-0.88]	p<0.002
UAE - European	1.53	[1.25-1.86]	p<0.002
UAE - Gulf countries	1.14	[0.89-1.46]	p=0.323

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table XI Seropositivity for toxoplasmosis according to the occupation distributions*

Variable	<i>Toxoplasma</i> Positive		<i>Toxoplasma</i> Negative		Relative risk & 95% CI	p value
	n=317	(%)	n=616	(%)		
Occupation:						
Professional	29	(48.3)	31	(51.7)	1.47 [1.11-1.93]	0.015
Office workers	40	(43.5)	52	(56.5)	1.32 [1.03-1.70]	0.042
Field workers	63	(33.3)	126	(66.7)	0.98 [0.78-1.22]	0.834
Farm labourers	19	(22.4)	66	(77.6)	0.64 [0.42-0.96]	0.017
Domestic workers	7	(31.8)	15	(68.2)	0.94 [0.51-1.75]	0.845
Skilled or semi-skilled	159	(32.8)	326	(67.2)	0.93 [0.78-1.11]	0.423
Total	317		616			

*Mantel-Haenszel test was performed for significance difference, Relative Risk (RR) and 95 % Confidence Interval (CI)

Table XII IgM seropositivity amongst blood donors

	IgG+	IgG-	Total
IgM+	2	0	2
IgM-	58	15	73
Total	60	15	75
%	3.3	0	2.7

Fig.14: Distribution of positive and negative cases according to sample source.

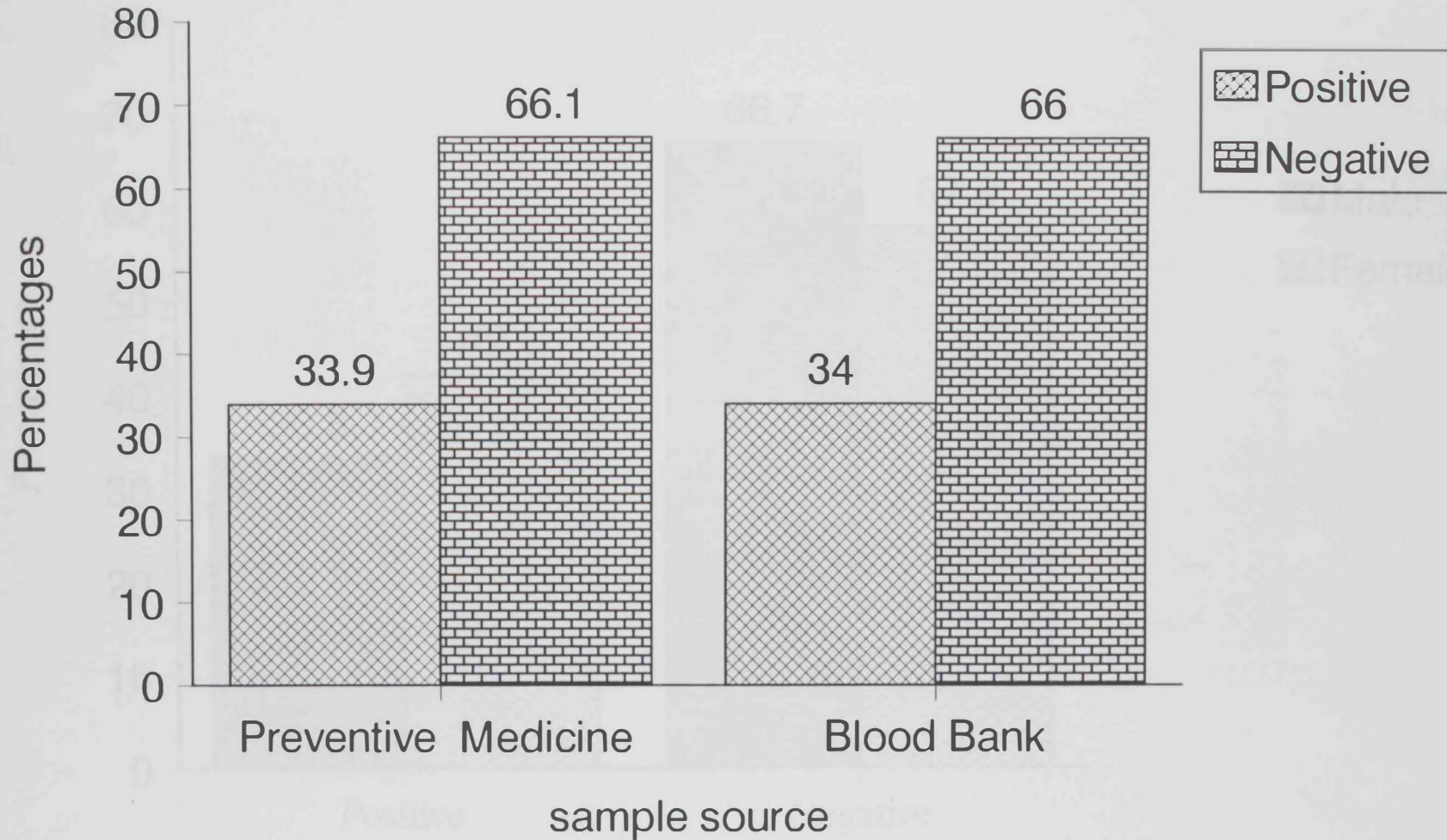


Fig.15: Sex distribution in the positive and negative cases

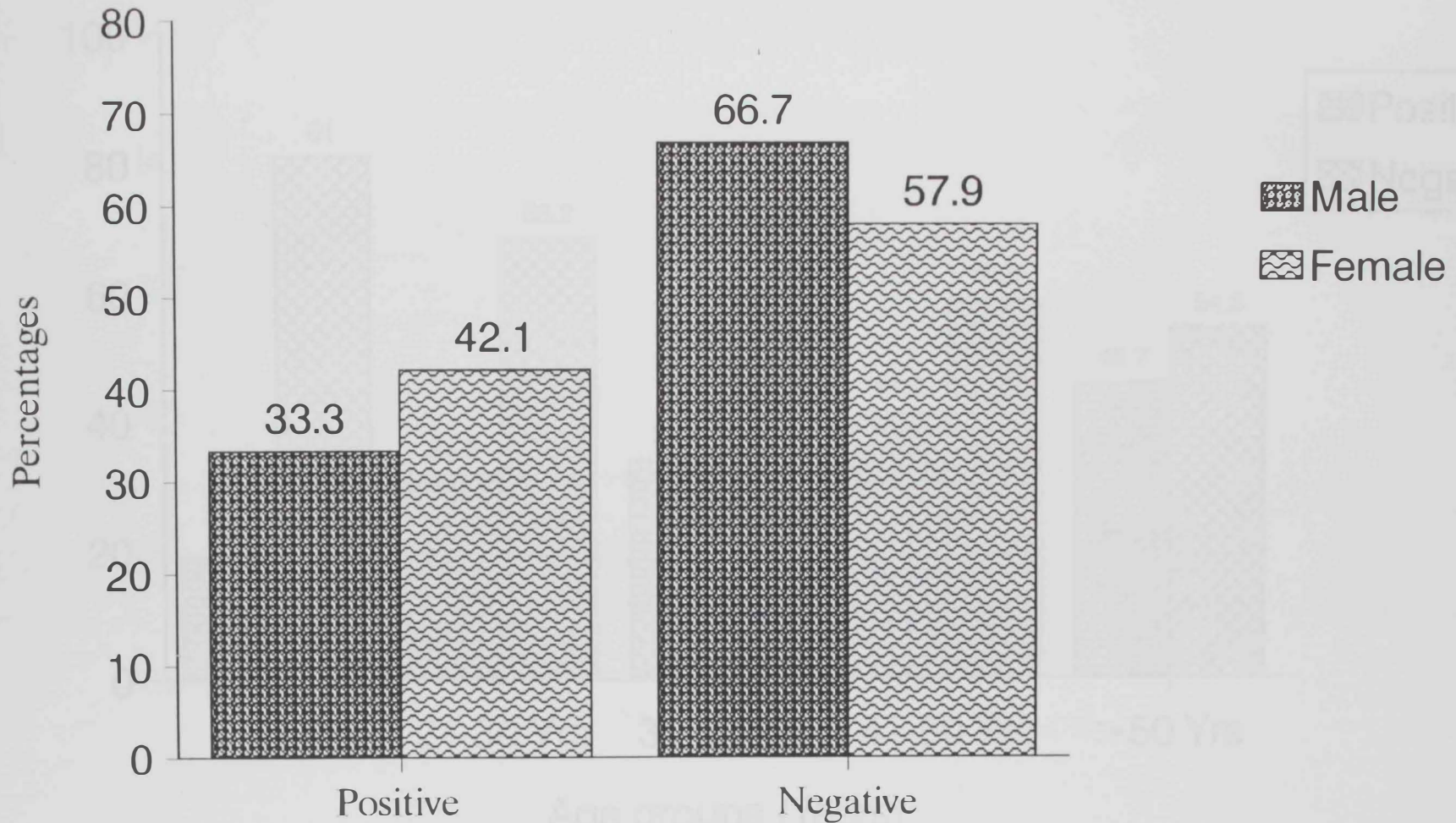


Fig.16: Distribution of positive and negative cases according to the Age Groups

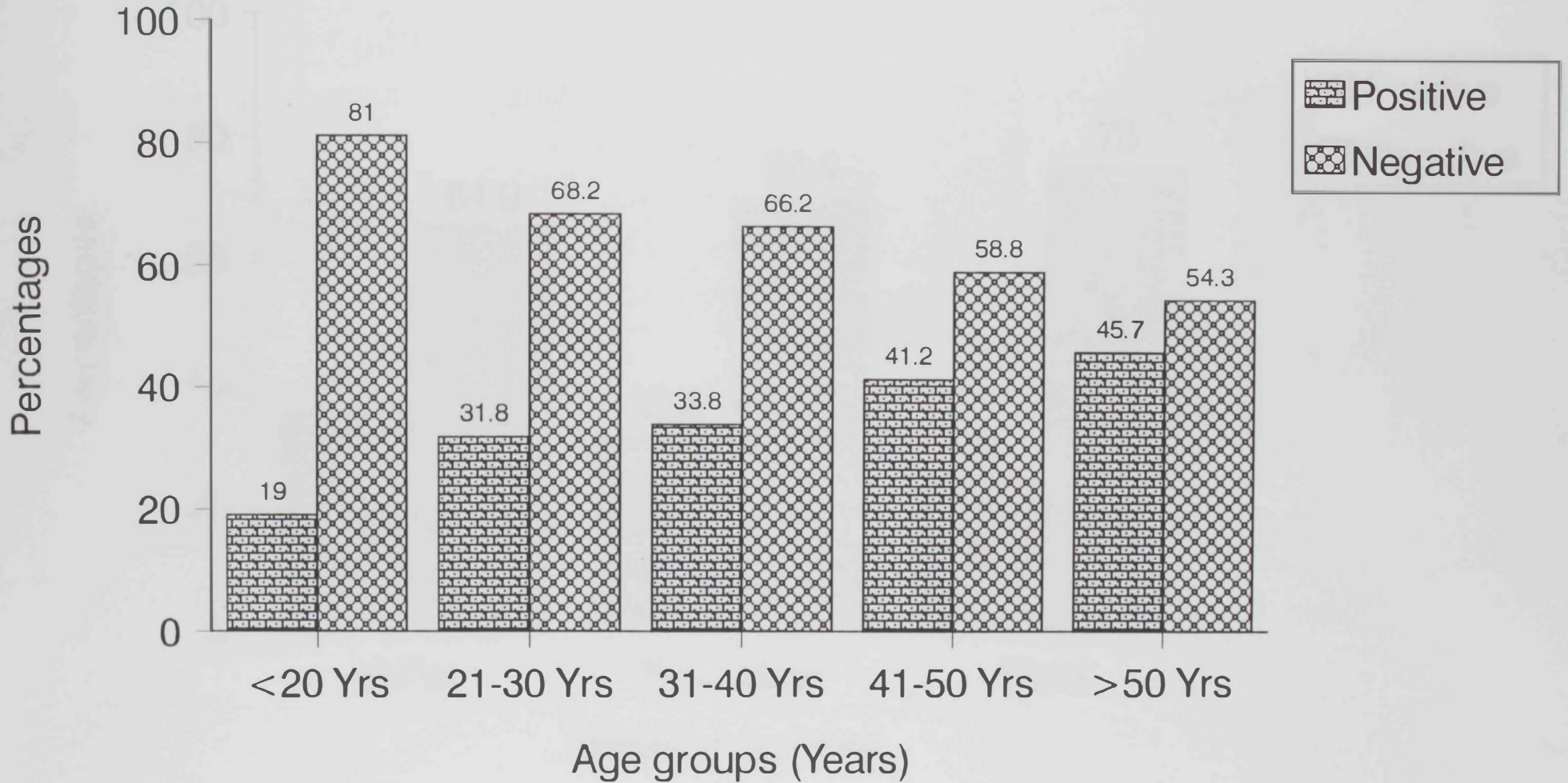


Fig.17: Distribution of positive and negative cases according to the areas of domicile

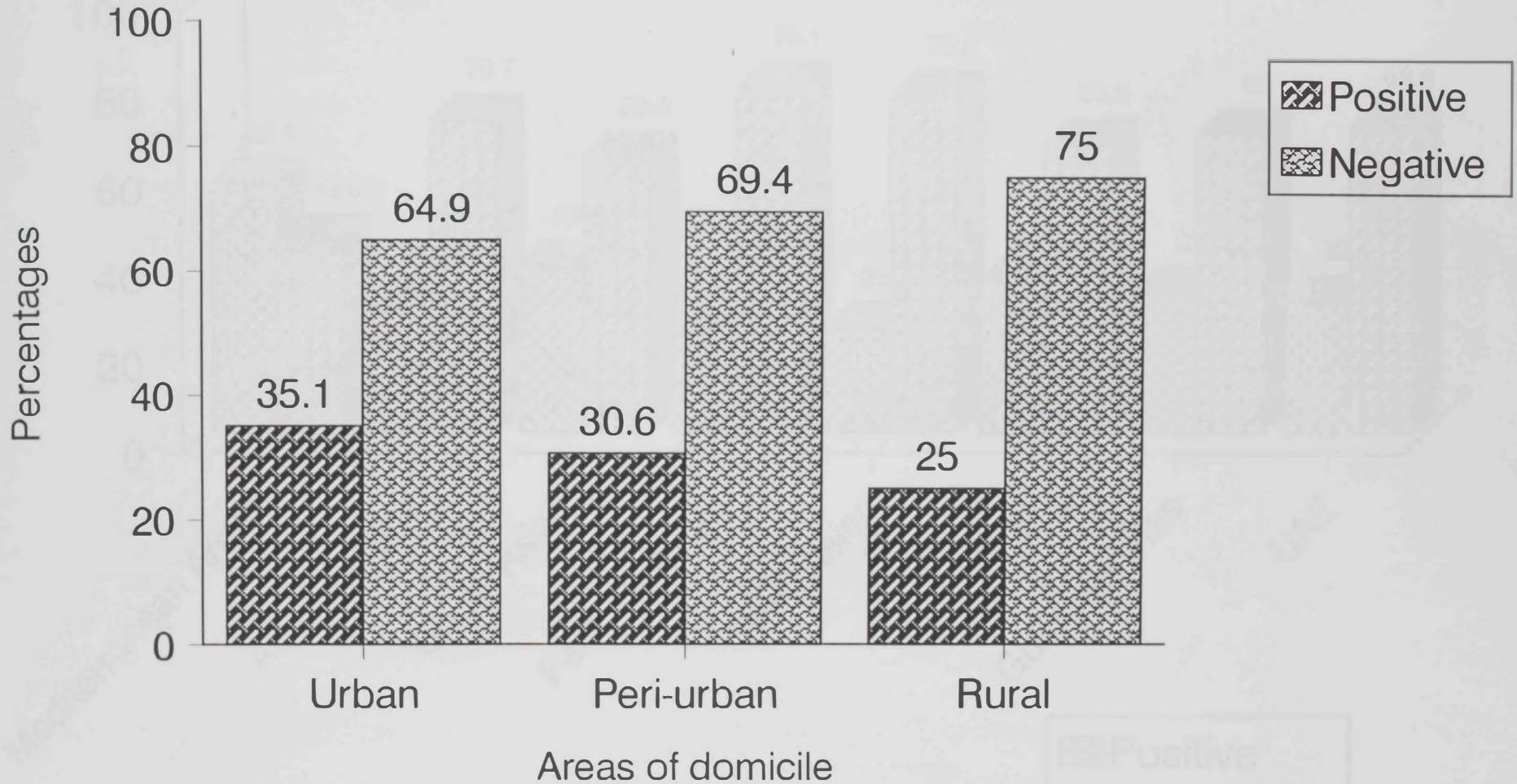


Fig.18: Distribution of Positive & Negative cases according to the Nationality Groups

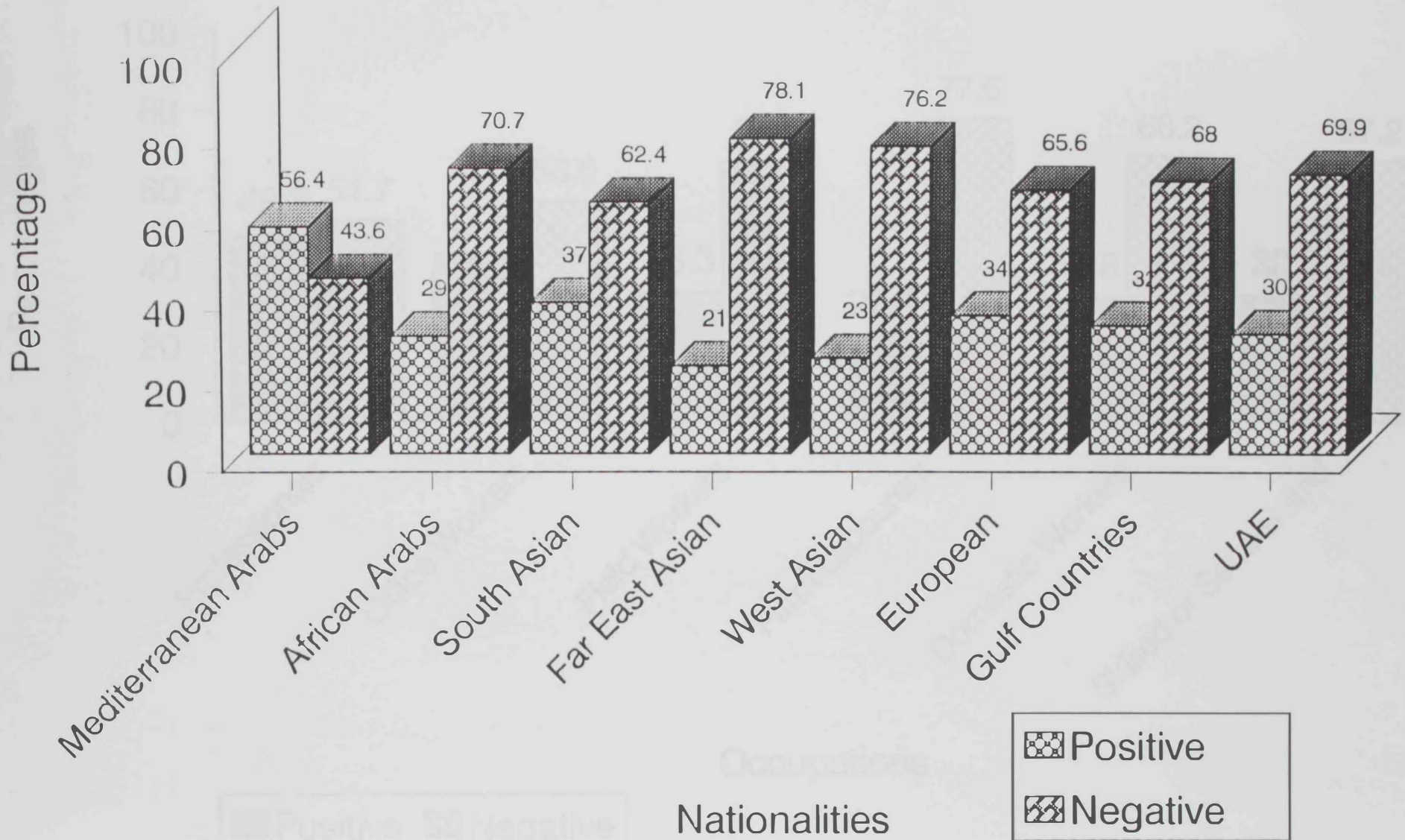
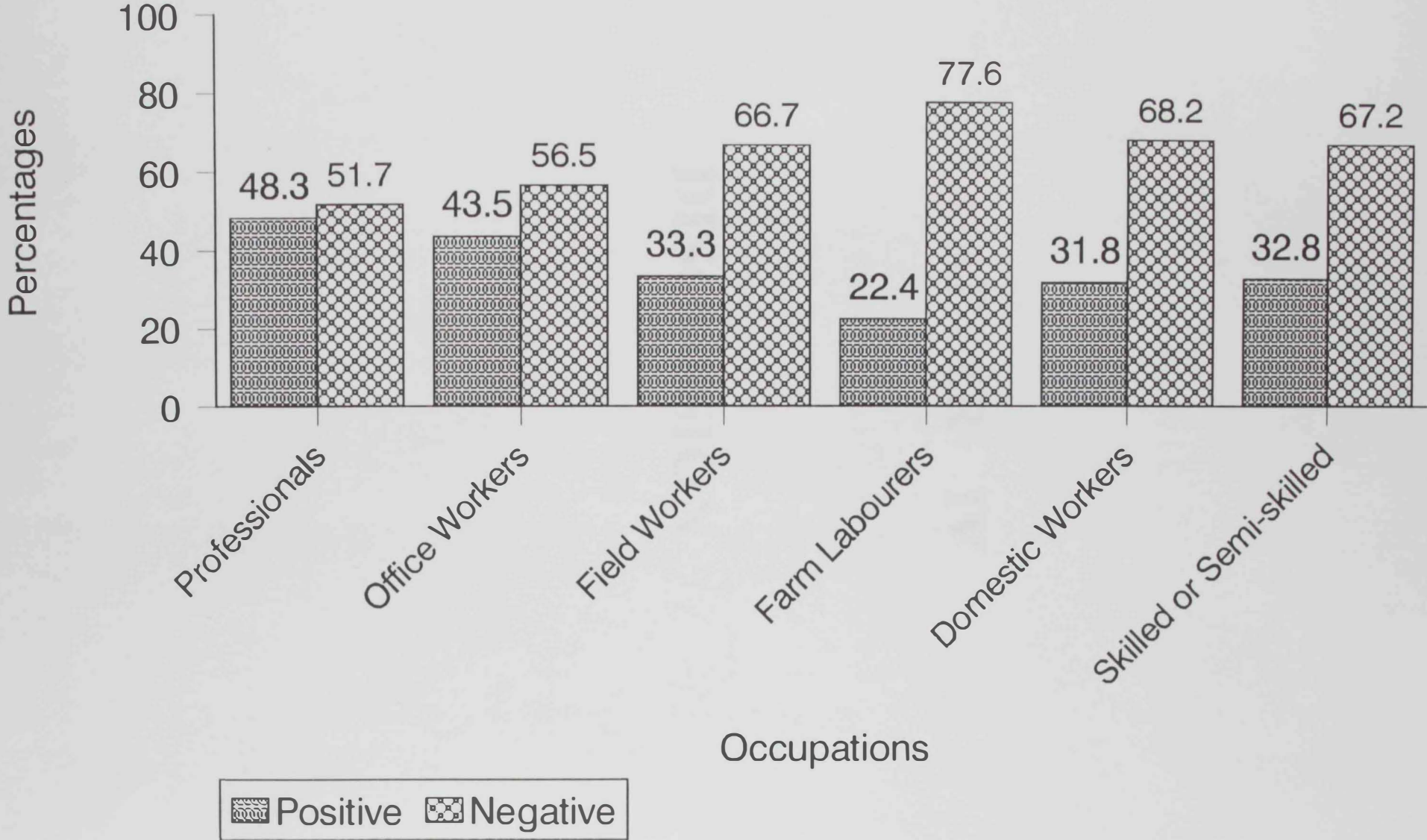


Fig.19: Distribution of Occupations between positive and negative cases



CHAPTER IV

DISCUSSION

IV. DISCUSSION

Toxoplasmosis is a wide-spread disease that occurs in most countries. It is a quite serious infection in both man and animals. It is a systemic disease which may end with grave CNS and ocular complications. Infection to man is through different routes, but mainly through contact with domestic animals (Stray-Pedersen, 1992).

Sero-epidemiologic data on *Toxoplasma* antibody prevalence is important because of its relevance to the strategic approach for preventing toxoplasmosis in the United Arab Emirates. There is very little information available on this subject in the UAE and to my knowledge a cross-sectional prevalence of toxoplasmosis has not been reported before in the UAE. This report is the first research focusing on the immune status to *Toxoplasma* in general UAE population. This study has established the prevalence rate of toxoplasmosis for our area. The incidence of acute toxoplasmosis in UAE population was defined as presence of specific anti *Toxoplasma gondii* IgM antibodies amongst *Toxoplasma* specific IgG positive samples. This study also showed a wide variation of seropositivity to *Toxoplasma gondii* exists amongst the different sections of the UAE population. This could be due to the varying back grounds of the different and many nationalities residing and working in this country. Also, geo-climatic conditions are variable

and may be a factor in the epidemiology *Toxoplasma* infection. The overall prevalence rate of 33.6% is comparable with Saudi Arabia (31.2%) previously reported for the Western Region of Saudi Arabia (Basalamah and Serebour, 1981). Also nearly 37% of all Saudi women possess IgG protective antibody (Abbas et al., 1986). This ranges from a low incidence of 22.8% in Hofuf to a high incidence of 51.2% in Abha. However, the 32.4% found in Jeddah compared with 31.2% previously reported, despite the use of different serological techniques.

The seroprevalence rate of 33.6% of *Toxoplasma* infection among the UAE population is relatively lower than the overall prevalence of 37.4% reported from Saudi Arabia (Abbas et. al., 1986), a neighbouring Arabian Gulf country. The immune status of the population for toxoplasmosis and the prevalence of the disease among subjects varies greatly from country to country. This is apparent even among different population groups of the same country as has been reported in Saudi Arabia by various studies, (Basalamah and Serebour, 1981; Abbas et al. 1986). It has been reported that more than one third of human beings in most areas of the world are infected with toxoplasmosis. Infection rates ranging from as low as 7% were recorded in some countries such as Norway to as high as 94% in other such as Guatemala (Morsy and Michael et al., 1980).

The overall 317 subjects (33.6%) seropositivity rate for *Toxoplasma* IgG antibodies in our region is similar to those reported in some parts of Western European countries, such as UK and USA (Remington and Desmonts, 1990). Our results are in agreement with the findings of Rifaat et al. (1978). However, our results are somewhat higher than those of Shoura et al. (1973), who reported a low prevalence rate of 21% in Saudi Arabia. (Morsy et al., 1977) who reported a high percentages among Jordanian (37.09%). Also, some authors reported higher seroprevalence figures of 87% for the French and 75% for El Salvadorians (Couvreur and Desmonts, 1988).

Differences in the varying prevalence rates may be explained by different serologic methods used in sampling techniques or the period of study. Infections occur through tissue cyst consumption in undercooked meat or by ingestion of vegetables contaminated with oocyst from cat faeces. Risk factors such as the life style of the population studied, the prevailing climatic conditions, and cultural and culinary habits may therefore play a significant role in epidemiology of *Toxoplasma gondii* infection.

With regard to the prevalence rate differences of toxoplasmosis between males and females in the UAE population, the present study revealed that there was no significant difference between males and females.

According to the age groups the highest rates of infection were among more than 50 year age group (42.2%). The lowest percentage of infection was found among the age group less than 20 years (19%). There was a steady increase in the prevalence rate with increased age. This finding is in agreement with those of Frenkel and Ruiz (1981), Barbie et al. (1983), and Romia et al. (1988) who reported that prevalence rate of infection increases with age .

Additionally, social life and economic factor such as income, occupation, housing conditions, health behaviours and life styles may play a role in the infection of the disease. The distribution of our sample population according to domicile shows most of the sample population (86%) was resident in the city of Al-Ain. Results of IgG seropositivity rates amongst inner city (urban), outer city (peri-urban) or isolated (rural) dwellers however, showed no statistically significant differences.

The present study showed that the percentage of infection with toxoplasmosis among expatriates is higher than that among the natives (90.2% and 9.8% respectively). Such higher percentage of infection among expatriates could be attributed to the fact that the families from overseas who are resident in the UAE usually prefer the existence of pet animals in their homes. Natives prefer to keep animals outside their homes. Moreover, they prefer camels, goats, sheep and birds. Such animals do not live in direct contact with natives

and are bred in special areas reasonably far away from human accommodation. Additionally, the cooking methods among foreign families could be another contributing factor leading to infection with such parasites. Also expatriates and their families may have contracted the infection in their home country before arrival in the UAE.

The results of present study showed considerable differences in the seropositivity rates amongst the different sub population groups. Within the total of positive cases, we calculated the percentage of positivity by nationality groups as follows: Mediterranean Arab (26.5%), South Asian (21.1%), West Asian (18.6%), African Arabs (10.7%), UAE (9.8%), Gulf Countries (7.6%), European (3.5%) and Far East Asian (2.2%). However within the population groups the highest seropositivity rate was found amongst Mediterranean Arabs (56.4%) and the lowest among far East Asian (21.9%). Other subgroups like South Asian (37.6%), European (34.4%), Gulf Arabs (32%), UAE Nationals (30.1%) and African Arabs (29.3%) had higher rates than West Asian.

The present study showed a considerable difference in the rate of seropositivity related to different occupations. Results of the IgG seropositivity rates amongst the different occupation groups was surprisingly highest among the skilled or semi-skilled (50.2%), followed by field workers (19.9%), office workers (12.6%), professionals (9.1%), farm labourer (6%), and domestic workers (2.2%). There were very highly significantly differences between professional vs field worker ($p < 0.0001$), skilled-semiskilled vs professionals

($p < 0.0001$) skilled-semi skilled vs farm workers ($p < 0.0001$) and skilled-semi skilled vs office workers ($p < 0.0001$). Within the occupation groups, however, professionals had the highest seropositivity rate (48.3%) and farm labourers the lowest (22.4%).

CHAPTER V

CONCLUSION

V. CONCLUSION

An important finding of this study was the theoretical possibility of transmission of toxoplasmosis through blood transfusion. Previous reports of seroprevalence of toxoplasmosis amongst blood donors have ranged from the low 1.2-4.6% (Morakote et al, 1984) and 7.6-7.8% (Jackson et al, 1987) to a high rate of 42% amongst blood donors in Kenya (Griffin and Williams, 1983). Our own IgG seropositivity rate of 34% amongst blood donors was very close to our general healthy adult population rate of 33.6% suggesting that our blood donor sampling was not biased. Also, serum IgG positivity is regarded as an immune state in toxoplasmosis.

In order to determine the actual risk of transmission it was important to assess the IgM seropositivity amongst blood donors. Depending on the titre this would reflect either current or acute infection or recent infection. Although our sample size was small (75) because of shortage of research funds, the IgM seropositivity rate of 2.7% was significant in that one of the two positive samples showed a high titre. This suggested a significant risk of transmission of toxoplasmosis to susceptible recipients.

The risk of blood or blood products transfusion is well-known for toxoplasmosis (Siegel et al., 1971; Roth et al., 1971), particularly in immunocompromised subjects or pregnant women. Toxoplasmosis may take a

fatal course in the former and cause abortion, stillbirth or severe congenital defects of the baby in the latter case. Our results suggest that the present national policy on blood donors screening needs to be reviewed.

CHAPTER VI

RECOMMENDATIONS

VI. RECOMMENDATIONS

1. The base line data on Seroprevalence of toxoplasmosis amongst various sub groups of healthy adults should help health planners in understanding the magnitude of the problem and to take appropriate measures to save guard the community against the dangerous infection.

2. By establishing a clear risk factor of blood transfusion in transmission of toxoplasmosis, this study opens the doors for an extensive research to obtain definitive guidelines in the development of a national policy on blood bank screening procedures, particularly for recipients who are immunocompromised or women in pregnancy state.

CHAPTER VII

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REFERENCES

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التوصيات

تعتبر هذه الدراسة هامة في تحديد القاعدة الاساسية التي توضح مدى انتشار داء المقوسات بعيار الاضداد (IgG) (الاجسام المضادة لداء المصورات القوسية) بين البالغين . وهي تسهم في اشارة الطريق امام المخططين للمحافظة على سلامة المجتمع من الاصابة بها وذلك من خلال دراسة البيانات المختلفة والسلوكيات التي تنقل العدوى .

كما تفتح هذه الدراسة المجال امام المزيد من البحث العلمي المكثف والمركز للحصول على مؤشرات تتعلق بتطوير السياسة الوطنية الخاصة بفحص الدم في مراكز التبرع بالدم بعد ان كشفت الدراسة بوضوح وجود مخاطر نقل عدوى داء المصورات في الدم اثناء عملية التبرع . خصوصا في حالة المتلقي للدم ممن لديهم (قصور في المناعة) او في حالة السيدات الحوامل .

وترجع هذه الفروق بوضوح إلى الفروق بين البيئات الطبيعية والعادات الخاصة بالطبخ واعداد الوجبات الغذائية والفروق بين معدل التعرض للأخطار المختلفة في ممارسة المهن والانشطة البشرية .

هناك ملحوظة هامة تمخض عنها البحث وهي وجود أضداد (IgM) بين المتبرعين بالدم ، وعلى الرغم من انخفاض المعدل إلى ٢,٧٪ في الشريحة المفحوصة فإن الإحتمال وارد بانتقال الإصابة عن طريق التبرع بالدم .

كما كشفت هذه الدراسة أيضا عن أحد الجوانب الهامة وهو الاحتمال النظري لنقل العدوى ، بينما كانت التقارير السابقة في الماضي تشير إلى وجود نسبة قليلة تتراوح بين ١,٢ - ٤,٦٪ (الدراسة التي أجراها مور أكول وآخرين عام ١٩٨٤ م) . وتتراوح بين ٧,٦ - ٨,٨٪ (دراسة جاكسون وآخرين عام ١٩٨٧ م) لداء المصورات القوسية بين المتبرعين بالدم مع ارتفاع تلك النسبة إلى ٤٢٪ في حالة المتبرعين بالدم في كينيا لدراسة (جريفيتين وويليامز) عام ١٩٨٢ م) .

أما بالنسبة لدراستنا لمعدل الإصابة بداء المقوسات الذي يعكسه ارتفاع أضداد (IgG) فكانت النسبة ٤٢٪ بين المتبرعين وهي تقترب كثيرا من النسبة الموجودة في شريحة السكان التي تصل إلى ٢,٦٪ مما يرجح أن الشريحة التي فحصت بين المتبرعين بالدم كانت تمثل الموقف تماما، كما أن الإصابة بالعدوى (IgG) ينظر إليها على أنها مناعة بالنسبة لداء المصورات القوسية في حالة وجود الإصابة السابقة للمرض أو وجود أضداد المرض .

وكان الأمر المهم بالفعل هو تحديد حجم المخاطرة الحقيقية في انتقال العدوى من خلال أن تقييم نسبة أضداد (IgM) بين المتبرعين بالدم، وهي تعتمد على التركيز (كمية الاجسام المضادة من نوع IgM) في تحديد ما اذا كانت الإصابة حديثة أو مزمنة .. ومع أن العينة التي خضعت للبحث صغيرة (٧٥) بسبب محدودية المبلغ المخصص لتمويل البحث، إلا أن معدل الإصابة هي حالتان (٢,٧٪) وهو معدل محسوس في هذه الحالة حيث كانت نسبة الأضداد عالية .

لقد أصبح من المعروف أن هناك خطرا في نقل العدوى لداء المصورات القوسية من خلال نقل الدم (دراسة سيج ١٩٧١ م . وروث ١٩٧١ م) . خصوصا بالنسبة لأولئك المصابين بأمراض نقص المناعة والسيدات الحوامل ، ومن المحتمل أن تؤدي العدوى إلى نتائج قاتلة، أو تؤدي إلى الاجهاض أو تشوهات الجنين في حالات كثيرة . وترجع دراستنا إعادة النظر في السياسة التي تضعها مراكز التبرع بالدم بالنسبة لفحص دم المتبرع .

ملخص الرسالة

داء المصورات القوسية من أهم الامراض واسعة الانتشار في العالم
ويسببه طفيلي وحيد الخلية يسمى المصورة القوسية الغوندية.. ولانه ليس بالإمكان الحصول على معلومات وبيانات مسجلة وموثقة بخصوص مدى انتشار داء المقوسات في دولة الامارات العربية المتحدة ، فقد أجرينا هذه الدراسة لتشكّل الدعامة الأساسية في هذا المجال، واعتمدنا على فحص شريحة عشوائية من السكان والمقيمين البالغين أولئك الذين تقدموا للحصول على شهادة لياقة صحية بإدارة الطب الوقائي بالعين وأولئك المتقدمين للتبرع بالدم في مركزي التبرع بمستشفيات مدينة العين . وقد استخدمت المواد المعروفة تجارياً باسم (IgM , IgG VIDAS) في اختبار مدى انتشار المرض ، وقمنا بتوزيع استبيان لجمع المعلومات الشخصية من كل متبرع بعد الحصول على موافقته ، ثم قمنا بتغذية كافة البيانات الجديدة إلى الحاسب الالكتروني ومعها نتائج الاختبارات للحصول على تحليل احصائي للبيانات والنتائج .

ولقد وجدنا أن معدل ارتفاع الاضداد (IgG) على المستوى العام قد بلغ ٣٣,٦% مطبقاً على شريحة وصلت إلى ٩٤١ شخصاً، فيما بلغ معدل الإصابة الحديثة المقاسة بارتفاع أضداد (IgM) ٢,٧% مطبقاً على شريحة بلغ عددها ٧٥ شخصاً . نظراً لمحدودية الامكانيات المادية المتاحة لتمويل البحث .

ولم نلاحظ فروقا محسوسة في النتائج التي توصلنا إليها للإصابة بـ IgG من بين الشريحة التي وضعت تحت البحث في ادارة الطب الوقائي وتلك التي أخذت من بنك الدم في كلا من المستشفيات . كما لم نلاحظ فروقا بين الجنسين.. ومع أن سكان الريف قد ظهرت لديهم نسب أقل (٢٥%) مقارنة مع سكان المدينة (٣٥,١%) ، إلا أن الفرق بين النسبتين ليس محسوساً. ولقد زادت نسبة اضرار (IgG) بارتفاع الشريحة العمرية كل حقبة طولها ١٠ سنوات ، وكان المعدل ١٩% لمن هم أقل من ٢٠ سنة في العمر إلى ٤٢,٢% لمن تجاوزوا الخمسين .

كما وجدت علاقة احصائية بين اضرار (IgG) والجنسية في كلتا المجموعتين اللتين تعرضتا للفحص (النسبة ٥٦,٤% أعلى بين الأردنيين والفلسطينيين واللبنانيين والسوريين ، بينما بلغت النسبة بين مواطني الدولة ٢٢,٤%) . أما من ناحية المهنة ، فقد كان من المدهش حقاً ارتفاع نسبة الإصابة بين الذين يمارسون مهنة (الطب ، الهندسة ، التدريس) (٤٨,٢%) ، وانخفاضها بين الذين يعملون في مهنة الزراعة .

بإشراف الأستاذ الدكتور

فضل كـريم دار (دكتورة في الطب)

رئيس قسم علم الأحياء الدقيقة بالإناابة

كلية الطب والعلوم الصحية

جامعة الامارات العربية المتحدة

مدى انتشار داء المصورات القوسية الفوندية
بين الأشخاص البالغين

في مدينة العين
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رسالة مقدمة إيفاء جزئيا لدرجة الماجستير في علوم البيئة

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