TOXOPLASMOSIS IN SOUTHERN AFRICA

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1978

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A Thesis Submitted to the Faculty of Medicine,
University of the Witwatersrand, Johannesburg
for the Degree of Doctor of Philosophy in
Medicine

Johannesburg 1978

This is to certify that, except for portions of Chapter 5 published in collaboration with members of the Parasitology Department, this thesis, 'Toxoplasmosis in Southern Africa' presented for the degree of Doctor of Philosophy in Medicine at the University of the Witwatersrand, Johannesburg, is my own work and has not been presented at any other university.

Michael Roy Jacobs

MICHAEL ROY JACOBS

This thesis is dedicated to my wife,
my parents and my teachers, without
whose help and encouragement it could
not have been completed.

Publications

Parts of this thesis have been published in the following papers:

Serological survey of toxoplasmosis in the Transvaal.
Mason, PR, Jacobs, MR and Fripp, PJ (1974).
South African Medical Journal 48: 1707-1709.

Prevalence of *Toxoplasma* antibodies in Southern Africa.
Jacobs, MR and Mason, PR (1978).
South African Medical Journal 53: 619-621.

ABSTRACT

Since its discovery in 1908, the protozoan *Toxoplasma gondii* has been found to be one of the most versatile and prolific of all parasites. Infections with *T. gondii* have been reported from most parts of the world in humans, animals and birds. *Toxoplasma* has recently been shown to be a coccidian, which has developed from a one-host to a two-host cycle. The definitive hosts are felines, in whose gastro-intestinal tracts the isosporan phase of development takes place. The toxoplasmic phase occurs in many organs of other animal and bird hosts. Transmission occurs via ingestion of oocysts shed by cats or cysts contained in raw or inadequately cooked meat, particularly mutton and pork.

Infections in humans occur at rates of up to 6% per year or higher. The major complication of infection is congenital transmission if acute infection occurs during pregnancy. Maternal infection rates of 10 to 115 per 10 000 pregnancies occur in different parts of the world, with up to 40% of such infections resulting in congenital infections. Fortunately, only about 15% of foetal infections are severe and 20% mild, with the remainder being asymptomatic.

The prevalence of toxoplasmosis varies in different parts of the world, being highest in hot humid climates and lowest in cold or arid climates. In Southern Africa, the overall prevalence found in these studies was 21%, varying from 30% in Natal to 10% in the Orange Free State. Prevalence varied with ethnic group, from 9% in the San (Bushmen) of South West Africa and Botswana to 28% in Coloureds and Indians. Prevalence in Whites was lower than in other ethnic groups in South Africa. The annual incidence of toxoplasmosis in Southern Africa is about 1% up to the age of 25 years, falling thereafter to 0.5%.

The incidence of toxoplasmosis during pregnancy was studied, and 20 acute infections were detected in 6705 pregnancies (0.03%) in 3 Johannesburg hospitals. Congenital transmission occurred in 2 of these 20 patients, and resulted in 1 severely affected and 1 asymptomatic infant. This congenital transmission rate of 10% is low compared to up to 40% found in other countries.

Transmission of toxoplasmosis in Southern Africa appears to involve both cysts and oocysts, although oocysts appear to be more important in the younger age groups. Virulence and congenital transmission rates are low. The estimated number of maternal infections in South Africa is 2 500 per year, resulting in 250 congenital infections. Thirty-eight of these congenitally infected babies would have severe infection, 48 mild infection and 165 would be asymptomatic.

If toxoplasmosis could be prevented, 1 to 2 severe infections per 16 500 births could be avoided. Unfortunately, there are at present no measures such as immunization available, and the only control measures advocated are the avoidance of sources of cysts (raw meat) and oocysts (soil contaminated with cat faeces) during pregnancy.

Screening programmes for the detection of acute toxoplasmosis during pregnancy would be very difficult and expensive to undertake, and there are many far more important diseases which should receive public health pricrity. Fortunately, the incidence of toxoplasmosis during pregnancy and the congenital transmission rate are particularly low by world standards. Until vaccination or other practicable preventive measures are developed, little can be done to prevent or treat toxoplasmosis during pregnancy.

ACKNOWLEDGEMENTS

This thesis was completed in the Department of Microbiology (Parasitology) of the School of Pathology of the South African Institute for Medical Research and the University of the Witwatersrand under Professor H J Koornhof, who was my supervisor. Grateful acknowledgement is made to the many members of staff of the School of Pathology for their assistance. In particular I would like to thank Dr P J Fripp for his interest and Dr P R Mason who collaborated with me in undertaking the serological surveys.

Due acknowledgement is made to the South African Institute for Medical Research for supporting my work and the South African Medical Research Council for a 3-year grant in support of the study on toxoplasmosis during pregnancy.

The expert technical assistance of Mrs M S du Plessis is gratefully acknowledged, as well as that of Mrs E Ross and Mrs T Jagiello, who assisted me during various periods. The help of Mrs J Nkosi, Mrs P Chakane, Mrs Z Kumalo, Mrs G McStay, Mrs J Cansfield and Mr B Caydon in the collection of specimens was invaluable.

For the serological surveys I am indebted to the Directors of the Natal, Eastern Province and Western Province Blood Transfusion Services, and Prof T Jenkins and Prof M Isaäcson, who provided sera for examination. I am grateful for the assistance of Prof L van Dongen, Dr R Marcus and Dr M Russel in carrying out the pregnancy study, and for permission from the superintendents of the Queen Victoria, Baragwanath and Coronation Hospitals to undertake this study.

I wish to thank Drs J S Dahms, M D Davis, D Lewis, N Livni, A C Mauff and R Sacchi for referring the cases of congenital and acquired toxoplasmosis to me for investigation. I also wish to thank Dr G V S Turner of the Onderstepoort Veterinary Research Institute for providing specimens from sheep for examination.

For the production of this thesis I wish to thank Mrs C M Watson and Mrs M Anderson of the Epidemiology Department for drawing the figures and illustrations, Mrs M Ulrich and Mr M Anderson of the Photographic Department for the photography, Mrs G E Dique and Mrs K Myburgh for typing the manuscript and Mr H Peyper for the printing. I also wish to thank Dr J K Frenkel for permission to reproduce figures 7.2 and 7.3, which were published in BioScience 23:343, 1973.

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1.0 REVIEW OF TOXOPLASMOSIS

1.1 Introduction

Despite its still being considered an 'exotic', 'rare' or 'tropical' infection, toxoplasmosis is one of the commonest infections of man and animals throughout the world. These infections are with the protozoan parasite, *Toxoplasma gondii*, which is found in many species of animals and birds. The definitive host of *T. gondii* is the cat family, where the coccidian nature of this parasite was discovered (Cahill, 1974).

T. gondii is the only species in the genus *Toxoplasma*, and it takes its generic name from the Greek 'toxon' meaning bow or arc and its specific name from the small North African rodent, *Ctenodactylus gondi*, which was used as a laboratory rodent by the Pasteur Institute in Tunis. Following the first descriptions of the parasite early this century, the universal nature of *Toxoplasma* infections has become apparent, and much effort has been expended throughout the world on investigating this parasite (Frenkel, 1971).

Since the first reports of human and animal toxoplasmosis 70 years ago, much has been learned about this organism, its modes of transmission and the diversity of disease patterns produced in man. It has been estimated that 500 million humans have evidence of infection, and the fact that congenital transmission can occur, raises the problems of the extent of the risk of congenital infection and the possibility and practicality of the development of a vaccine for human use (Kean, 1972).

1.2. History

In 1908 *T. gondii* was described by workers in both North Africa and Brazil. Gondis used at the Pasteur Institute of Tunis as laboratory rodents for leishmaniasis research were found in 1907 to be dying of a new infection by Nicolle and Manceaux (1908). The organism was also described by Splendore (1909) in laboratory rabbits in São Paulo, Brazil. These workers considered the parasites to be either *Piroplasma* or *Leishmania*.

Also in 1908, the first probable case of human toxoplasmosis was reported from Panama (Darling, 1908). Parasites were observed in muscle biopsies, but were mistaken for *Sarcocystis* in Darling's report 'Sarco-

sporidiosis: with report of a case in man'. This case was regarded by Kean (1972) as being classical of asymptomatic toxoplasmosis of muscle. In 1923, the first human case of congenital toxoplasmosis was described by Janku (1923) in Prague. The diagnosis was made by the observation of parasites in retinal sections of an infant who had microphthalmia. Large numbers of rabbits were bred on the farm of this child's parents, and the mother had frequently eaten rabbit meat during her pregnancy. Janku considered the parasites seen in the child's eye to be coccidia resembling *Eimeria*, a pathogen of rabbits, and that transmission had been transplacental.

In 1939, the first report of the isolation of the parasite by animal inoculation of tissues from a neonate with encephalitis was published by Wolf *et al.* (1939). Until this report, *Toxoplasma* was considered to be somewhat of a nuisance in laboratory rodents used for virological studies, and this report firmly established the role of *Toxoplasma* in human disease. It was also the first example of an infectious agent producing foetal disease *in utero*, the association of rubella with intrauterine infection only being considered two years later (Feldman, 1974). Toxoplasmosis of childhood was characterised by Sabin (1942), and in adults by Pinkerton and Henderson (1941). *Toxoplasma* retinochoroiditis was recognized in adults by Wilder (1952), and organisms first isolated from the eye by Jacobs *et al.* (1954). Relapsing toxoplasmosis due to immunosuppression was recognized in corticosteroid treated hamsters, and later described in man (Frenkel, 1956).

Sabin in 1942 described the first serological test for toxoplasmosis using inhibition of rabbit skin necrosis by organisms exposed to sera containing antibodies (Sabin and Ruchman, 1942). In 1948, the Sabin-Feldman dye test (Sabin and Feldman, 1948), complement fixation test (Warren and Russ, 1948) and toxoplasmin skin test were described (Frenkel, 1948).

Despite the disease in man being well characterized, the modes of transmission remained largely obscure until 1969, when *Toxoplasma* was recognized by Frenkel *et al.* (1969) as an intestinal coccidian of cats, which are the definitive hosts, with many intermediate hosts, including man. This discovery followed the work of Hutchison (1967), who suggested that transmission occurred in nematode ova from the intestinal tract of cats. Although transmission was later shown to occur independently of

nematode ova, Hutchison's work led to the establishment of cats as the definitive host of *Toxoplasma* and to the role of cats in the maintenance of infection in herbivores. Evidence suggestive of oocyst transmission to humans was obtained in an outbreak of toxoplasmosis traced to cats in a horse-riding stable (Francis *et al.*, 1977).

Some of the above and other historical highlights of toxoplasmosis, modified from Frenkel (1973a), are shown in Table 1.1.

TABLE 1.1. Highlights of the history of *T. gondii* and toxoplasmosis, based on Frenkel (1973a)

1908	Discovered in gondi and rabbit (Nicolle & Manceaux; Splendore) First probable human case (Darling)
1923	Identified in eye of child at autopsy (Janku)
1937	Intrauterine transmission and neonatal encephalitis recognized (Wolf & Cowan)
1942	First comprehensive review (Sabin)
1945	Cysts recognized in asymptomatic humans (Kean & Grocott)
1948	Dye test described (Sabin & Feldman) Toxoplasmin skin test described (Frenkel)
1949	*Toxoplasma* retinochoroiditis in adult described (Frenkel)
1952	*Toxoplasma* identified in retinas of 50 human cases (Wilder) Glandular toxoplasmosis recognized (Siim)
1953	Synergism of sulphadiazine and pyrimethamine recognised (Eyles & Coleman)
1954	*Toxoplasma* isolated from eye with retinochoroiditis (Jacobs *et al.*)
1957	Folinic acid used to counteract side effects of therapy (Frenkel & Hitchings)
1959	Repeated congenital transmission described in mice (Beverley)
1960	Meat suggested as important source of infection (Jacobs *et al.*)
1965	Human infection rate increased by raw meat diet (Desmonts *et al.*)
1968	Cat nematode ova dissociated from transmission (Frenkel *et al.*, Hutchison *et al.*)
1970	Sexual cycle in cats discovered (Frenkel *et al.*, Hutchison *et al.* and others)
1972	Confirmation of epidemiologic role of cats from island studies (Wallace; Munday)
1974	Definitive information on the role of *Toxoplasma* in pregnancy and the risk of congenital infection (Desmonts & Couvreur)

1.3. Classification

Although the taxonomic status of *Toxoplasma* has not been finalized, provisional classification is as follows:

Phylum	-	PROTOZOA
Subphylum	-	SPOROZOA
Subclass	-	COCCIDIA
Family	-	TOXOPLASMIDAE
Genus	-	*TOXOPLASMA*
Species	-	*gondii*

T. gondii is the only species recognized in the genus *Toxoplasma*.

Other families in the subclass Coccidia include Besnoitiidae and Sarcocystidae, and they can be differentiated as follows (WHO, 1969):

Toxoplasmidae. Cysts with thin membranes and pseudocysts. Naked zoites possess a vesicular nucleus, a conoid, toxonemes and a micropyle but no flagella or cilia. Reproduction is by endodyogeny. Locomotion is by subpellicular fibrils.

Besnoitiidae. Cysts with thick, laminated, nucleated walls and pseudocysts. Naked zoites, reproduction and locomotion as for toxoplasmidae.

Sarcocystidae. Elongated cysts, often septate and with cytophaneres. Elongated naked zoites possess a vesicular nucleus, a conoid, toxonemes, a zone of central granules, and a micropyle, but no flagella or cilia. Reproduction and locomotion as for toxoplasmidae.

1.4 Definition of terms

Terms used are based on recommendations contained in the report of a WHO meeting of investigators (WHO, 1969) as well as those of Zigas and Benfante (1972), Hoare (1972) and Jacobs (1974).

1.4.1 Tissue stages

Endozoite (tachyzoite). The individual organism inside the pseudocyst produced as the result of asexual division (endodyogeny).

Cystozoite (bradyzoite). The individual organism inside a cyst resulting from endodyogeny.

Pseudocyst. The collection of endozoites within a vacuole inside the host cell.

Cyst. A collection of cystozoites enclosed within a tough, resilient, dense wall laid down on the internal membrane of the host cell vacuole.

Cysts may eventually become extracellular because of distension and rupture of the host cells.

Endodyogeny. The production of two merozoites within the original surface membrane of the mother organism, commencing with the formation *de novo* of two conoids and the division of its nucleus.

1.4.2 Intestinal epithelium stages

Trophozoite. The form of parasite which grows and prepares for schizogony in intestinal epithelial cells.

Schizont. The form which produces merozoites by multiple formation of cytoplasmic components and nuclear replication.

Merozoite. Individual product of schizogony.

Gametocyte. Precursor of male or female gamete.

Gamete. Male or female product of gametogony.

Zygote. Product of fertilization of female gamete by male gamete.

Oocyst. Zygote with a heavy protective wall.

Sporont. Zygote undergoing division into sporoblasts.

Sporoblast. Derivative of sporont undergoing further division into sporozoites.

Sporozoite. Product of division of sporoblast.

1.4.3 Hosts

Host. Vertebrate susceptible to infection with *T. gondii*.

Definitive host. Host in which sexual reproductive cycle occurs in intestinal tract.

Intermediate host. Host in which only asexual reproductive cycle occurs in extra-intestinal tissues.

Apparent host. Animal in which *T. gondii* produces a clinical syndrome.

Dead-end host. Man, who does not usually serve as a source of infection for other animals.

1.5 Parasitology

1.5.1 Morphology

T. gondii proliferative forms (endozoites) under the light microscope resemble those of other Sporozoa. However, unlike other Sporozoa which are host-specific, *Toxoplasma* is capable of parasitising not only a wide range of hosts but also a wide range of organs and cell types in these hosts. Like other Sporozoa, *Toxoplasma* has a sexual cycle in a definitive host, which for *Toxoplasma* is the cat family (Frenkel, 1970).

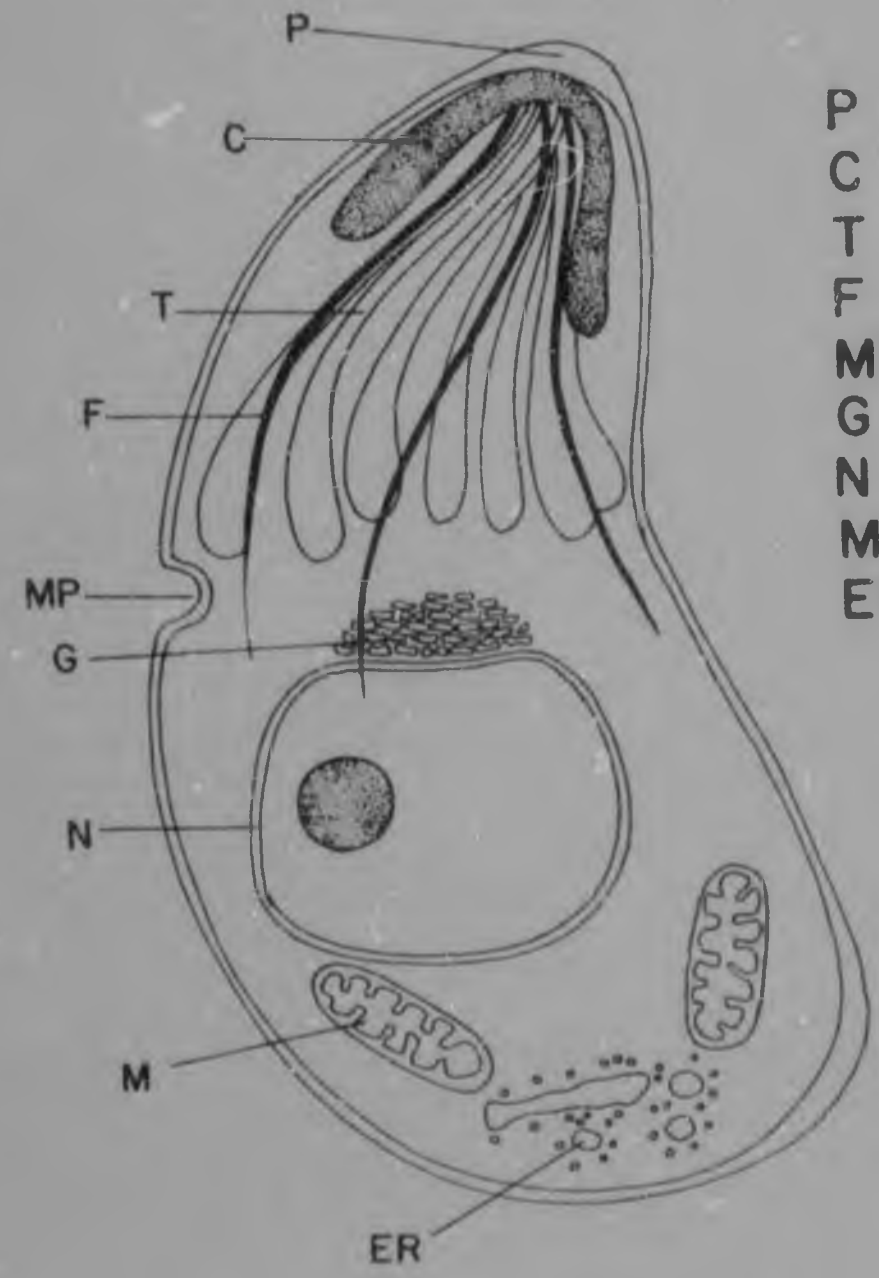
The form of *T. gondii* found during the acute infection of a host is a slender, arc-shaped endozoite, 2 to 4 µm wide and 4 to 7 µm long, with one end more tapered than the other. It can move slowly with its narrow anterior end describing a circular motion, and resembles a merozoite of malaria when stained with Giemsa. A reddish nucleus and light blue cytoplasm with occasional cytoplasmic granules can be seen.

Electron microscopy shows a complicated system of organelles. The parasite is enclosed in a double-unit membrane. The inner membrane is continuous except for a slight thickening called the polar ring which surrounds the conoid at the anterior end and a similar thickening at the posterior end. Fibrils extend longitudinally between these thickenings and are probably responsible for the gliding movements of the parasite. Within the anterior polar ring is the conoid, a conical organelle made up of tubules wound in a spiral. The posterior end of the conoid appears open, and 4 to 8 paired organelles called toxonemes extend posteriorly to the Golgi body just anterior to the nucleus. A number of micronemes also extend posteriorly from the conoid to the nucleus. The nucleus is in the posterior half of the organism, surrounded by a double membrane with pores in it. The cytoplasm contains rough endoplasmic reticulum, free ribosomes, mitochondria and glycogen vacuoles. At the level of the anterior end of the nucleus is a micropyle which may function as a cytostome (Jacobs, 1974). A diagramatic illustration of the organism is shown in Fig. 1.1.

1.5.2 Life Cycle

Toxoplasmic phase: *T. gondii* endozoites reproduce by a process called endodyogeny, originally described by Goldman *et al*, (1958) as an internal budding process. Two cones of heavily staining material consolidate near the nucleus, and 2 new nuclei form underneath these cones. Eventually the cones and daughter nuclei are surrounded by new cell membranes and break out of the maternal cell, destroying it. This process resembles the type of reproduction seen in Sporozoa, and is considered to be a special form of schizogony called endodyogeny in which only 2 progeny are formed.

In chronic infections, organisms persist for years as cystozoites in cysts. The cyst wall is a dense matrix of material originally deposited on the inner membrane of the host cell vacuole containing the parasites. Rough endoplasmic reticulum and mitochondria of the host cell are concen-



P Polar ring
C Conoid
T Toxonemes
F Fibrils
MP Micropyle
G Golgi body
N Nucleus
M Mitochondria
ER Endoplasmic reticulum

Fig. 1.1 Schematic diagram of the electron microscopic morphology of *Toxoplasma gondii* showing intracellular organelles.

trated outside the vacuole and may contribute to the cyst wall substance. The cyst wall substance eventually extends between multiplying parasites, and becomes a true parasitic cyst. Organisms within a cyst continue to reproduce by endodyogeny, but more slowly, and cysts can reach sizes of 100 µm or more in diameter. Cyst development is independent of the host's immune response. Cystozoites are resistant to digestion by gastric juice, whereas proliferative forms of organisms derived from infections in the acute phase are immediately destroyed by gastric juice. Cysts dissolve rapidly in gastric juice, but the cystozoites released are capable of survival for up to 3 hours.

Within its many hosts, *T. gondii* proliferates intracellularly in almost every organ. Parasites may appear briefly free in the blood, and may later appear in leukocytes. In chronic infection, cysts may be demonstrated in brain tissue, skeletal, cardiac and smooth muscle, and in various other organs.

After ingestion, cyst digestion occurs in the stomach with the release of gastric juice-resistant forms which can invade the intestinal epithelium. In animals other than the cat family, endodyogeny occurs, while in felines both endodyogeny and schizogony can occur.

Isosporan Phase: The development of intestinal forms proceeds in the epithelium of the feline jejunum, ileum and colon, in 2 stages - asexual development and gametogony (Jacobs, 1974).

Asexual Development: This starts from 12 hours after cyst ingestion and continues for up to 15 days. Organisms penetrate intestinal epithelial cells, grow and become rounded trophozoites which develop into schizonts containing 4 to 24 merozoites. The formation of cytoplasmic elements lags behind nuclear division in this process. Merozoites are released from host cells and can re-invade new cells and repeat the progress of schizogony. The number of generations of merozoites produced is unknown.

Sexual Development: Gametogony then occurs with the invasion of host cells by merozoites which develop into micro- and macrogametocytes. Microgametogony occurs with nuclear multiplication; each nucleus migrates into surface protruberances in which 2 flagella develop from a basal body. Six to 32 microgametocytes are produced. Microgametocytes are 3 to 5 µm long with flagella 6 to 10 µm long. Macrogametocytes are produced from merozoites, and are 13 µm in diameter when mature.

Microgametocytes are released into the intestinal lumen with fertilization of macrogametocytes in the small bowel epithelium. After fertilization, the zygote lays down the oocyst wall and sporogony begins. The oocyst is shed unsporulated, and measures 10 by 12 µm. After a period in the environment, 2 sporoblasts form, which develop into 2 sporocysts each containing 4 sporozoites.

After ingestion of *Toxoplasma* cysts by a cat, oocysts are shed after a prepatent period of 4 to 5 days, and shedding occurs for 9 to 20 days. After ingestion of endozoites the prepatent period is 5 to 19 days, and shedding occurs for 2 to 12 days. Cats are relatively resistant to infection with oocysts, and large numbers are required to produce infection. Prepatent period with oocysts is 21 to 49 days, and shedding occurs for 5 to 8 days (Wallace, 1973).

As well as the domestic cat, the bobcat, ocelot, jaguarundi, cougar and Asian leopard have been shown to shed oocysts. Oocysts are infectious to many species of animals and birds, and probably account for infection of herbivores (Frenkel, 1973a).

Toxoplasma appears to represent the development from the usual one-host coccidium to a two-host cycle. Infection can be perpetuated by carnivorism, with only the toxoplasmic phase of reproduction occurring. The life cycle of *T. gondii* is shown schematically in Fig. 1.2.

The isosporan phase of asexual development and gametogony has only been described in the gut of cats, and is host specific (Frenkel, 1971). However, two other intestinal coccidia of cats, *Isospora felis* and *I. rivolta* have also been shown to infect extra-intestinal tissues in cats and to parasitise extra-intestinal tissues of rodents (Frenkel and Dubey, 1972). The life-cycle of *Toxoplasma* is certainly not unique among the coccidia, and strengthens its inclusion in this subclass of Sporozoa.

1.5 3 Transmission

Toxoplasma appears to have originated as an intestinal coccidian of cats, with faecal-oral transmission. The formation of persisting cysts in tissues such as brain and muscle has made it possible for carnivorism to become an additional means of transmission. Animals and birds can become infected by either oocysts or cysts, the cat being the definitive host, while other animals and birds are facultative intermediate hosts (Frenkel, 1971).

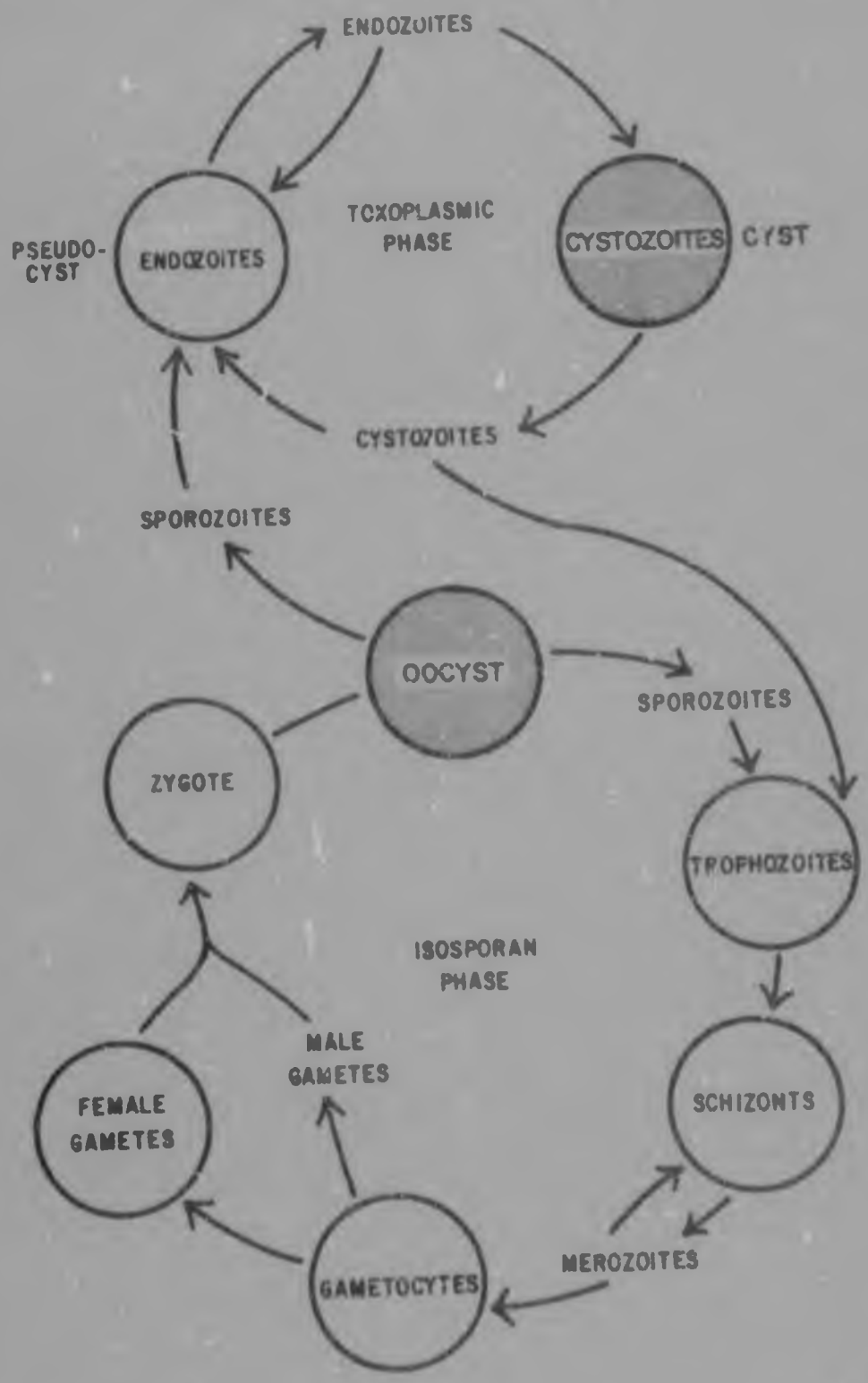


Fig. 1.2. Life cycle of *Toxoplasma gondii*, showing isospo[illegible]nase which only occurs in felines and toxoplasmic [illegible]e which occurs in all animals and in birds. Intrace[illegible]ular stages are in circles and resistant stages are in shaded circles.

Carnivorism. This appears to be an important mode of transmission. Toxoplasmosis has been shown to be rapidly acquired in institutions serving undercooked beef or horse meat, and an epidemic of toxoplasmosis from eating undercooked hamburgers was described by Kean *et al.* (1969) in New York. In this outbreak five students who all ate rare hamburgers at the same place and at the same time developed acute lymphadenopathic toxoplasmosis with high and rising Sabin-Feldman dye test titres and positive complement fixation tests. The meat involved was ground beef which could have been contaminated with pork. The incubation period in this outbreak was 8 to 13 days, and the patients presented with fever, rash and lymphadenopathy. A further 24 seronegative students who ate hamburgers at the same time did not become infected, either as their meat was adequately cooked or the number of cysts was scanty or irregularly distributed. In France, institutionalized children fed undercooked beef or mutton for therapeutic purposes seroconverted at a very high rate, indicating these as sources of infection and probably providing the explanation for the high seropositivity rate in France (Desmonts *et al.*, 1965a).

Transmiss[illegible] by carnivorism appears to be due to ingestion of cysts from chronically infected animals, and pork and mutton appear to be infected more frequently than beef (Jacobs, 1967; Work, 1971). T[illegible] prevalence of *Toxoplasma* antibodies in sheep varies, with a maximum of 90 percent positives in Yorkshire, England. In swine, 11 to 66 percent have been found to be seropositive, and up to 50 percent of cattle are seropositive, cattle tending to have lower titres. Digestion of skeletal muscle samples from animals with artificial gastric juice and subsequent mouse inoculation has resulted in isolation of *Toxoplasma* from sheep and swine in many parts of the world. Beef is less l[illegible]y to contain *Toxoplasma* cysts, but the epidemic reported by Kean *et al.* (1969) was probably transmitted via beef.

Faecal. The oocyst is now known to be the method of transmission from cats. Hutchison (1967) first transmitted the infection from cat faeces in the presence of ova of *Toxocara cati*, in which he thought the *Toxoplasma* was transmitted. In 1969, Frenkel *et al.* (1969) separated *Toxoplasma* from *Toxocara*, and in 1970 several workers described the oocyst of *T. gondii* and demonstrated it to be the infective form of this parasite (Frenkel, 1973a). Oocysts are infectious to all animals and survive passage through the stomach.

In 1977, one of the largest outbreaks of acute toxoplasmosis in the USA occurred in Atlanta, Georgia, and further evidence of direct trans-

mission from cats to man via oocysts was postulated (Francis *et al* 1977). In this outbreak, 29 riding-stable patrons developed fever, headache and lymphadenopathy over an 8-week period, and 28 showed serological evidence of acute toxoplasmosis. Five asymptomatic infections were also diagnose[illegible]logically. Cats from the stable were bled, and 7 out of 3 were se[illegible]sitive. Transmission was thought to be by inhalation or ingestion o[illegible]ocysts from cat faeces in the extremely dusty conditions in the riding r[illegible] (Walls, 1978).

Transplacental: This [illegible] first recognized in man (Sabin, 1942), and has also been observed in mice, rats, guinea pigs, dogs, sheep and mink. Transplacental spread in man usually only occurs if primary maternal infection occurs during pregnancy. Discussion of the importance of this method of spread is presented in Chapter 2.

Accidental: Laboratory workers have become infected after accidental needle inoculation or splashing infective material into the eyes (Frenkel, 1973b).

Organ transplantation and blood transfusion: Occasional cases have been described, mainly in immuno-suppressed donors or recipients (Frenkel, 1973b).

Role of arthropods: Various arthropods have been shown to be capable of mechanically transmitting oocysts on their feet or disseminating them after ingestion, but their role in transmission is considered to be minor (Frenkel, 1971).

Birds and eggs: Chicken eggs have rarely been found to be infected with *Toxoplasma*, and organisms can be isolated from the ovary and oviducts of hens (Jacobs and Melton, 1966). The meat and eggs of birds, eaten in some Alaskan communities, may be a source of human infection (Peterson *et al.*, 1974).

Milk: Organisms have been isolated or transmitted from milk in mice, cows, pigs, dogs, cats, goats, guinea pigs, rabbits and sheep (Frenkel, 1973b). Infected milk may occasionally transmit toxoplasmosis in animals, but pasteurization will destroy all forms of the organisms (Frenkel, 1973b).

Other: Whether additional methods of transmission occur and are important, especially in man and herbivores, is still unknown. Feldman (1974) has stated that 'after we have considered meat and [illegible]ats, we are left with a large, if not major, proportion of human infections which are unexplained'.

1.6 Resistance of *Toxoplasma* to physical and chemical agents

The ability of *T. gondii* to survive in the environment is of critical importance with regard to its spread. In studies conducted by Work (1971), naturally infected pork was studied. He found that normal cooking procedures such as frying and roasting as well as slight salting and low temperature smoking killed cysts. Freezing at -20°C for 24 hours was also sufficient to kill the cysts. In the animals studied, all parts of the raw carcass were infective. Tasting of raw meat during food preparation by housewives is thought to be important in transmission from infected animals.

Survival of *Toxoplasma* endozoites and cysts has been studied by many workers, and details are shown in Table 1.2 (Work, 1971). Oocysts are the most resistant forms, but heating to over 45°C, as well as drying, formalin, ammonia and iodine will kill oocysts or prevent them from sporulating and becoming infectious (Frenkel, 1973b).

1.7 Pathology

The range of infections caused by *T. gondii* varies from asymptomatic to fatal. Factors contributing to pathogenicity include the capacity to invade cells and to persist causing chronic infection. *Toxoplasma* is capable of infecting most nucleated cells, including nucleated red cells of birds. The parasite can multiply after invading cells as well as after being phagocytosed. Dissemination occurs directly from cell to cell, via the blood stream and lymphatics, via serosal spread and in monocytes and macrophages (Frenkel, 1971).

The effects of infection vary with age - asymptomatic infections occur in adult rats and chickens, but baby rats and chick embryos are extremely susceptible to infection. Mice, hamsters and rabbits usually develop overt disease. In man, the most severe effects of infection are produced in the foetus.

Acquired immunity to *Toxoplasma*

With the development of humoral and cellular immunity to infection, a state of premunition is reached, with the persistance of cysts for years. Immunity results in a striking reduction in the numbers of parasites, and mononuclear cells appear to develop a specific capacity to digest *Toxoplasma*. Antibody assists in lysis of extracellular organisms in the presence of the accessory complement-activating (properdin) system. Human sera lacking any of complement factors C5 to C8 also lack this

TABLE 1.2. Survival of *Toxoplasma* endozoites, cysts and oocysts

	Endozoites	Cysts	Oocysts
Heat	Killed by 10-15 mins at 50°C	Killed by 10 mins at 60°C or 1 hour at 50°C	Killed by 60°C for 10 mins
Freezing	Survive for up to 200 days at -70°C in 5-10% glycerol	Usually killed by -15°C for 24 hrs	Survive freezing at -21°C for more than 4 weeks
4°C	Survive for up to 18 days in skim milk	Survive for up to 68 days	Survive
Osmotic changes	Killed by water after 30 mins at 37°C. Survive 30 mins in saline 0.2-5% at 37°C	Killed by water after 30 mins	Survive
Drying	Killed in 2½ hrs at room temperature	Killed after 1 day at 18-20°C	Killed
Chemical	Killed in 10 mins by 70% alcohol and 5% phenol		Resistant to 2% sulphuric acid, sodium hypochlorite (24 hrs), and 10% formalin (6 hrs). Destroyed after 24 hrs by 10% formalin and in 10 mins by 10% ammonium hydroxide and 7% iodine
Gastric juice	Killed in 30 mins	Survive for 2-3 hrs	Survive
Household cooking	-	Killed by normal procedures	-

activator activity (Feldman and Schreiber, 1978). However, antibody does not affect intracellular multiplication, and widespread dissemination can occur in immunosuppressed persons and animals with chronic infection, and the main defence against *Toxoplasma* is cell-mediated immunity.

Hypersensitivity

Delayed hypersensitivity can occur in toxoplasmosis with release of antigenic material in chronic infection, leading to exaggerated necrosis and inflammatory response. Delayed hypersensitivity is important in some manifestations of disease due to toxoplasmosis, such as retinochoroiditis.

Virulence

Strains of *Toxoplasma* show marked differences in virulence or relative pathogenicity in a single species, and this may change with host adaptation. Virulence in one species does not necessarily correlate with virulence in another species, and differences in strain virulence in different parts of the world are difficult to assess (De Roever-Bonnet, 1966).

Mice have often been used to study the virulence of strains, but do not provide much information as they are highly susceptible to infection. Different strains of mice have marked differences in susceptibility to infection (Araujo *et al.*, 1976) and a strain of mouse highly susceptible to *Toxoplasma* has been reported (Kamei *et al.*, 1976).

1.8 Clinical Features of Toxoplasmosis in Man

1.8.1 Acute acquired toxoplasmosis

The prevalence of *Toxoplasma* antibodies increase with increasing age, indicating that the majority of cases of toxoplasmosis are acquired. The majority of these infections are completely asymptomatic, but a small number of cases present clinically.

Features of acute toxoplasmosis are not characteristic or pathognomonic, and may mimic commoner diseases. Described features include lymphadenopathy, myocarditis, pericarditis, hepatitis, encephalitis, pneumonitis, myalgia, arthralgia and maculopapular rash. These features often lead to an incorrect diagnosis, and toxoplasmosis as the diagnosis is either rarely considered (Remington, 1974) or is over-diagnosed in patients with serological evidence of past infection.

The lymphadenopathic form of infection is the commonest clinical manifestation of toxoplasmosis, and has been recognized in many parts of the world. This presentation can mimic infectious mononucleosis with generalized lymphadenopathy, and hepatomegaly and splenomegaly may occur. Lymphocytosis can occur and atypical lymphocytes may be detected in acute toxoplasmosis. Lymphadenopathy may also be localized, often being cervical only. Toxoplasmic lymphadenopathy results in a characteristic and almost diagnostic histopathologic picture originally described by Piringer-Kuchinka (1952).

Treatment of immunologically normal persons with toxoplasmic lymphadenopathy is rarely indicated as this form of infection is self-limited and has no untoward sequelae (Remington, 1974).

In a recent analysis of 100 cases of the glandular fever syndrome of fever, lymphadenopathy and pharyngitis (Paublini, *et al*, 1977), 65 cases were found to be due to infectious mononucleosis, 22 caused by *T. gondii* and 13 by cytomegalovirus. Differentiating features of these infections were assessed. Throat involvement was prominent in infectious mononucleosis and toxoplasmosis but not in cytomegalovirus. Pyrexia was uncommon in toxoplasmosis, and hepatomegaly and splenomegaly less prominent. Liver function tests were usually normal in toxoplasmosis and abnormal in the other two conditions. Lymphocytosis and atypical lymphocytes were commonly found in infectious mononucleosis and cytomegalovirus but rarely present in toxoplasmosis.

1.8.2 Recrudescent toxoplasmosis in the compromised host

Together with certain fungi, Gram-negative bacteria, DNA viruses and *Pneumocystis carinii*, *Toxoplasma* is an important pathogen in patients with impaired immunologic mechanisms (Remington, 1970). Host resistance can be compromised either by underlying diseases such as malignancies and/or by therapy with corticosteroids and cytotoxic agents. This form of infection has been reported in leukaemias, Hodgkin's disease, multiple myeloma and other lymphomas, as well as in other malignancies. Cases have also occurred following renal and cardiac transplants (Remington, 1970).

The predominant features of recrudescent toxoplasmosis are necrotising encephalitis, myocarditis and pneumonitis. These infections are life-threatening and potentially treatable, and it is therefore important to establish the diagnosis as early as possible. Most patients had had their malignancy for more than a year, and usually had received extensive therapy with cytotoxic agents, steroids and irradiation. Features of toxoplasmic encephalitis are coma, hemiparesis, headache, drowsiness, disorientation, cranial nerve palsies, papilloedema and convulsions. Fever is often present, and less commonly rash, myocarditis, pneumonitis or lymphadenopathy may be associated. Cerebrospinal fluid studies have shown elevated protein levels with up to 100 mononuclear cells per µl. Electroencephalograms are usually abnormal, showing diffuse slowing and disorientation. Treatment is effective in arresting the disease, and intraventricular pyrimethamine has been used in a few cases with apparent success. Many immunosuppressed patients with recrudescent toxoplasmosis have concurrent cytomegalovirus or herpes simplex virus infections, and the prognosis in such cases is extremely poor (Vietzke, *et al.*, 1968).

An interesting case of chronic lymphadenopathic toxoplasmosis was reported by Sheagren *et al.* (1976). An 18-year old patient developed recurrent *Toxoplasma* lymphadenitis, and *T. gondii* was isolated from lymph nodes on each occasion over a 6-year period. During successive recurrences, the patient showed generalized anergy to cell-mediated immune stimuli. This patient subsequently was diagnosed as having Hodgkin's disease, and the depression of his cell mediated immunity was reflected in both his cell mediated anergy to skin test antigens and his prolonged *Toxoplasma* infection.

Treatment if begun early enough can prove lifesaving, and recommended treatment is a loading dose of 100 to 200 mg pyrimethamine in divided doses on the first day, followed by 25 mg daily for at least one month, combined with 6 to 8 g sulphadiazine or triple sulphonamides daily after a loading dose of 4 to 6 g. Folic acid and/or yeast may be given to protect the bone marrow (Remington, 1974).

1.8.3 Ocular toxoplasmosis

Since Janku's discovery of ocular toxoplasmosis (Janku, 1923), much has been done to clarify the pathogenesis of this form of *Toxoplasma* infection. The characteristic feature of ocular toxoplasmosis is the atrophic pigmented retinal scar of focal necrotising retinitis (O'Connor, 1974).

Toxoplasmic cysts are known to occur in the retinas of chronically infected persons, but spontaneous breakdown of such cysts has not been observed in man, and recurrent retinal lesions are thought to be the result of the multiplication of organisms and not the result of antigen release from cyst rupture (O'Connor, 1974). Lapses in cell-mediated immunity may permit proliferation of parasites in the retina and elsewhere, resulting in recurrent retinochoroiditis.

Anterior uveitis can also be caused by *Toxoplasma*, presumably from the release of antigens into the intraocular fluids. The iris and ciliary body are infiltrated by lymphocytes and plasma cells with release of proteins into the anterior chamber. Recurrence of posterior uveitis (retinochoroiditis) can stimulate an anterior reaction, but anterior uveitis can occur in the absence of posterior uveitis, possibly due to circulating antigens or immune complexes released from distant sites.

Ocular toxoplasmosis is a manifestation of both congenital and acquired infection, although some workers feel that many cases in adults

are the end result of previously asymptomatic congenital infection (Perkins, 1973). However, 9 well documented cases of acquired retinochoroiditis have been reported (Masur *et al.*, 1978).

The diagnosis of ocular toxoplasmosis depends on finding a morphologically acceptable lesion, on obtaining a positive serological result and on excluding other infective causes. The characteristic lesion is a focal necrotising retinitis. In the acute stage, the lesions appear as yellowish-white, cotton-like patches in the fundus. They are usually in small clusters, and exudate may be cast off obscuring the fundus. Retinal oedema is commonly obser ec, and oedema of the macula is present if lesions occur above the macula. The optic nerve can be involved, and lesions can result in segmental atrophy of the nerve. Peripheral retinal lesions can also occur.

In anterior uveitis, cells, protein and keratic precipitates are found in the anterior chamber, with posterior synechiae and nodules on the iris. Posterior uveitis is almost always present.

The clinical picture of ocular toxoplasmosis is virtually specific, but serum antibody titres are often very low, and the titre bears no relation to the activity or the ocular disease (O'Connor, 1974). Any dye test (or equivalent test) titre is significant in the presence of characteristic ocular lesions, even if titres are below those generally considered significant.

In atypical cases, precipitating antibodies can be detected in anterior chamber fluid. Other causes of necrotising retinochoroiditis such as syphilis and tuberculosis should be excluded.

Pyrimethamine and sulphonamides have produced disappointing results in the treatment of ocular toxoplasmosis, but are often effective in early lesions. Treatment rarely affects chronic elevated lesions. If lesions are a threat to vision or likely to result in retinal detachment, oral corticosteroids should be given with pyrimethamine and sulphonamides. Sulphonamides or tetracycline with corticosteroids may be used if pyrimethamine cannot be tolerated or causes haemopoeitic depression. Steroids should never be given alone (O'Connor, 1974).

Clindamycin has been shown to be effective in experimental ocular toxoplasmosis in rabbits (Nozik and O'Connor, 1968), but no human studies have been performed. Local and intravenous BCG has also been effective in limiting experimental ocular toxoplasmosis, but awaits human trial (O'Connor, 1974).

1.8.4 Congenital toxoplasmosis

The foetus is infected *in utero* during acute maternal toxoplasmosis, and organisms reach the foetus from the placenta via the umbilical vein. Lesions are usually more severe in the immunologically immature infant than in the mother, and infection remains active longer. Encephalitis, retinochoroiditis and hepatitis characteristically occur. Intraventricular dissemination of the organisms may occur leading to hydrocephalus from obstruction of the aqueduct. Periaqueductal and periventricular necrosis occur as a result of antigen-containing ventricular fluid coming into contact with blood vessels carrying antibody and resulting in immune complex reactions (Frenkel, 1974). These lesions produce the characteristic clinical features of classical congenital infection.

The classical triad of retinochoroiditis, hydrocephalus and intracranial calcification was originally described by Wolf *et al.* (1939). However, clinical features are extremely variable, and the diagnosis should be considered in a wide range of neonatal and infantile illnesses (Eichenwald, 1959). Table 1.3 summarises these findings in 152 clinically affected cases. Two types of diseases were described, the neurologic type being more common, while the generalized type occurred less frequently but was recognized earlier in life, usually in the first month.

TABLE 1.3. Signs and symptoms occurring in 152 cases of clinical congenital toxoplasmosis reported by Eichenwald (1959)

Type of disease:			
Neurologic	69%		
Generalized	28%		
Retinochoroiditis	80%	Pneumonia	21%
Abnormal spinal fluid*	69%	Diarrhoea	16%
Anaemia	64%	Hydrocephalus	14%
Splenomegaly	56%	Rash	13%
Jaundice	54%	Hypothermia	11%
Fever	51%	Eosinophilia	11%
Hepatomegaly	47%	Abnormal bleeding	11%
Lymphadenopathy	43%	Microcephaly	7%
Convulsions	34%	Glaucoma	1%
Vomiting	32%	Optic atrophy	1%
Intracranial calcifications	27%	Microphthalmia	1%

* Lymphocytosis and/or raised protein level

Retinochoroiditis was the commonest finding, but only occurred with hydrocephaly and intracranial calcification in about a quarter of cases. Many features such as anaemia, splenomegaly, jaundice and lymphadenopathy are too nonspecific to be useful diagnostically. Four of Eichenwald's cases were asymptomatic at birth, but two developed severe neurological disease later (Eichenwald, 1959).

Subclinical or very mild infection appears to be much more frequent than symptomatic infection. Twenty to thirty percent of infants born with congenital infection have severe disease, 10 percent have ocular involvement only, and the remaining 70 percent are asymptomatic (Alford *et al.*, 1974).

Screening for congenital toxoplasmosis is best achieved by determining cord blood total IgM levels and *Toxoplasma* IgM-IFA titres. Premature delivery was associated with 50 percent of cases. Cerebrospinal fluid abnormalities were prominent, with lymphocytosis and disproportionately elevated protein levels. These abnormalities persisted for up to 4 months even in cases with less severe forms of infection, and I.Q. development was impaired (Alford *et al.*, 1974).

Treatment of congenital toxoplasmosis should be given in view of the central nervous system sequelae of infection, even though the efficacy of treatment is uncertain. Pyrimethamine and sulphadiazine should be used, although spiramycin may be useful. Treatment schedules are:

Pyrimethamine - 1 mg/kg/day given at 12 hourly intervals. Maximal dose 25 mg/day. Dosage may be halved after 4 days and maintained for a month. Dosages may be doubled in severe cases. Can be given orally or intravenously.

Sulphadiazine or multiple sulphonamide - 100-150 mg/kg/day given 6 hourly for 1 month.

Blood and platelet counts should be monitored twice weekly and folinic acid supplements given to minimise toxicity due to folate antagonism.

1.9 Diagnosis of Toxoplasmosis

Diagnosis depends on demonstration of organisms or cysts in blood, sputum, cerebrospinal fluid or tissues, isolation of organisms by culture in animals or in tissue cultures, and on serology. Serology is the mainstay of diagnosis as material and techniques for demonstration and isolation of organisms are not readily available.

1.9.1 Demonstrations of organisms

Endozoites are occasionally seen in peripheral blood smears inside mononuclear cells, and cysts may be seen in biopsy or autopsy material, particularly in muscle and brain tissue. Organisms and cysts seen must be identified as *Toxoplasma* by special stains, electron microscopy or direct fluorescent antibody staining. Organisms are difficult to distinguish from *Sarcocystis*, *Besnoitia*, *Nosema* and some yeasts, and pollen and fungal spores can be mistaken for cysts (Frenkel, 1971). Direct fluorescent antibody testing must be adequately controlled to avoid false-positive reactions (Frenkel and Piekarski, 1978).

1.9.2 Diagnosis of *Toxoplasma* lymphadenitis

During acute toxoplasmosis with lymphadenopathy, lymph node biopsy is not uncommonly performed to exclude a lymphoma. The distinctive histopathologic changes that occur in toxoplasmosis are well recognized, following the original description of this picture by Piringer-Kuchinka (1952). Lymph nodes are only moderately enlarged (10 to 20 mm in diameter), the capsule may be slightly thickened and is usually infiltrated by histiocytes and lymphocytes. The basic lymph node architecture is retained, and the peripheral sinus is usually distended. Lymphocytes, macrophages, some polymorphonuclears and cells resembling monocytes pack the peripheral sinuses. Lymphoid follicles are enlarged and irregular and have prominent reaction centres with plentiful mitoses and phagocytosed nuclear debris. The characteristic feature is the presence of numerous clusters of epithelioid cells. These cells are scattered throughout the node but are more common at the periphery of the node. Individual cells are eosinophilic with ill-defined borders and vesicular nuclei with clear nucleoli. Cells may phagocytose nuclear debris. Giant cells do not occur, necrosis is rare and caseation has not been seen. The sub-capsular arteries in the node are enlarged by endothelial swelling (Harrison, 1966).

These histopathologic features correspond remarkably with the level of dye-test antibodies and the presence of IgM-IFA antibodies. Dye test titres are greater than 1:4096 in over 80% of cases of *Toxoplasma* lymphadenitis, and the IgM-IFA is positive in 97% of cases. These features should enable a confident histopathological diagnosis of acute toxoplasmosis, and emphasises the need for clinicians to consider toxoplasmosis in the differential diagnosis of lymphadenopathy (Dorfman and Remington, 1973).

1.9.3 Isolation of *Toxoplasma*

Material suspected of containing *Toxoplasma*, such as blood, cerebrospinal fluid, and tissues such as lymph nodes, placenta, muscle and brain, can be used for isolation. Isolation can be attempted in animals and in tissue culture, although the success of tissue culture is much poorer (Abbas, 1967). For animal inoculation, tissue may be ground and if necessary trypsinized. Mice, hamsters or rabbits can be used, and material is inoculated intraperitoneally. Passage of peritoneal washings and brain may be necessary to obtain enough organisms for diagnosis, and animals may be monitored for seroconversion. Cysts may be demonstrated in the brains of seroconverters, and their identity must be confirmed. Pre-treatment of animals with steroids facilitates isolation. The identity or presence of oocysts in cat faeces may be demonstrated by mouse inoculation after sucrose gradient centrifugation and allowing time for sporulation (Frenkel, 1971).

1.9.4 Serological diagnosis

The Sabin-Feldman dye test is the classic reference serologic test. Dye test antibody appears within 8 to 10 days of infection in man, and titres reach 1:1000 or higher within a few weeks. Sera must be inactivated for 60 minutes at 56°C. The test depends on the observation that antibody-coated live organisms, in the presence of accessory factor, undergo partial cytoplasmic lysis which can be seen under phase contrast microscopy. Methylene blue stains the cytoplasm, and loss of cytoplasm leaves nothing to be stained by the dye. The test is regarded as positive when there are more than 50 percent unstained parasites in the preparation. A micromodification of the test has been described, and this generally gives 4-fold higher titres than the original method. The dye test has the major disadvantages of requiring live organisms, and accessory factor, which can be obtained from seronegative humans or gerbils (gerbils have been found to provide a good source of accessory factor by the Tropical Disease Department of the South African Institute for Medical Research). During acute infection, titres can reach very high levels (greater than 1:16 000), and titres gradually fall to low stable titres of 1:16 to 1:64, at which level they can remain positive for life. Patients with chronic toxoplasmosis may have high or low titres, and sera should be tested from a 1:2 dilution rather than the conventional 1:16. No non-specific reactions or cross-reactions have been observed in inactivated human sera (Frenkel, 1971).

The indirect fluorescent antibody (IFA) test has been applied very

successfully to the serology of toxoplasmosis. This method does not require live organisms or accessory factor and can be performed with commercially-available killed organisms and anti-human fluorescein-labelled conjugates. Titres agree well with those obtained in the dye test and sensitivity and specificity of these techniques compare well. The availability of commercial lyophilized organisms and reference sera has enabled the IFA technique to become the standard method in many laboratories (Remington, 1970). False positive reactions in the IFA test can occur in patients with antinuclear antibodies (Araujo *et al.*, 1971). The immunofluorescence pattern in such false positive tests is indistinguishable from true positives, and is not abolished by absorption of sera with DNA. The antibodies causing false positive reactions are thought to attach to nuclear antigens of *T. gondii* (Araujo *et al.*, 1971). Another problem in the IFA test is polar staining caused by nonspecific IgM antibodies (Renterghem and Nimman, 1976; Hobbs *et al.*, 1977).

The IFA test has been adopted for the demonstration of IgM anti-*Toxoplasma* antibodies (IgM-IFA) based on the work of Remington (1970). This technique uses fluorescein-labelled anti-human IgM conjugates, and is useful in distinguishing maternal IgG from foetal IgM in suspected cases of congenital toxoplasmosis at birth. Unfortunately, the IgM-IFA test has several technical difficulties. Many commercially available anti-human IgM conjugates do not work well and are not standardized, and many contain anti-IgG giving false positive results. At present, readily available standardized reagents for IgM-IFA are still not available (Alford *et al.*, 1974). Another limitation of this test is that IgM antibody can persist for more than three years, although this is not common (Remington, 1970).

The indirect haemagglutination (IHA) test described by Jacobs and Lunde (1957) in general also gives similar results to the dye test, but tends to give more positives and higher titres, and its use is usually restricted to survey work or testing of animal sera when other methods are not available. This test is now available commercially from several sources, and will be useful if other methods cannot readily be used.

The complement fixation test (CFT) becomes positive later than the dye, IFA and IHA tests, and titres reached are low (1:8 to 1:128). The CFT usually only remains positive for 6 to 24 months, and generally

indicates recent infection if positive. However, the CFT can remain positive for several years. Antigen for this test can be prepared from peritoneal exudate from mice or from infected chick embryos. The CFT generally reflects IgM antibodies, and is generally positive in parallel with the IgM-IFA test. Problems associated with the CFT are the possibility of sera being anti-complementary or giving false-positive reactions, and the difficulty in obtaining antigen of good quality commercially. A positive CFT in a child of between 2 and 24 months of age is particularly useful in supporting a diagnosis of congenital toxoplasmosis (Kean and Kimball, 1977].

Various immunoenzyme methods of antibody detection have been described using enzyme activity to read the test instead of fluorescence of fluorescein in the IFA test. One example is the use of peroxidase-conjugated antisera instead of fluorescein-conjugated antisera in the IFA test. Slides are stained for peroxidase activity with 3-3-diamino-benzidine tetrachloride in the presence of hydrogen peroxide, and in a positive result the parasite is stained brown with a dark outline. In one study, the immunoperoxidase method compared well with the dye test and IFA test, but has the same possibility of false-positive reactions due to anti-nuclear antibodies as does the IFA test (Patramanis *et al.*, 1973).

Another promising immunoenzyme technique is the enzyme-linked immunosorbent assay (ELISA) which has been applied to *Toxoplasma* in microplate form (Voller *et al.*, 1976), and adapted to detect IgG and IgM (Camargo *et al.*, 1978). The ELISA technique uses antigen absorbed onto microtitre plate wells, to which antibody attaches if present in serum. After washing, enzyme-labelled antiglobulin is added and the wells washed again. Enzyme substrate is then added and the amount of enzyme hydrolysed is measured spectrophotometrically or visually and reflects the amount of antibody present. Horseradish peroxidase is usually used with p-nitrophenylphosphate or 5-aminosalicylic acid as the substrate. This technique compares well with the dye test and IFA test, and has the added advantage over the dye test of being able to detect IgM.

Radioimmunoassay for toxoplasmosis has also been described, and is potentially applicable to detect antibody to purified organism components (Gehle *et al.*, 1976).

Another modification of the IFA technique is the FIAX technique, using plastic dipsticks on which antigen is absorbed to a cellulose acetate

disc, which is placed in a patient's serum, washed, placed in conjugated antiserum, washed and the intensity of fluorescence read in a special fluorometer. This technique is showing considerable promise by virtue of its speed and simplicity, but awaits detailed evaluation (Walls and Barnhart, 1978).

Latex particle *Toxoplasma* agglutination tests have been used, but have usually been lacking in sensitivity and difficult to read, and have very little place in *Toxoplasma* serology (Beverley *et al.*, 1973).

A microprecipitation method in agar gel has been described by HÜbner and Uhlikova (1973), and was found to be positive when the CFT was positive. This test does not appear to have any advantages over the CFT or the IgM-IFA techniques.

From the above, the status of an individual regarding *Toxoplasma* infection can be assessed by performing the dye test or one of its equivalents (IFA, IHA), and either the CFT or IgM-IFA. In acute infections, dye test seroconversion, rise in titre or single high titre (≥1:4096) in the presence of a positive CFT or IgM-IFA is found. In patients with chronic toxoplasmosis, dye test titres are low and stable, and CFT and IgM-IFA tests are negative. In recrudescent infection, the dye test titres rise, and CFT and IgM-IFA tests may become positive again. The ELISA and FIAX techniques for toxoplasmosis appear promising, and may become the procedures of choice in the future.

1.9.5 Toxoplasmin skin test

This measures delayed hypersensitivity and usually appears within 3 months of infection, and remains positive for several decades. It is a useful test for screening surveys for the prevalence of chronic toxoplasmosis, and may be useful in patients with retinochoroiditis. Test and control materials are injected intradermally and reactions read after 48 hours (Frenkel, 1971).

1.10 Treatment

The mainstay of treatment is the use of pyrimethamine and sulphadiazine which synergistically inhibit folate synthesis (Eyles and Coleman, 1953). Dosage of pyrimethamine is 75 mg/day for 3 days, then 25 mg/day in adults; 1 mg/kg/day in infants. Dosage of sulphadiazine is 2 to 3 g/day in divided doses in adults, and 100 mg/kg/day in infants (Frenkel, 1971).

Chemotherapy in toxoplasmosis is only suppressive until immunity is acquired. Therapy is generally recommended for up to a month. Platelet and leukocyte counts should be performed twice weekly to monitor for

folate deficiency, and folinic acid or yeast should be given to prevent thrombocytopaenia and leukopaenia. Dosage of folinic acid is 3 to 10 mg/day orally or intramuscularly, and of yeast is 10 to 20 g/day orally.

Treatment during pregnancy presents a major problem, as pyrimethamine has been reported to be teratogenic. Spiramycin, a macrolide, has been used in pregnancy and appears to be effective in preventing foetal transmission if used early enough. It does not cross the placenta or affect the severity of disease in the foetus (Desmonts and Couvreur, 1974a).

Trimethoprim-sulphamethoxazole (cotrimoxazole) has been used experimentally, and trimethoprim has been shown to potentiate the action of sulphamethoxazole in mice (Sander and Midtvedt, 1970) and in tissue culture (Nguyen and Stadtsbaeder, 1975). Cotrimoxazole has been used in man in a few cases of acute infection in adults, with equivocal results as therapy is not usually indicated in such cases (Nørrby *et al.*, 1975).

Clindamycin has been found to be effective in mice with acute infection (McMaster *et al.*, 1973) but does not eliminate brain cysts during chronic infection in mice as it does not cross the blood-brain barrier, although significant clearing of organisms in other organs occurs (Araujo and Remington, 1974). No reports of the use of clindamycin in man are available, but this agent appears promising, particularly in view of its experimental success in ocular infection (Nozik and O'Connor, 1968) and in combination with sulphamethoxypyridazine in acute infection in mice (Thiermann *et al.*, 1978).

Tetracyclines have also been investigated for anti-toxoplasmic activity, but results have shown that levels required would generally be above those obtainable therapeutically (Eyles and Coleman, 1954; Perea and Daza, 1976). Oxytetracycline has been used with apparent success in one human case (Fertig *et al.*, 1977), but further studies are required.

1.11 Epidemiology

The prevalence of toxoplasmosis varies from zero in isolated Pacific atoll inhabitants (Wallace, 1969) to over 97% in the over 50-year age group in El Salvador (Remington *et al.*, 1970). Prevalence of antibodies increases with age, and the annual incidence of seroconversion varies from zero to 6 percent or more in different parts of the world. Prevalence is high in hot, humid climates such as Guatemala, El Salvador, Easter Island and Tahiti, and low in Navajo Indians and in Eskimos living in cold, dry climates (Frenkel, 1973b).

The age of maximal seroconversion reflects contact with cats and ingestion of raw meat. In El Salvador, the acquisition rate is high in children under the age of 5 years, indicating probable oocyst transmission as food is well cooked. In the USA, acquisition is higher in adults than in children, indicating probable transmission in meat, which also occurs in France, but in all age groups due to eating of raw meat by all age groups (Frenkel, 1973b).

The relationship between cats and animal and human infection has been studied, and has shown the absence of transmission in the absence of cats. In a study by Wallace *et al.* (1972), where cats lived on 2 of 3 islets, *Toxoplasma* antibody was only found on the 2 islets where cats were found, and eating habits of the people on the islets suggested that oocyst transmission was responsible for human infection. Similar conclusions were found in a study by Munday (1972) in sheep on islands between Australia and Tasmania. Island-bred sheep on cat-free islands had virtually no seropositive animals, whereas those on cat-infested islands had 32 percent seropositive animals.

Antibody studies in animals have shown variable results depending on the susceptibility of the species to infection and duration of antibody response, which is only a few months in some animals (Frenkel, 1973b). Epizootics of toxoplasmosis causing ovine abortion are well known, and transmission is thought to be via oocysts or infected products of conception. Prevention of ovine abortion can be achieved by mixing new stock with the parent flock soon after the end of the lambing season to enable new stock to become infected before they become pregnant in the following lambing season (Watson and Beverley, 1971).

From the above studies, cats appear to be important in maintaining infection in domestic animals used for meat production, and that human infections can occur from ingestion of raw or undercooked meat from such animals.

1.12 Prevention

Human infection occurs via ingestion of cysts or oocysts, and transplacental infection can occur during acute infection contracted during pregnancy. The main problem is the prevention of infection during pregnancy because of the risk of congenital transmission.

Toxoplasma cysts have been found in pork, mutton, beef, chicken and eggs. Pork and mutton are most often involved, but this depends on the degree of contact of pigs and sheep with cats.

Prevention of transmission via meat depends on adequate cooking of meat, which should be heated to above 66°C throughout. Freezing reduces the number of viable organisms, but does not always kill all organisms. Handling of raw meat can also increase the rate of transmission, and hands should be washed before touching the eyes or mouth (Frenkel, 1974).

Cats, after primary infection, shed oocysts for about 2 weeks, and on reinfection with the same strain, briefly or not at all. Oocysts sporulate in 1 to 5 days and remain infectious for at least 12 months. Control of the risk of transmission from cats depends on prevention of cats becoming infected and on safe disposal of cat faeces. Domestic indoor cats are easily controlled by feeding only cooked meat and by daily disposal of faeces before sporulation can occur. Cat faeces can be disposed of by flushing down the toilet, or treating with boiling water or dry heat (Frenkel, 1974).

Stray and outdoor cats are a major problem as they acquire infection from hunting birds and rodents, and shed oocysts in their faeces, which are buried in soil. Oocysts remain viable after sporulation in soil for at least 12 months, and will survive freezing for more than 4 weeks at -21°C (Ruiz *et al.*, 1973; Frenkel and Dubey, 1972). Wearing of gloves and washing hands after working with soil should decrease the risk of infection. Children's sand pits should be covered when not in use, and sand replaced if contaminated as effective sterilization cannot be achieved (Frenkel, 1974).

Particular attention to these measures should be observed during pregnancy. Pregnant women should avoid eating raw or undercooked meat, and should wash their hands after handling raw meat, cats or soil. Gloves should be worn for gardening.

The development of a safe vaccine for use in humans and cats is a distinct possibility, and work is in progress to determine whether different *Toxoplasma* strains exist for inclusion in a vaccine (Frenkel, 1974).

Recommendations for prevention of toxoplasmosis have been summarized by Frenkel (1974) as follows:

1. Feed cats only dried, canned or cooked meat
2. Keep cats from foraging
3. Change cat's litterboxes daily and disinfect them with boiling water

4. If pregnant, wear gloves if handling litterboxes or gardening
5. Cover children's sandboxes when not in use
6. Control stray cats
7. Control flies and cockroaches
8. Avoid eating raw or undercooked meat
9. Wash hands before meals and before touching the face.

1.13 Coccidia related to *Toxoplasma*

The provisional classification of *Toxoplasma* in section 1.3 was proposed before the coccidian nature of *Toxoplasma* was known. Following this finding, and with recent findings concerning other poorly classified coccidia, a reclassification of the cyst-forming isosporid coccidia has been proposed by Frenkel (1977). This classification is based on modes of transmission, presence of intermediate hosts in life-cycle, and whether oocysts are shed sporulated or unsporulated. Modes of transmission can be homoxenous-faecal (oocyst to same definitive host), heteroxenous-faecal (oocyst to intermediate host), heteroxenous-carnivorous (cyst to definitive host), or homoxenous-carnivorous (cyst to intermediate host).

The isosporid coccidia classified in this way include the following (table 1.4):

Family EIMERIIDAE

Homoxenous. Tissue cysts are absent or unknown, and sporulation occurs after oocysts are shed. Examples of genera in this family are *Isospora* (found in birds; oocyst has two sporocysts, each containing four sporozoites), and *Eimeria* found in birds, rodents and ruminants; oocyst has four sporocysts, each containing two sporozoites).

Family SARCOCYSTIDAE

Heteroxenous isosporid coccidia with cysts occurring in intermediate hosts. Sporulation occurs before or after oocysts are shed. Two subfamilies are proposed - Sarcocystinae and Toxoplasmatinae. Oocysts have two sporocysts, each containing four sporozoites.

Subfamily SARCOCYSTINAE

Cysts only in intermediate host. Cystozoites develop directly into gametes in gut of definitive host. Oocysts usually shed as sporocysts. Obligatorily heteroxenous, with carnivores as definitive hosts and omnivores or herbivores as intermediate hosts.

Genus *Sarcocystis*

Cysts typically occur in skeletal and cardiac muscle, and are elongated, often with septa. This parasite has been described in mammals, birds and reptiles. The life cycle of *Sarcocystis* is still largely unknown. Shedding

Table 1.4. Characteristics of the Isosporid Coccidia (modified from Frenkel, 1977)

Family	EIMERIIDAE (Homoxenous)	SARCOCYSTIDAE (Heteroxenous)					
Subfamily	EIMERIINAE	TOXOPLASMATINAE				SARCOCYSTINAE	
Genus* / Species*	*Isospora ezrini*	*Cystoisospora felis, rivolta*	*Toxoplasma gondii*	*Besnoitia besnoiti, wallacei*	*Hammondia hammondi*	*Sarcocystis muris*	*Frenkelia glethionomyobutesonis*
Enteric stages	3+	3+	5 types	1+ types	3-5 types	None	None
Oocyst shed sporulated	No	No	No	No	No	Yes	Yes
Definitive hosts*	Birds	Cat	Felids	Cat	Cat	Cat	Hawk
Intermediate hosts*	None	Rodents, dog	Mammals, birds	Rodents	Rodents	Mouse	Vole
Transmission [Homoxenous faecal	+++	+++	+	-	-	-	?
Transmission [Heteroxenous faecal or carnivorous	-	+	+++	+++	+++	+++	+++
Transmission [Homoxenous carnivorous	-	-	+++	+/-	-	-	-
Cyst location in intermediate host	-	Lymph nodes	Many cell types	Fibroblasts	Skeletal muscle	Skeletal or cardiac muscle	Brain or spinal cord

*Examples only are given where many genera and/or species have been described.

of sporocysts and oocysts has been reported in cats fed *Sarcocystis tenella* from sheep and cattle, and in man, cats, and dogs fed *S. fusiformis* from cattle (Rommel *et al.*, 1972; Heydorn and Rommel, 1972). These authors suggested that the oocysts of *Isospora hominis* and *I. acti* may be those of *Sarcocystis*, but further confirmation is required. Human infections have been described, usually as *S. lindemanni* infections, but the source of infection is not known. *Sarcocystis*-infected muscle does not transmit infection, and little is known about this group of organisms (Frenkel, 1974).

Genus *Frenkelia*

Cysts occur in brain and spinal cord of rodents, are thin-walled and often septate.

Subfamily TOXOPLASMATINAE

Cysts are aseptate, and oocysts are shed unsporulated. A propagative cycle precedes gametogony in gut of definitive host.

Genus *Toxoplasma*

Cysts occur in many cell types in both intermediate and definitive hosts. Seven species have been included by Levine (1977):

T. gondii - type species, felids as definitive hosts.

T. alencari - frog)
T. brumpti - iguana) very little information available,
T. colubri - snakes) and complete life cycles are not known.
T. rajae - [illegible])
T. serpai - [illegible])

T. hammondi - cat is definitive host and house mouse intermediate host. Classified as *Hammondia hammondi* by Frenkel (1977) as it is obligatorily heteroxenous (*T. gondii* is facultatively heteroxenous).

Genus *Besnoitia*

Cysts occur in fibroblasts and possibly other cells, but only in intermediate hosts. Definitive hosts, when known, are felids. Described species are: *B. wallacei, B. besnoiti, B. jellisoni, B. darlingi, B. tarandi* and *B. bennetti.*

Genus *Hammondia*

Cysts typically occur in skeletal muscle. Obligatorily heteroxenous. Species: *H. hammondi* (classified as *T. hammondi* by Levine) with rodent-cat cycle); *H. bigemina* (?) with cattle-dog cycle.

Genus *Cystoisospora*

Monozoic cysts are found in many tissues, e.g., mesenteric lymph nodes. Cysts only infect the definitive host, but oocyst can infect both intermediate

and definitive hosts. Known species are *C. felis, C. rivolta, C. vulpina, C. ohioensis* and *C. canis.*

In the above proposed classification, generic distinctions are based on cyst rather than oocyst characteristics, subfamilial distinctions on whether asexual reproduction takes place in the definitive host, and familial distinctions on existence or not of tissue cysts. Serologic comparisons have shown no significant cross-reactions in the dye test between *Besnoitia* spp., *Sarcocystis muris* and *Toxoplasma*, although the IFA test shows some cross-reactivity.

ADDITIONAL REFERENCES

Frenkel, JK (1977): *Besnoitia wallacei* of cats and rodents: with a reclassification of other cyst-forming isosporid coccidia. Journal of Parasitology 63:611-628.

Levine, ND (1977): Taxonomy of *Toxoplasma*. Journal of Protozoology 24:36-41.

Peterson, DR, Cooney, MK and Beasley, RP (1974): Prevalence of antibody to *Toxoplasma* among Alaskan natives: Relation of exposure to the Felidae. Journal of Infectious Diseases 130:557-563.

2.0 TOXOPLASMOSIS AND PREGNANCY

Since most *Toxoplasma* infections in adults are asymptomatic, seroconversion of the Sabin-Feldman dye test (or alternative test such as the IFA test) is the *sine qua non* in the diagnosis of the infection. Passively transferred antibodies in the infant have generally disappeared by the age of 6 months, and it is generally agreed that any infant with persistent antibodies over this age has congenitally acquired toxoplasmosis (Hume, 1972).

Many authors believe that congenital infection can only occur during primary maternal infection, and that transmission does not occur thereafter. Others feel that congenital transmission can and does occur during chronic maternal toxoplasmosis. A problem complicating assessment of the position is that many congenital infections are asymptomatic at birth and are detected by persistent positive serology. However, the possibility that asymptomatic seronegative cases at birth can occur has been suggested, but no definite cases have been described (Hume, 1972).

The question of how much of congenital infection is due to acute and how much due to chronic maternal infection remains a controversial point. Most studies have shown that acute infection during pregnancy can result in abortion or congenital infection, there have usually been cases in these series with high or rising titres where serologic evidence of acute infection is not absolute, and the possibility, however remote, of chronic maternal infection could be considered. Since wide titre fluctuations may occur in chronic toxoplasmosis, interpretation in individual cases can be extremely difficult.

2.1 Animal studies

Although the applicability of animal work to man is always suspect, many observations have been made. Mice have often been used, but their use is problematical as they are immunologically deficient against *Toxoplasma*.

Chronically infected mice have been shown to transmit congenital infection for at least 5 generations (Beverley, 1959). Spontaneous parasitaemia has been demonstrated in mice and rabbits by Remington *et al.* (1961).

The possibility of transmission from uterine cysts has also been shown in mice, with congenital infections during chronic infection occurring mainly in animals with cyst-containing uteri (Werner and Egger, 1969).

The role of immune tolerance has also been investigated. In some mice, congenitally infected from chronically infected mothers, parasites could be isolated despite these animals being seronegative. Some of these animals also failed to produce antibody on injection with formalinized organisms, but parasites could not be recovered from these animals (Jacobs, 1967).

Strain virulence has also been investigated. The less virulent Beverley strain has a greater capacity to produce uterine cysts in mice and spontaneous parasitaemia in rats. It is probably that conflicting reports in humans could be on the basis of strain and virulence differences (Hume, 1972).

2.2 Human studies

Spontaneous parasitaemia has been documented by Prior *et al.* (1953) and Miller *et al.* (1969). One of these patients had parasitaemia demonstrable over a 14 month period despite several courses of therapy. Viable uterine cysts in humans have also been demonstrated by Remington *et al.* (1960).

2.2.1 Habitual abortion

Despite categorical denials by most authorities, it is possible that occasional cases of congenital infection can occur in chronic toxoplasmosis. Much evidence for this form of transmission has come from Langer (1963) who reported isolation of *Toxoplasma* from lochia, menstrual blood, milk or foetal tissues in 23 of 79 women with habitual abortion. However, this work has been the subject of much dispute as to the validity of the isolation methods used (Frenkel and Piekarski, 1978), and Langer himself admitted some doubt concerning possible misidentification of pollen grains for *Toxoplasma* cysts (Langer, 1966). Four of Langer's cases were reported to be seronegative. Remington *et al.* (1964) described 3 patients with chronic infection, 2 of whom aborted and 1 who had a congenitally infected infant.

In France, however, studies on 15 000 women with chronic infection have not shown a single congenital infection (Desmonts *et al.*, 1965b), and similar results have been reported by other workers (Jacobs, 1967).

Although by no means settled, it seems probable that, except for the very rare occurrence of recurrent transmission in successive pregnancies, toxoplasmosis is not a cause of habitual abortion.

2.2.2 Sporadic abortion

It is generally accepted that acute infection during early pregnancy will frequently result in spontaneous abortion (Hume, 1972).

2.2.3 Congenital infection

Congenital toxoplasmosis has been extensively studied in France by Desmonts and Couvreur (1974a, 1974b), and their results have defined the extent of the risk of acute infection during pregnancy, the risk and severity of congenital transmission, and the complete lack of evidence of transmission during chronic infection.

Their results showed 84% of patients were seropositive, and that the seroconversion rate was 6.3 per 100 per year. In the patients studied, high antibody titres (≥1:1000 dye test titres) or seroconversion was detected in 378 cases, who were classified into 2 groups. The first group (183 cases) had acute infection documented during pregnancy (Table 2.1), while the second group (195 cases) had evidence of acute infection prior to pregnancy, or where time of infection was unknown (Table 2.2).

TABLE 2.1. Data of patients who had acute toxoplasmosis during pregnancy (Group 1)

Finding	Number of cases	
Seroconversion	121	66%
Lymphadenopathy with rising titre	21	11%
Lymphadenopathy and high titre	24	13%
Asymptomatic with rising titre	17	9%
TOTAL	183	100%

TABLE 2.2. Data of patients with acute toxoplasmosis before pregnancy or where time of infection could not be determined (Group 2)

	Number of cases	
Acute infection before pregnancy	15	8%
Acute infection around conception	14	7%
High stable titres	150	77%
Seroconversion between previous pregnancy and first trimester of present pregnancy	16	8%
TOTAL	195	100%

Isolation of *Toxoplasma* was attempted from the placentas of these patients, and was positive in 24 of 96 (25%) in Group 1 and in 2 of 105 (2%) in Group 2. The 2 positive isolates in Group 2 had high stable titres at 6 months' gestation, and were probably acute infections during pregnancy. Isolation of organisms from placentas correlated closely with the presence of congenital infection.

Of the 378 pregnancies, 11 ended in abortion and 7 were stillborn or died soon after birth. Excluding the 11 abortions (6 were induced), the outcome in the remaining cases was as follows - definite congenital infection in 59 (16%), possible congenital infection in 15 (4%), and no congenital transmission in 293 (78%). Sixty-six of the congenital cases were in Group 1 and 8 in Group 2 (Table 2.3).

TABLE 2.3. Outcome of 369 pregnancies with high antibody titres at delivery

	No. in Group	Congenital infection Definite		Possible*	
Group 1	176	55	(31%)	11	(6%)
Group 2	191	4	(2%)	4	(2%)
	367	59	(16%)	15	(4%)

* Possible cases, not proven due to neonatal deaths, foetus lost to examination or inadequate follow-up

To assess the effect of the stage of pregnancy when acute infection occurred and of treatment of mothers with spiramycin, the remaining 176 mothers in Group 1 plus the 4 definite congenital cases in Group 2 were analysed (Table 2.4).

TABLE 2.4. Outcome of 180 pregnancies according to trimester material infection was acquired

Trimester	Number		Congenital infection Definite		Possible		No. presenting clinically
1st	30	(17%)	5	(17%)	1	(3%)	5
2nd	84	(47%)	20	(24%)	5	(6%)	13
3rd	39	(22%)	24	(62%)	2	(5%)	2
Undetermined	27	(15%)	10	(37%)	3	(11%)	4
TOTAL	180	(100%)	59	(33%)	11	(6%)	24

The effect of spiramycin treatment is shown in Table 2.5, and severity of disease in Table 2.6. Spiramycin does not affect the severity of disease in the foetus as it does not cross the placenta, but is effective in preventing spread to the foetus if used early enough.

TABLE 2.5. Outcome of 180 pregnancies according to spiramycin treatment

	Number	Congenital infection Definite	Possible	No. presenting clinically
Treated	98 (54%)	22 (22%)	2 (2%)	12
Untreated	82 (46%)	37 (45%)	9 (11%)	12
TOTAL	180 (100%)	59 (33%)	11 (6%)	24

TABLE 2.6. Severity of disease in 59 definite congenital infections

Severe - still birth, neonatal death, serious cerebral damage	9	(15%)
Mild - retinochoroiditis	11	(19%)
Asymptomatic	39	(66%)

This study by Desmonts and Couvreur has shown that congenital infections occurred exclusively in mothers with acute infection during that pregnancy. However, acute infection only resulted in 33% definite and a possible further 6% possible congenital cases, and that severe disease only occurred in 15% of the cases, or 6% of maternal infections during pregnancy.

Transmission occurs less frequently during early pregnancy, but disease produced is more severe. Spiramycin if used early enough, is effective in reducing transmission from 48% to 24%.

In their studies, Desmonts and Couvreur found that dye test titres in mothers with infected infants were ≥1:1000, and that among over 800 congenital infections seen over a 15-year period there were no siblings other than twins in their series. They concluded that infection can only be transmitted during acute maternal infection and not during chronic infection.

In a study by Kimball *et al.* (1971) in New York, 6 out of 4048 obstetric patients seroconverted, and 2 of the 6 patients, and a further 1 of 17 patients with a substantial rise in titre transmitted the infection. In England, 9 of 3187 patients seroconverted during the second half of pregnancy, but there was no congenital transmission (Rouss and Bourne, 1972). In Germany, Kraübig (1966) reported 17 congenital infections from 3213 patients, and in Brazil, Castilho (1976) estimated the incidence of congenital toxoplasmosis in Sao Paulo to be 16 per 1000 live births, with 4 symptomatic cases per 1000 live births.

From the above data, there appear to be marked differences in the incidence of congenital infection in different parts of the world, being high in Europe, lower in the USA and lowest in England, despite an acquisition rate of 0.5 to 1% per year in women of child-bearing age. These differences have been ascribed to possible differences in virulence, but other factors may be involved (Fleck, 1973).

3.0 TOXOPLASMOSIS IN SOUTHERN AFRICA

Relatively little attention has been paid to toxoplasmosis in Africa, but prevalence surveys for seropositivity have been conducted in various parts of Africa, and have yielded prevalence rates of from 9% in the San (Bushmen) of South West Africa (Jacobs and Mason, 1978) to 77% in the Hazda of Tanzania (Bennett *et al.*, 1970). A toxoplasmin skin test survey in Blacks in Johannesburg conducted by Schneider *et al.* (1955) showed a positive reaction in 31% of 209 subjects. Examples of prevalence rates in other parts of Africa are given in Table 3.1 (Jacobs, 1977). Details of prevalence studies in Southern Africa will be presented in Chapter 5.

TABLE 3.1. Prevalence of *Toxoplasma* antibodies in Africa

Uganda	12%
Egypt	17%
Southern Africa	20%
Rhodesia	35%
Kenya	60%
Sudan	61%
Nigeria	65%
Tanzania	77%

3.1 Cases of clinical toxoplasmosis in man

A small number of cases of clinical toxoplasmosis has been reported in South Africa. The first reported case was by Klenerman (1951) who described a 6-week old Black infant with congenital infection. The brain contained *Toxoplasma* cysts and pseudocysts, and the mother's dye test titre was 1:1024. A further 4 congenital cases have been described by Rabkin and Javett (1952), Tasser (1955) and Edge and Wallace (1961). Two post-mortem cases in children were reported by Becker (1954), but there was very little to substantiate the diagnosis in one of these cases. A few cases in adults and children have also been reported (Edge and Wallace, 1961; Saunders and Thatcher, 1963; Te Groen, 1971 and van der Horst *et al.*, 1972). A summary of these cases is given in Tables 3.2 and 3.3.

TABLE 3.4 Summary of reported cases of congenital toxoplasmosis in South Africa

Author	Age	Sex	Race	Presentation	Serology	Other features	Comments
Klenerman (1951)	6 wks	M	Black	Hydrocephalus	Dye test 1:1024 (maternal)	Cysts and pseudocysts seen in brain sections	Confirmed congenital case
Rabkin & Javett (1952)	4 yrs	M	White	Microcephaly, ataxia, mental retardation, retinochoroiditis and cerebral calcification	Dye test 1:1024 (child) 1:256 (mother)	-	Typical congenital case. Child born in Congo
Becker (1954) Johannesburg	5 mths	F	White	Hydrocephalus with death from purulent meningitis at 5 months	Not done	Organisms not described	Unlikely case. Child born in Northern Rhodesia
Passer (1955) Pretoria	15 mths	M	Indian	Hydrocephalus, mental retardation	Dye test 1:256 and CFT positive for mother and child	No retinochoroiditis or cerebral calcification	Probable congenital case
Edge & Wallace (1961) Durban	4 mths	F	White	Convulsions, mental retardation, microcephaly, cerebral calcification, hydrocephalus	Dye test 1:64 CFT negative	-	Possible congenital case
	4 mths	M	White	Pyrexia, irritability, raised intracranial pressure, retinochoroiditis, raised CSF protein	Not done	-	Probable congenital case

TABLE 5.3. Summary of reported cases of acquired toxoplasmosis in South Africa

Author	Age	Sex	Race	Presentation	Serology	Other features	Comments
Becker (1954) Johannesburg	4½ yrs	M	White	Pyrexia, coma, convulsions, raised intracranial pressure	Not done	Cysts and pseudocysts seen in brain	Probable case of encephalitis - ?acquired
Edge & Wallace (1961) Durban	12 yrs	F	White	Retinochoroiditis for 4 yrs with recurrent lymphadenopathy	Dye Test 1:64 CFT 1:4	-	Probable *Toxoplasma* retinochoroiditis
	11 yrs	M	White	Pyrexia, cervical lymphadenopathy, hepatosplenomegaly, pneumonitis, old bilharzia. Developed retinochoroiditis	Dye test 1:1024 CFT 1:4 (serology done 1 yr after acute illness	-	Possible acute case
Saunders & Thatcher (1963) Cape Town	22 yrs	M	White	Retinochoroiditis and generalized lymphadenopathy	Dye test 1:4096 CFT negative	Lymph node histology not suggestive of toxoplasmosis	Probable *Toxoplasma* retinochoroiditis
	24 yrs	M	White	Pyrexia and lymphadenopathy over 9-month period	Dye test 1:65536 (constant 5 mths later) CFT negative	-	Probable recrudescent case
	18 yrs	M	White	Pyrexia for 1 yr, with retinochoroiditis	Dye test 1:1024, CFT anticomplementary	-	Possible recrudescent case
	38 yrs	F	White	3-week history of pyrexia, malaise and chest pain. No lymphadenopathy	Dye test 1:4096 CFT negative	-	Unlikely acute case in view of negative CFT
Te Groen (1971) Pretoria	26 yrs	F	White	Previous abortions or infants with congenital defects. Treated for toxoplasmosis and had 2 normal infants	IFA 1:32 CFT 1:8	-	Insufficient data but toxoplasmosis unlikely
	35 yrs	F	White	Habitual mid-trimester aborters due to cervical incompetence. Shirodkar stitch successfully used to obtain full-term pregnancies (patients also treated for toxoplasmosis)	CFT 1:32	-	Two patients with cervical incompetence. No evidence of toxoplasmosis affecting these cases
	31 yrs	F	White		'Positive'	-	
Van der Horst *et al.* (1972) Durban	10 yrs	F	White	Mental retardation since birth. Died of cardiac failure	3 yrs previously IFA was 1:400 and CFT 1:8	Diffuse chronic myocarditis but no parasites seen at autopsy	Diagnosis uncertain

The cases of toxoplasmosis reported show that the disease in most of its forms occurs in South Africa, the only form not being described being recrudescent infection in malignancy. However, many cases of infection are probably missed, and to date no successful laboratory isolates of *Toxoplasma* have been made from human material, although organisms have been isolated from animals.

3.2 Toxoplasmosis in animals in South Africa

Dye test antibodies have been found in 12% of 50 dogs (SAIMR, 1957), 100% of 20 cattle, 2 of 3 donkeys, 1 of 5 horses, 80% of 20 pigs, 70% of 10 sheep (SAIMR, 1959), and in almost all sheep tested (Bigalke, 1978).

Post-mortem diagnoses of toxoplasmosis have been made in a Cape Hunting Dog (Hofmeyr, 1956) and in 7 dogs seen in Pretoria (Smit, 1951).

The first reported isolation of *Toxoplasma* in South Africa was from ferrets maintained in a colony at the Onderstepoort Veterinary Research Institute, Pretoria. Five out of 6 ferrets examined were found to be infected on mouse inoculation, but the source of infection and the reasons for spontaneous illness occurring in the colony could not be explained (Bigalke *et al.*, 1966).

An outbreak of toxoplasmosis in chinchillas in the Western Cape was reported by Du Plessis *et al.* (1967). Over a 3-month period in 1966, 17% of the 600 animals in the colony died with features including lethargy, anorexia, diarrhoea and dyspnoea. Organisms and cysts were seen in histological material, and isolation of organisms in mice confirmed the diagnosis.

From the above reports, toxoplasmosis in rodents and meat-producing animals occurs in animals in South Africa, and this risk of infection via raw or undercooked meat would be similar to that found in other parts of the world. The strains isolated from ferrets and chinchillas are the only published reports of isolates of *Toxoplasma* in South Africa from human or animal sources, but the strains were unfortunately not retained for comparison with strains isolated elsewhere (Bigalke, 1978).

4.0 METHODS

The methods described were used for the studies described in Chapters 5 to 8, and details of the methods employed are given in this Chapter.

4.1 Serology

4.1.1 Indirect fluorescent antibody (IFA) test

The IFA test was the standard test used for the studies as the techniques involved are simple, equipment and reagents are readily available commercially, results are generally accepted as being equivalent to the dye test and the prozone phenomenon in the dye test does not occur (Fletcher, 1965). The only disadvantage of the IFA test is the possibility of false positive reactions caused by sera containing anti-nuclear antibodies (Araujo *et al.*, 1971), and the necessity to have the required anti-species conjugate.

The IFA test was employed for human, sheep and mouse sera using fluorescein isothiocyanate labelled anti-human conjugate, anti-human IgM conjugate, anti-sheep conjugate and anti-rat conjugate (all obtained from Wellcome Reagents, Beckenham, England). All conjugates, except for the anti-human IgM conjugate, were titrated out with control sera to obtain the titre for use in the test. Control positive and negative human and sheep sera were obtained commercially (Wellcome Reagents; Behringwerke, Marburg, West Germany; and Roche Diagnostics, Basel, Switzerland). Negative mouse serum was obtained from normal mice, and positive mouse serum from mice injected with the antigen used in the IFA test; these sera were tested with the dye test to confirm the negative serum and obtain the titre of the positive serum. Typical data on conjugate titrations are shown in Table 4.1.

Control human serum for anti-*Toxoplasma* IgM antibody determination by the IFA technique was not available commercially and could not be obtained, and the anti-human IgM conjugate was standardized as follows. Sera from a patient with acute toxoplasmosis were obtained at intervals over a 14-month period after presentation, and were tested with different dilutions of anti-IgM conjugate, with results as shown in Table 4.2. As IgM titres generally are low or become negative after a year, the conjugate dilution in which this occurred with the patient's sera was assumed to be at a

suitable dilution for testing other sera for anti-*Toxoplasma* IgM antibodies. For the conjugate used, which was from the same batch throughout, a 1:20 dilution fulfilled these requirements. In view of the problems reported with some anti-IgM conjugates (Remington and Desmonts, 1973), the IgM-IFA test was only performed on selected sera, and titres of ≥1:16 were regarded as significant.

TABLE 4.1. Data on fluorescein-labelled conjugates used in the IFA test. All conjugates were obtained from Wellcome Reagents

	Anti-human immunoglobulin	Anti-human IgM	Anti-sheep immunoglobulin	Anti-rat immunoglobulin
Species produced in	Sheep	Sheep	Rabbit	Rabbit
Lot Number	K8872 & K9070	K2934	K2537	K1378
Fluorescein/protein ratio:				
optical density	0.646	0.72	0.92	0.76
molar	2.8	3.2	4.5	3.5
Protein (mg/ml)	9.85	7.2	8.2	6.3
Antibody protein (mg/ml)	3.1	Not stated	1.2	1.7
Gel diffusion reactivity against human:				
IgG	+	-		
IgM	+	+		
I	+	-		
Significant cross-reaction with animal sera:				
Monkey	++	-	-	-
Cow	-	-	++	-
Goat	-	-	++	-
Sheep	-	-	+++	-
Mouse	-	-	-	++
Rat	-	-	-	+++
Rabbit	-	-	-	-
Working dilution	1:80-1:120	1:20	1:40	1:10

TABLE 4.2. Standardization of anti-human IgM conjugate with sera from a patient with acute toxoplasmosis

Time after presentation (months)	IFA titre	Titre with anti-human IgM conjugate used in dilutions of 1:10	1:20	1:40	1:80
2	1:16384	1:512	1:512	1:256	1:128
14	1:8192	1:16	1:8*	1:8*	1:8*

* IgM-IFA test regarded as positive at titres ≥1:16 only

The antigen used was killed lyophilized whole organisms obtained commercially from Wellcome Reagents and later from Roche Diagnostics. The reason for the change was the finding of several vials of antigen containing very few parasites, while the number of organisms from the new source was found to be as stated. The antigen was reconstituted from the lyophilized state according to the manufacturers' instructions, and the number of organisms was counted in a Fuchs-Rosenthal counting chamber. The antigen was, if necessary, diluted with phosphate buffered saline to contain approximately 2.5×10^6 organisms per ml, and was not used if there were fewer than this number in the reconstituted vial. This number of organisms was found to give 40 to 60 single organisms per high power field. In the antigen obtained from Wellcome Reagents, organisms tended to clump together, and this clumping was removed by squirting the antigen through a 26-gauge needle a few times, and this was routinely done on all vials of antigen. The correctly diluted antigen was applied onto 2.5 mm spots on multi-spot slides with a 1 ml syringe with a 26-gauge needle. Slides were allowed to air-dry and were kept in air-tight containers at -20°C until used. The contents of the whole vial were applied to slides after reconstitution. Multi-spot slides were initially made by hand using glycerine drops sprayed with teflon aerosol spray, and later obtained commercially from Dynatech Laboratories (Surrey, England) with the same specifications. Multi-spot slides contained 26 spots, each 2.5 mm in diameter, as shown in Figs. 4.1 to 4.3. Eight sera could be screened in 3 dilutions or 3 sera titrated for 8 dilutions per slide, while the 2 extra spots could be used for positive and negative controls on each slide to check microscope function. No differences in performance were found between the antigens from the 2 different sources.

Phosphate-buffered saline (PBS) pH 7.2 was used as the diluent for

all IFA test procedures such as antigen, serum and conjugate dilution, and all washing stages. PBS in powder form was obtained commercially (Difco Laboratories, Detroit, USA) in vials containing 7.65 g NaCl, 1.2688 g disodium phosphate, 0.1 g monosodium phosphate and 0.2113 g monopotassium phosphate per vial. Each vial was made up to 1 litre in a volumetric flask with reagent-grade water (reverse osmosis water processed through deionizing resins), and the pH measured and if necessary adjusted. Particular attention was paid to checking the pH on each litre of PBS made up. PBS was only used on the day it was made up, and left-over solution was discarded at the end of the day.

After conjugates had been titrated out with control sera, the IFA test was performed as follows:

1. Suitable doubling dilutions of test sera (inactivated at 56°C for 30 minutes) were made in microtitre plates using microtitre droppers and diluters (Dynatech).
2. Sufficient serum of each dilution was placed onto each spot with a Pasteur pipette. The same pipette was used for the same serum, starting with the highest dilution. Sera were routinely screened at dilutions of 1:8, 1:16 and 1:32, and titrated out further if positive at 1:32.
3. Positive and negative control sera were placed on a slide as shown in Fig. 4.2, and the 2 left-hand spots on further slides used for positive and negative controls on each slide as shown in Fig. 4.3.
4. Slides were incubated at room temperature for 30 mins in a humid chamber and serum spots then rapidly flushed off with PBS.
5. Slides were placed in a staining rack and washed in PBS on a magnetic stirrer at slow speed for 30 mins, with a change of PBS after 15 mins, and then blotted dry.
6. A drop of conjugate in working dilution (conjugate working dilution made up fresh each day) was placed on each spot with a Pasteur pipette and slides incubated at room temperature for 30 mins in a humid chamber. Conjugate was diluted with PBS containing Evans' Blue in 1:10 000 dilution.
7. Conjugate was flushed off slides with PBS, and slides washed and dried as in 5.
8. Slides were mounted in buffered glycerol (9 parts glycerol to 1 part PBS), covered with a cover-slip, and examined under 600 X magnification on a fluorescent microscope.

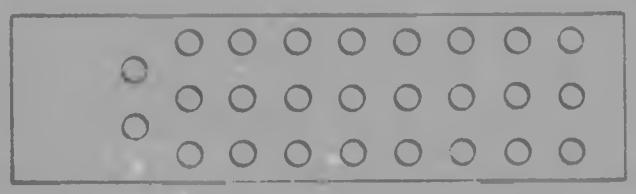


Fig. 4.1. Layout of multispot slides used for the IFA test. Eight sera could be screened in 3 dilutions, using spots vertically, or 3 sera titrated for 8 dilutions using spots horizontally, using the 2 left-hand spots for positive and negative controls.

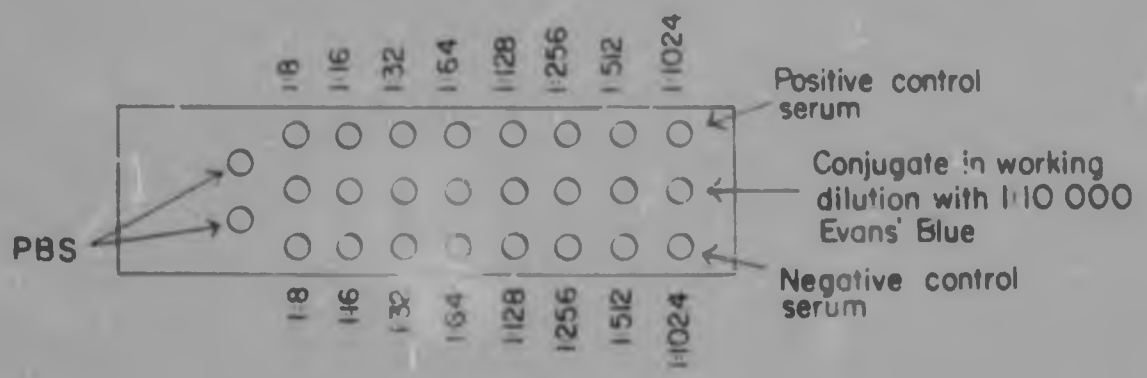


Fig. 4.2. Layout of control slide used in duplicate for each batch of IFA tests. In this example, titre of positive control serum is 1:64 and serum dilutions were placed on the slide so that the change from positive to negative should occur between the 4th and 5th spots. Control slides were satisfactory if the 2 left-hand spots and the centre row showed no fluorescence, the positive control showed 1+ to ± fluorescence within 1 doubling dilution of the expected titre and the negative control no more than ± fluorescence at a 1:8 dilution.

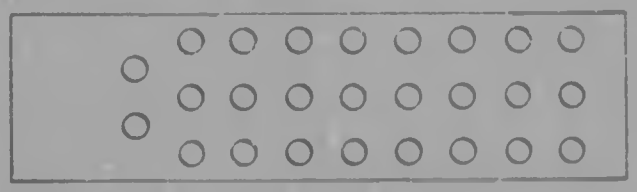


Fig. 4.1. Layout of multispot slides used for the IFA test. Eight sera could be screened in 3 dilutions, using spots vertically, or 3 sera titrated for 8 dilutions using spots horizontally, using the 2 left-hand spots for positive and negative controls.

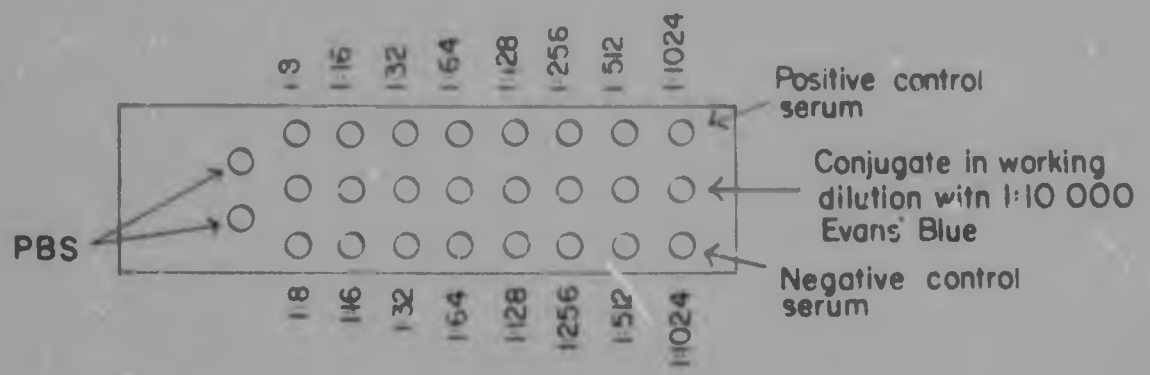
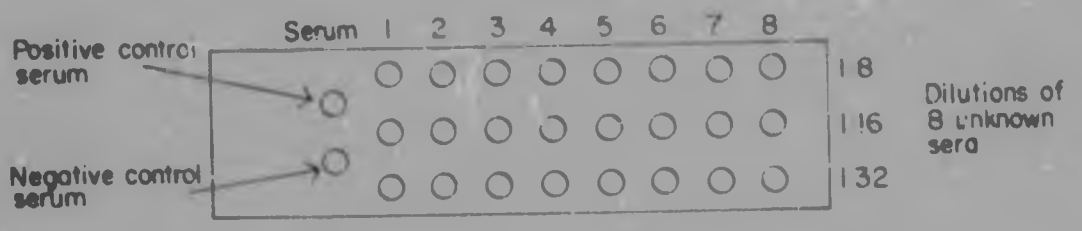


Fig. 4.2. Layout of control slide used in duplicate for each batch of IFA tests. In this example, titre of positive control serum is 1:64 and serum dilutions were placed on the slide so that the change from positive to negative should occur between the 4th and 5th spots. Control slides were satisfactory if the 2 left-hand spots and the centre row showed no fluorescence, the positive control showed 1+ to ± fluorescence within 1 doubling dilution of the expected titre and the negative control no more than ± fluorescence at a 1:8 dilution.



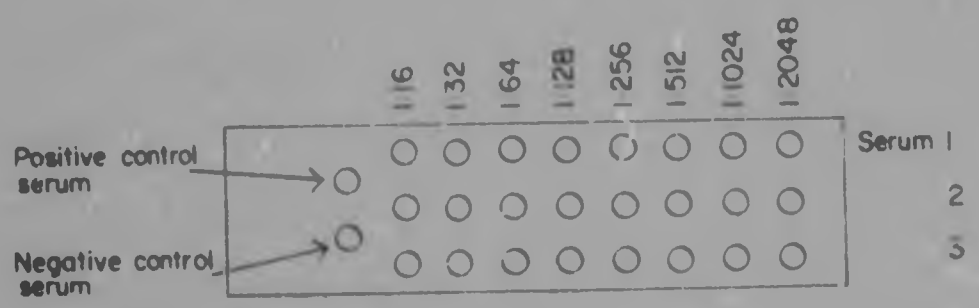


Fig. 4.3. Layout of test slides used for screening 8 unknown sera in 3 dilutions (upper figure) or titration of 3 screened sera for 8 dilutions (lower figure). Sera still positive at 1:2048 were titrated out to their end-point. Positive and negative control sera on the 2 left-hand spots enabled checking of microscope function for each slide.

After the reconstitution of a vial of lyophilized conjugate, the conjugate was kept at 4°C if expected to be used within a few weeks, or frozen in aliquots at -20°C if infrequently used. All conjugates used performed satisfactorily and were used in dilutions of 1:10 to 1:120. Conjugates were checked for anti-*Toxoplasma* activity, which was absent, but the anti-human IgM conjugate tended to cause polar staining with many sera. Conjugates were diluted in Evans' Blue in 1:10 000 dilution to reduce non-specific fluorescence and to enable the presence of unstained organisms to be seen. Evan's Blue was kept as a 1:1 000 stock solution in distilled water at 4°C.

Slides were read with a Reichert transmitted light microscope, with a halogen light source, filters for fluorescein fluorescence, dark field condenser and glycerol immersion objectives. During the later part of this study, a Leitz Ortholux incident light microscope was used. This microscope had a halogen light source, filter modules for fluorescein fluorescence and oil immersion objectives. The Leitz microscope gave more powerful fluorescence and required re-titration of conjugates to obtain equivalent results to that obtained with the same controls on the Reichert microscope.

The IFA test was read as positive if organisms showed a complete ring of fluorescence, and intensity of fluorescence was graded from 4+ to ±. Readings of ± fluorescence were regarded as the end-point. Polar fluorescence, which was particularly noticeable with the anti-human IgM conjugate, was disregarded.

Random sera tested by the IFA method were sent for checking with the dye test by the Tropical Diseases Department of the South African Institute for Medical Research, and all dye test results were within 1 doubling dilution of the IFA result. Sera checked with the dye test included high and low titre positive as well as negative sera.

4.1.2 Complement Fixation Test (CFT)

This test was carried out in microtitre plates according to the method described in Public Health Monograph No. 74 (US Public Health Service, 1965), using microtitration methods and a 50 percent haemolysis (CH_{50}) end-point for complement titration. The method used the following ratios of reagents - 1 volume each of patient's serum, antigen and sensitised red cells, and 2 volumes of CH_{50} complement, as follows:

Buffer

Veronal buffered saline (VBS) pH 7.2 to 7.4, containing 8.3 g NaCl,

1.02 g sodium barbital, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.022 g $CaCl_2 \cdot 2H_2O$ and 3.46 ml 1 N HCl per litre, was made up from commercial tablets (Oxoid Ltd., Basingstoke, England) with the addition of 0.1% gelatin (Difco Laboratories). VBS was made up on the day before use, autoclaved for sterility, kept at 4°C and always used cold.

Inactivation of test sera

Sera were inactivated for 30 mins at 56°C prior to testing. If sera were to be stored, they were inactivated before freezing and re-inactivated when thawed prior to testing.

Sheep red blood cells

Sheep blood was collected aseptically in an equal volume of Alsever's anticoagulant solution (containing 20.5 g dextrose, 4.2 g NaCl, 8.0 g sodium citrate, and 0.55 g citric acid per litre). Alsever's solution was sterilized by filtration through a 0.22 µm Millipore membrane. For use, red cells were spun down and washed twice in VBS, and then suspended to approximately their original volume in VBS. Samples of this suspension were placed in 2 capillary tubes and spun in a microhaematocrit centrifuge to obtain the haematocrit. The cell suspension was then diluted to give a 2.8% red cell suspension, using the formula:

$$\frac{\text{haematocrit \%}}{2.8} = \text{dilution factor of cell suspension.}$$

Haemolysin titration

This was performed for each bottle of sheep haemolysin used, as follows:

1. Haemolysin (Wellcome Reagents) was diluted in doubling dilutions from 1:100 to 1:6400 and from 1:150 to 1:4800 in 0.2 ml volumes.
2. 0.2 ml of 2.8% sheep red cells was added to each tube.
3. 0.8 ml complement at $1CH_{50}$ was added to each tube (see complement titration).
4. 0.8 ml VBS was added to each tube.
5. Tubes were incubated for 30 mins at 37°C, being shaken after 15 mins and then centrifuged.
6. Percentage haemolysis was read on a Unicam spectrophotometer at a wave-length of 540 µm, using VBS as the zero point and 0.2 ml of 2.8% red cells with 1.8 ml distilled water as 100% haemolysis.
7. Percentage haemolysis was plotted against haemolysin dilution, and haemolysin dilution read on plateau of graph as shown in Fig. 4.4. For the actual test, 2 haemolysin units were used.

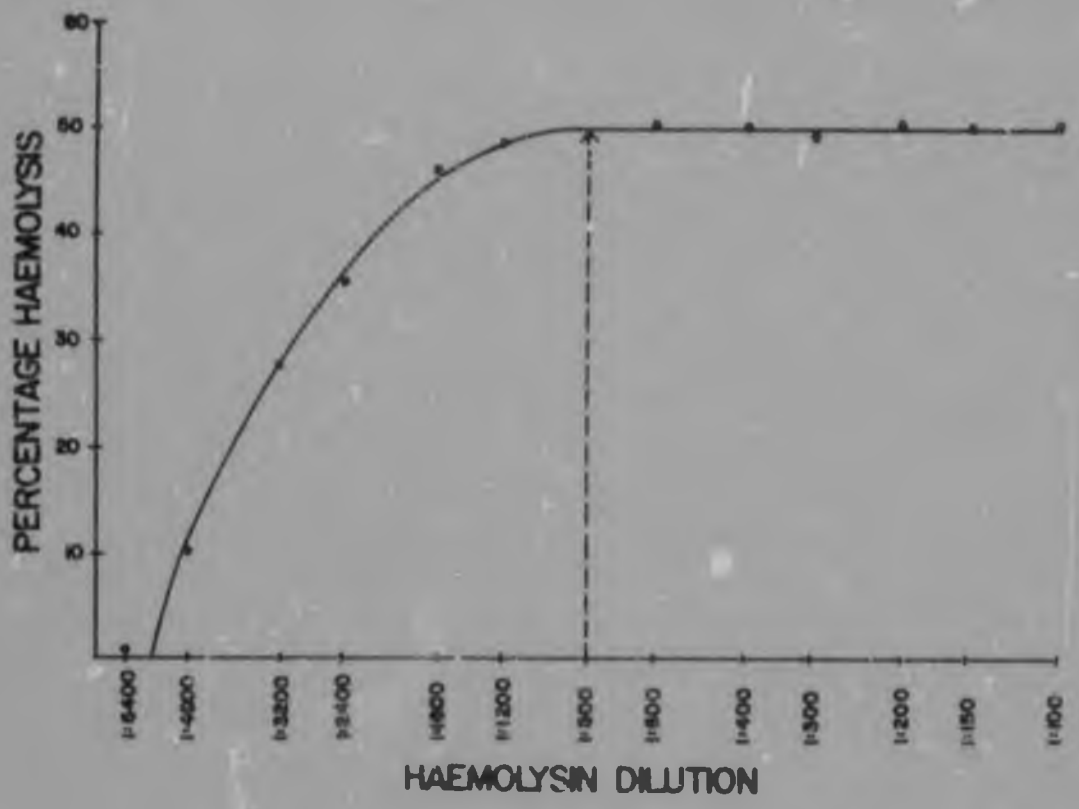


Fig. 4.4. Haemolysis titration. A 1:800 dilution is 1 haemolysin unit, and a 1:400 dilution (2 haemolysin units) final dilution should be used for the CFT.

Haemolysin titration was only necessary for each bottle of haemolysin opened, and does not need to be performed each time a CFT is done.

Sensitisation of Sheep Red Cells

Standardised 2.8% sheep red cells were added to an equal volume of haemolysin diluted in VBS to give a final concentration of 2 haemolysin units with 1.4% red cells. Red cells and haemolysin were mixed slowly and thoroughly, and allowed to stand at room temperature for 10 minutes to sensitise. Sensitised cells can be kept at 4°C for use the next day.

Complement Titration

If haemolysin titration has not been performed, excess haemolysin (eg 1:100 dilution) should be used until haemolysin titration is performed. Complement titration must be performed each time a CFT is performed.

Complement used was fresh guinea pig serum used on day of collection or within 3 weeks kept at -20°C in aliquots and thawed immediately prior to use. All manipulations of complement were carried out as quickly as possible on ice.

The method used was performed in tubes as follows:

1. Sensitised red cells were prepared as above.
2. Complement was diluted 1:5 and kept at 4°C.
3. Complement was diluted from 1:5 to 1:100, and a further series of dilutions in duplicate in 0.4 ml volumes made from the 1:100 dilution. These dilutions made were 3 doubling dilutions each from dilutions of 1:200, 1:250, 1:300 and 1:350, giving dilutions of 1:200 to 1:1400.
4. To each complement dilution, 0.4 ml VBS and 0.2 ml sensitised cells were added.
5. All tubes were mixed and then incubated for 30 mins at 37°C, shaking after 15 mins, centrifuged, and percentage haemolysis read as described under haemolysin titration.
6. The average percentage haemolysis from each pair of tubes was calculated, and the percentage haemolysis (Y-value) determined to the nearest 5% for 2 values above and 2 below 50% haemolysis. Y-values were converted to Y/100-Y values according to the conversion table (Table 4.3).
7. Complement dilutions were plotted against converted Y-values on 3-cycle log/log paper as shown in example in Fig. 4.5. The CH_{50} at the converted Y-value of 1 (50% haemolysis, was read from the line plotted and the slope of the line determined. The slope of the line should ideally be 0.18 to 0.22 for reproducibility, and fresh complement should have a CH_{50} of 1:400 to 1:600. For the CFT, $5CH_{50}$ units were used.

TABLE 4.3. Converted Y-values of percentage haemolysis (converted Y-value is % lysed /% non-lysed cells)

Percentage haemolysis (Y-value)	Converted Y-value (Y/100-Y)	Percentage haemolysis (Y-value)	Converted Y-value (Y/100-Y)
10	0.111	55	1.22
15	0.176	60	1.50
20	0.25	65	1.86
25	0.33	70	2.33
30	0.43	75	3.0
35	0.54	80	4.0
40	0.67	85	5.7
45	0.82	90	9.0
50	1.0		

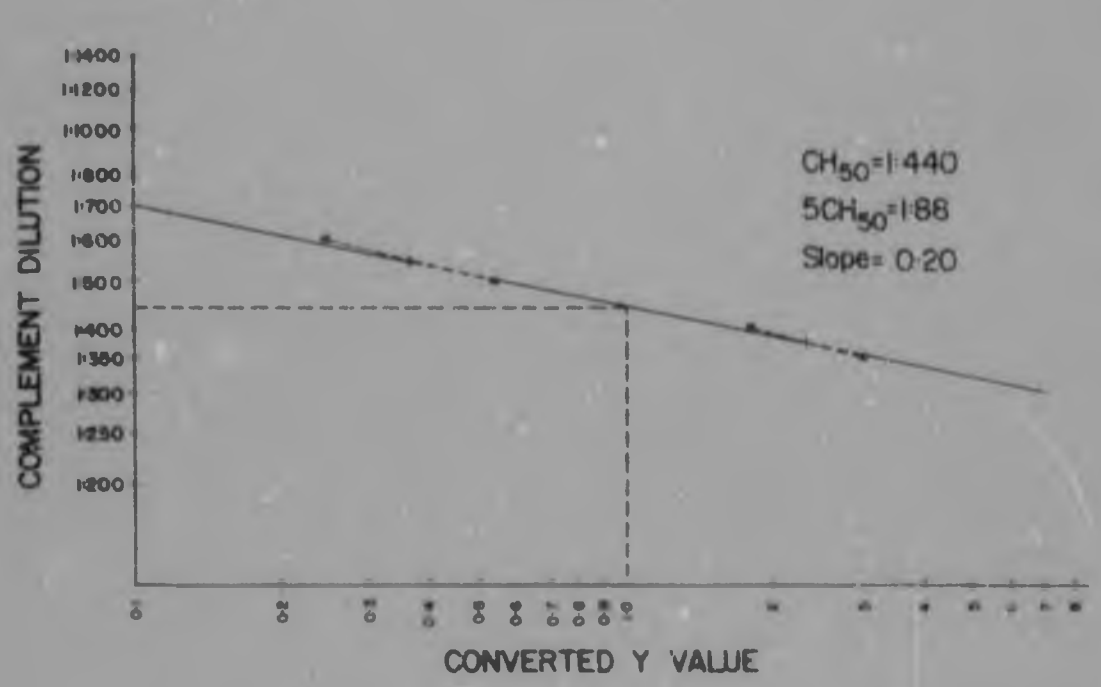


Fig. 4.5. Complement titration. The CH_{50} is 1:440, and $5CH_{50}$ (1:88) should be used.

Antigen Titration

Block antigen titration must be performed on all batches of antigen used, and typical results are shown in Fig. 4.6. Antigen titration was performed in microtitre plates as follows:

1. 0.025 ml positive control serum was added to wells 1 to 8 in row F, and negative control serum to wells 9 and 10 in row F with 0.075 ml VBS.
2. Sera were diluted with VBS in rows A to E, obtaining dilutions from 1:8 to 1:128 in 0.025 ml volumes.
3. 0.025 ml antigen was added to rows A to E and F and G as follows - antigen in 1:2 dilution to row 1, 1:4 to row 2, 1:8 to rows 3 and 9, 1:16 to row 4, 1:32 to row 5 and 1:64 to row 6. Control antigen in 1:8 dilution was added to row 7.
4. 0.05 ml of $5CH_{50}$ complement was added to all wells in row A and to rows 1 to 10 from B to E and G 0.05 ml $2.5CH_{50}$ was added to wells 1 to 10 in row H and to B 11 and 12, $1.25CH_{50}$ to C11 and 12, and 0.6 CH_{50} to D11 and 12.
5. Volumes in all wells were made up to 0.1 ml with VBS where necessary.
6. Plates were covered, mixed gently and incubated overnight at 4°C.
7. Plates were removed from the refrigerator on the next day and allowed to reach room temperature.
8. 0.025 ml sensitised red cells were added to all wells (including H12 with 0.1 ml VBS).
9. Plates were sealed, mixed well and incubated at 37°C for 30 mins, shaking after 15 mins.
10. Cells were left to settle for 2 hours, or centrifuged and read.

Plates were interpreted as follows:

4+	-	no haemolysis
3+	-	25% haemolysis
2+	-	50% haemolysis
1+	-	75% haemolysis
+	-	90% haemolysis
0	-	100% haemolysis

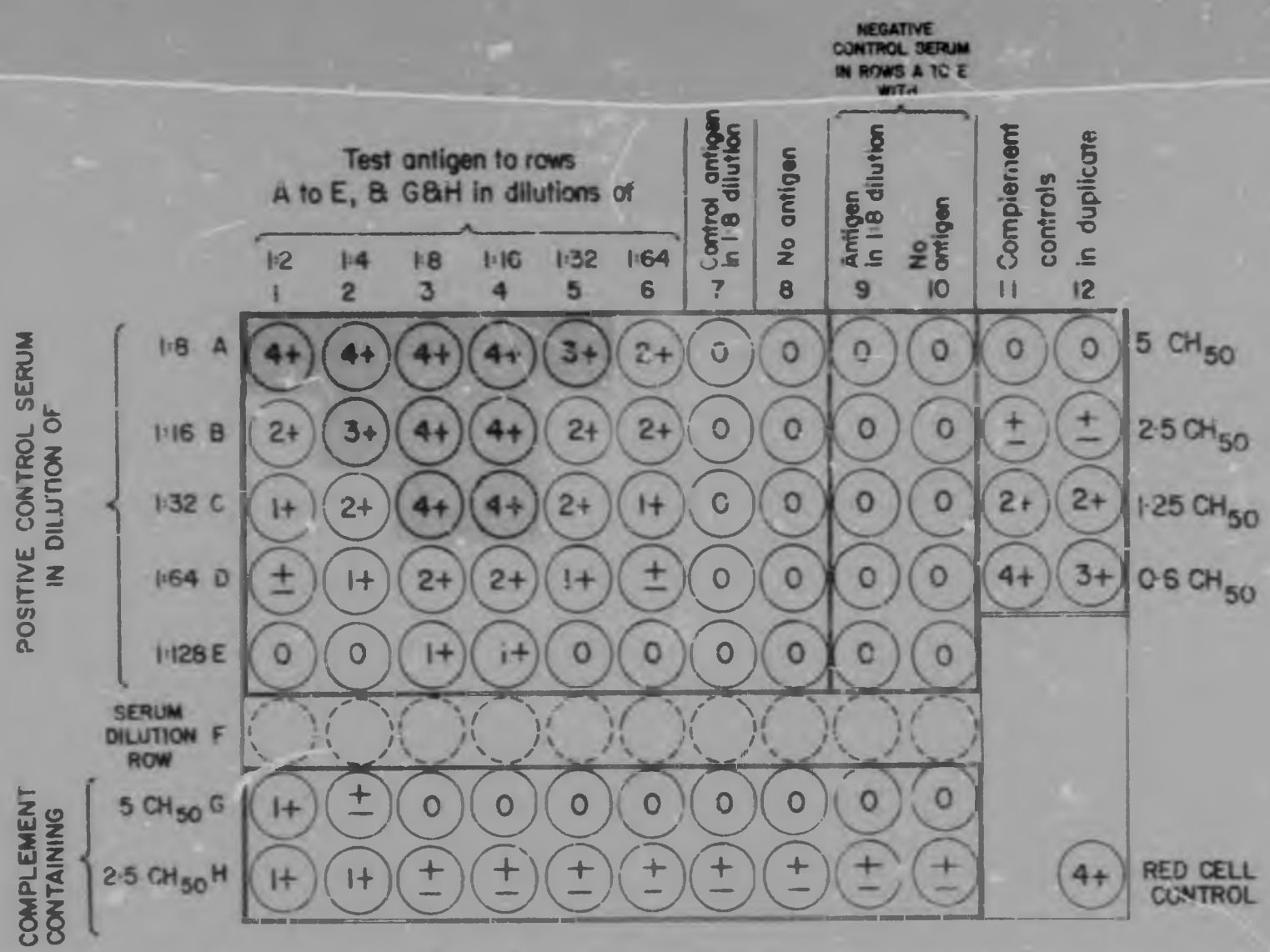


Fig. 4.6. Antigen titration performed in microtitre plates. Titration was performed in wells in the block A to E rows 1 to 6. The remainder of the wells were used for controls. Wells A to D in rows 11 and 12 show the adequacy of the amount of complement used, rows G and H the presence of any anti-complementary activity of the antigens, and rows B to 10 the presence of any anti-complementary activity of the control sera. The shaded area indicates positive results obtained with the positive control serum.

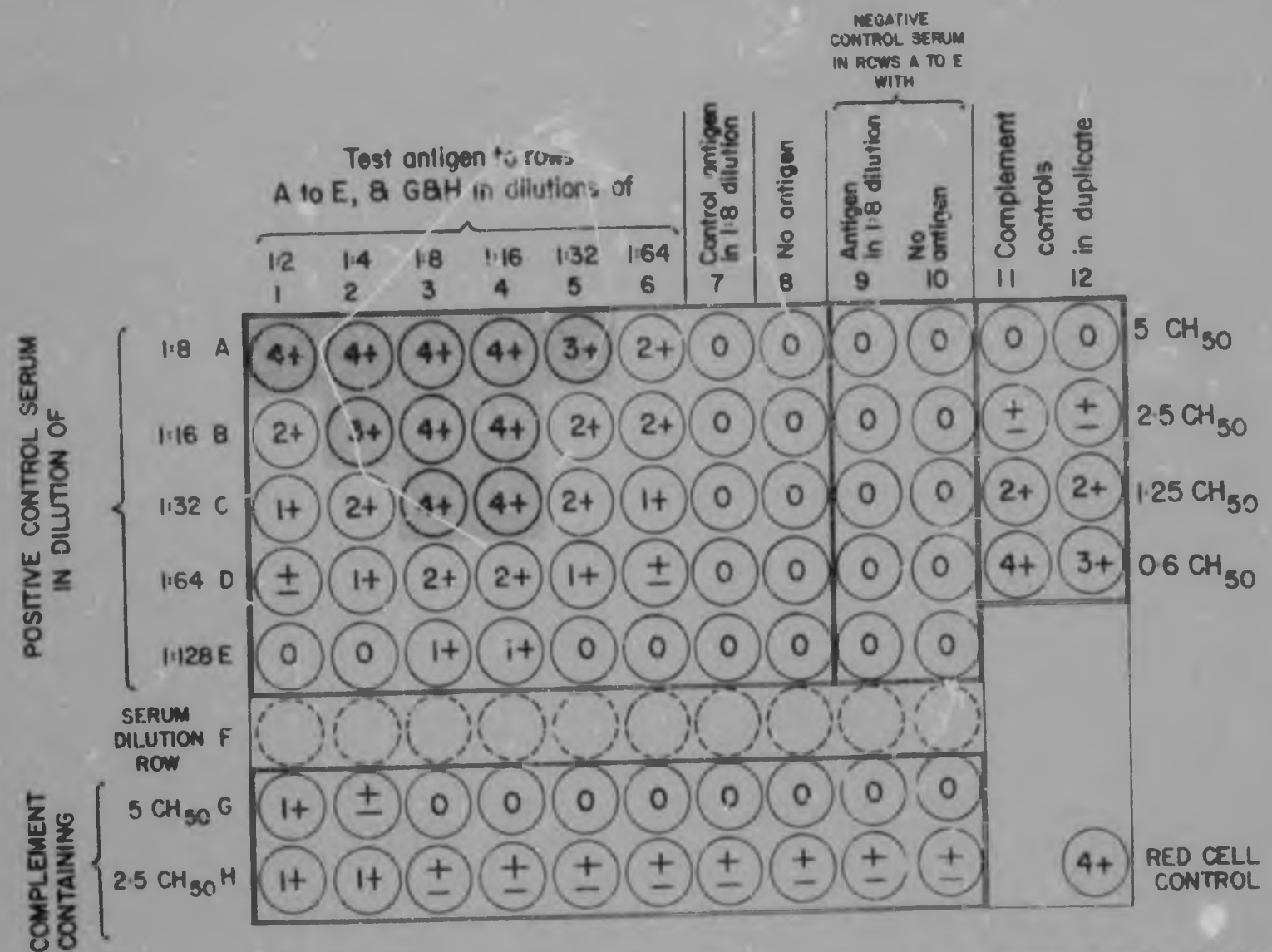


Fig. 4.6. Antigen titration performed in microtitre plates. Titration was performed in wells in the block A to E rows 1 to 6. The remainder of the wells were used for controls. Wells A to D in rows 11 ard 12 show the adequacy of the amount of complement used, rows G and H the presence of any anti-complementary activity of the antigens, and rows 8 and 10 the presence of any anti-complementary activity of the control sera. The shaded area indicates positive results obtained with the positive control serum.

In the example shown in Fig. 1.6, interpretation is as follows:

1. Red cell control (12 H) shows no haemolysis.
2. Complement controls (11 and 12 A to D) - amount of complement used was adequate and complement has not deteriorated.
3. Complement and antigen controls (G and H 1 to 7 and 9) - antigen and control antigen show no significant anti-complementary activity.
4. Serum anti-complementary controls (8 and 10 A to E) - control sera not anti-complementary.
5. False positive control (7 A to E) - positive control does not give false positive reaction with control antigen.
6. Negative control serum gives negative result with antigen in 1:8 dilution (9 A to E).
7. Positive control serum gives titre of 1:32 at antigen dilutions of 1:8 and 1:16, 1:16 at 1:4 antigen dilution and 1:8 at 1:2 and 1:32 antigen dilutions. As the expected titre is 1:32, system is working correctly and antigen should be used at 1:8 dilution.

Complement Fixation Test Performance

In microtitre plates, the CFT was performed using 0.025 ml volumes each of diluted serum, antigen and red cells, and 0.05 ml volumes of complement. Plates were set up as shown in Fig. 4.7. as follows:

1. 0.025 ml inactivated serum and 0.075 ml VBS were added to wells in row F (1:4 dilution). Positive and negative control sera were included.
2. 0.025 ml VBS was added to wells in rows A to E, and G and H.
3. Using microtitre diluters, sera were diluted from row F to rows G and H, producing 1:8 and 1:16 dilutions respectively.
4. Diluters were blotted and sera diluted from row F again into rows A to E, producing dilutions of 1:8 to 1:128.
5. 0.025 ml antigen in optimal dilution was added to wells in rows A to E.
6. 0.025 ml VBS was added to row G.
7. 0.025 ml control antigen in same dilution as test antigen was added to row H.
8. 0.05 ml complement containing $5CH_{50}$, made up from the 1:5 complement dilution was added to all wells in rows A to E and G and H.

9. Complement controls - $2.5CH_{50}$, $1.25CH_{50}$ and $0.6CH_{50}$ complement dilutions were prepared. In row A 0.05 ml $5CH_{50}$ was added to 3 wells (eg rows 10 to 12) and similarly in rows B, C and D 2.5, 1.25 and $0.6CH_{50}$ respectively were added. 0.05 ml VBS was added to the wells used in row 10, and 0.025 ml VBS to rows 11 and 12. 0.025 ml antigen was added to row 11 and 0.025 ml control antigen to row 12.
10. Plates were covered, mixed gently and incubated overnight at 4°C.
11. Plates were removed from the refrigerator on the next day and allowed to reach room temperature.
12. 0.025 ml sensitised red cells were added to each well and to a control well with 0.1 ml VBS (eg row 12 H).
13. Plates were sealed, mixed well and incubated at 37°C for 30 mins, shaking after 15 mins.
14. Cells were left to settle for 2 hours, or centrifuged, and read. CFT microtitre plates were read as follows:

4+	-	no haemolysis
3+	-	25% haemolysis
2+	-	50% haemolysis
1+	-	75% haemolysis
±	-	90% haemolysis
0	-	complete haemolysis

Interpretation of CFT:

1. Red cell control (12 H) should read 4+ (no haemolysis).
2. Complement controls (row 10)
   - Row A ($5CH_{50}$) should read 0 (complete haemolysis)
   - Row B ($2.5CH_{50}$) should read 0 to ±
   - Row C ($1.25CH_{50}$) should read ± to 2+
   - Row D ($0.6CH_{50}$) should read 3+ to 4+
3. Antigen and complement controls in rows 11 and 12 should read the same as row 10 if antigen and control antigen are satisfactory and are not anti-complementary.
4. Serum anti-complementary controls - all wells in row G should read 0. Any sera showing 1+ to 4+ are anti-complementary.
5. Serum false positive controls - all wells in row H should read 0. Any sera showing 1+ to 4+ are giving false positive results.
6. Positive control serum should give 3+ to 4+ reading within 1 dilution of correct titre, and negative control serum should be 0 throughout.

7. Unknown sera can then be read as positive at the highest dilution showing a 3+ to 4+ reading.
8. Anti-complementary sera can be treated with an equal volume of 1:5 complement at 37°C for 30 mins and inactivated again at 56°C for 30 mins, and then retested.

Interpretation of the example shown in fig. 4.7:

Red cell control is correct.
Complement controls are correct (10 A to D) and antigens are not anti-complementary (11 and 12 A to D).
Anticomplementary controls - serum in row 5 is anti-complementary.
False positive controls - serum in row 8 is giving a false positive result.
Control sera are correct (expected titre of positive serum is 1:32).
Serum in row 3 is positive, titre 1:16.
Sera in rows 4, 6, 7 and 9 are negative.

Many problems were encountered in the performance of the CFT. Reagents other than antigens and control sera were readily available, but antigens and control sera could only be obtained from Behringwerke, the only other manufacturer being Italdiagnostics which was not represented in South Africa. Initially, when the CFT was set up the positive control serum was found to be highly anticomplementary. This problem was overcome by treating the control serum with complement as described in 8 above, and the test then performed well and gave clear results. At that stage antigen batch A31B, expiry July 1975, was being used, and was still giving good results up to early in 1976. At that stage batch 3539C, expiry May 1977, was obtained and tested in parallel with the old batch. The new antigen was found to be significantly anticomplementary.

Early in the study, the husband of one of the patients who seroconverted in the pregnancy study was also found to be CFT positive at a titre of 1:16, and from then on his serum, which was frozen in small aliquots, was used as a positive control, and remained satisfactory for 3 years before becoming anticomplementary.

The manufacturers of the unsatisfactory antigen and control serum, Behringwerke, were informed of these problems and this led to my visiting their manufacturing plant in Marburg. Behringwerke agreed that the products were anticomplementary in the microtitre CFT, but stated that

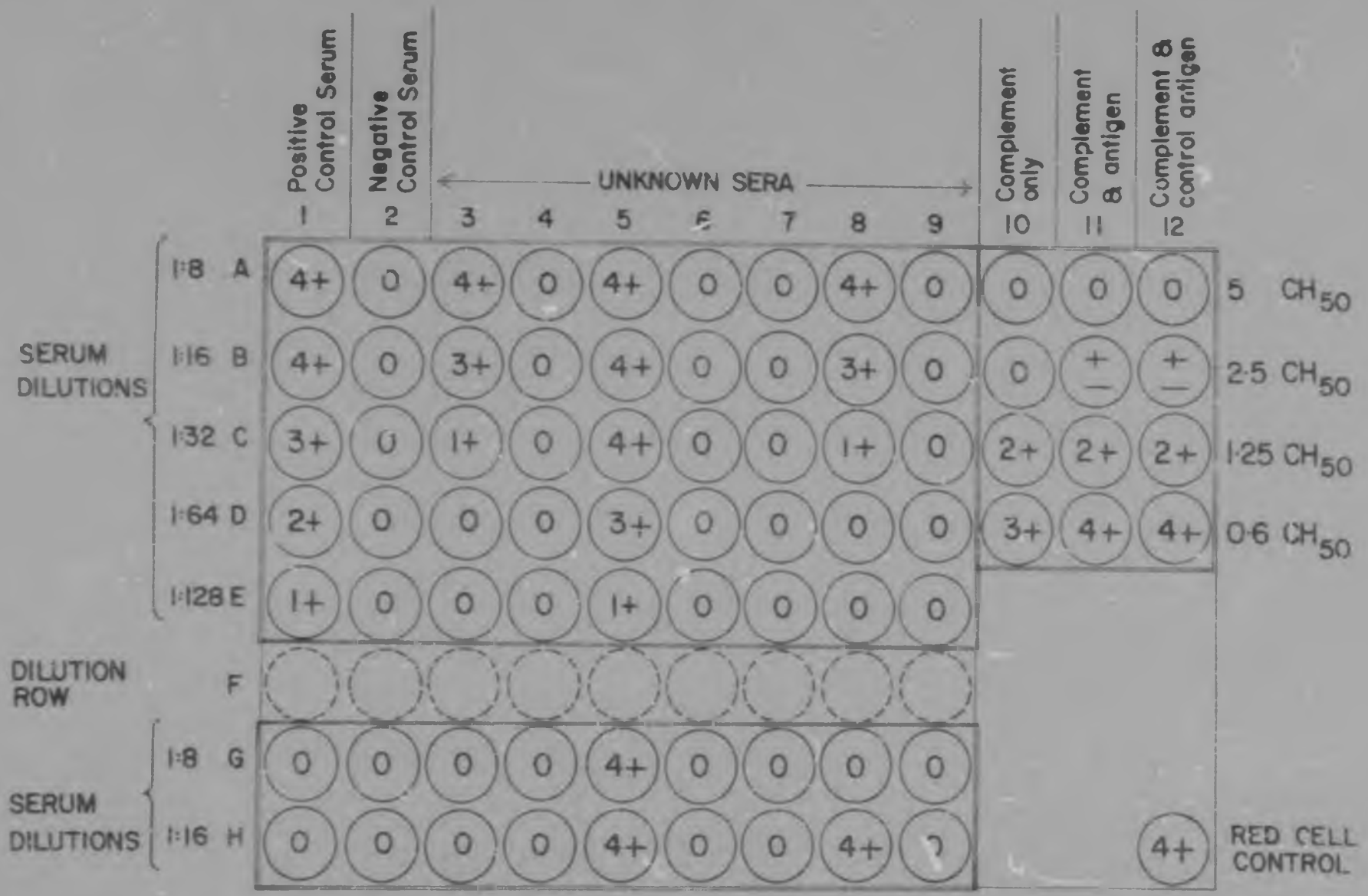


Fig. 4.7. Layout of microtitre CFT plate, showing the testing of 7 unknown sera with all necessary controls.

this did not occur when they used their antigen in the macro method. They could not explain the differences between antigen batches on their manufacturing procedures, and could not explain either why the macro and micro methods should give different results. They did not find the positive control serum to be anticomplementary in their laboratories, and they considered this to be caused by problems in transport to South Africa.

Fortunately, the manufacturers had a supply of the A32B batch of antigen, and this was used and remained satisfactory despite being used for 3 years past expiry date.

4.1.3 Haemagglutination Test

The indirect haemagglutination (IHA) test was performed using the 'Tox HA test' haemagglutination kit (Wellcome Reagents). The test uses sonicated soluble extracts from endozoites to sensitise formalin-treated tanned turkey red blood cells. The turkey red cells were provided lyophilized, and unsensitised cells were provided as controls for non-specific haemagglutination. Positive and negative control sera and buffer were also provided. After reconstitution, the red cells must be used within 8 hours, or they can be frozen at -20°C for up to a month and thawed before use. Sera to be tested should be inactivated at 56°C for 30 mins.

The test was performed in V-bottom microtitre plates as follows:

1. Four-fold dilutions of test and control sera were prepared in wells of V-bottom plates in 0.1 ml volumes. Two wells containing serum dilutions of 1:16, and 1 well containing serum dilutions of 1:64, 1:256, 1:1024 and 1:4096 were prepared.
2. 0.025 ml test cells were added to all wells from 1:16 to 1:4096 and to a blank well.
3. 0.025 ml control cells were added to the second 1:16 serum dilution row and to a blank well.
4. Plates were tapped to suspend cells evenly and left covered for at least an hour.
5. Positive results showed an agglutination pattern forming a carpet or thin ring of cells. Negative results showed a button of cells in the centre of the well. Control cells should show no agglutination, and if agglutination occurs, the test cannot be read.

6. Positive and negative sera should give correct results and cell controls should show clear buttons.

The haemagglutination test was used to test sheep and mouse sera as problems with the IFA technique were expected. The test did not, however, perform well and difficulty was experienced in reading end-points, which tended to fade out indistinctly rather than giving a clear-cut end titre. This was particularly noticeable for sheep sera, and the kit does not appear to be very suitable for testing sera from these animals. In view of the high cost of the IHA kits, human sera were not tested by this method.

4.2 Total IgM Determination

IgM levels were determined by radial immunodiffusion according to the method described by Shulman (1973). Wells, 2 mm in diameter, were cut in 9 cm disposable petri dishes, and 52 wells could be cut per plate. Plates contained 8 ml of 1% purified agar (Behringwerke) made up in barbitone buffer (1.38 g barbitone and 4.3 g sodium barbitone per litre) and containing a 0.1 ml of anti-IgM serum (Dako Immunochemicals, Copenhagen, Denmark). 0.1 ml was found to be the optimal volume of anti-IgM serum for use when different volumes were tested against control sera. Wells were filled with 5 µg of serum and plates read after 24 hours at room temperature.

Ring diameters of unknown and control sera (6 dilutions of a reference serum applied twice per plate) were read on a viewing box. Callibration curves of the standards on each plate were drawn up on 3 cycle semilogarithmic paper, and values of test sera read off (Fig. 4.8).

4.3 Isolation in Mice

Isolation of *Toxoplasma* in mice was attempted on specimens of human placenta and sheep diaphragm.

Specimens were refrigerated in saline containing penicillin G (25 units/ml) and streptomycin (25 µg/ml) for a maximum of 3 days before being processed.

For placental specimens, a small piece of placenta was ground in a Griffith's tube in a small volume of saline, and 1 ml injected intraperitoneally into 4 mice.

For sheep diaphragms, about 50 g was cut up finely with scissors and digested with 500 ml of artificial gastric juice containing 1.3 g of pepsin powder 1:10 000, 2.5 g NaCl and 3.5 ml concentrated HCl (Work, 1971).

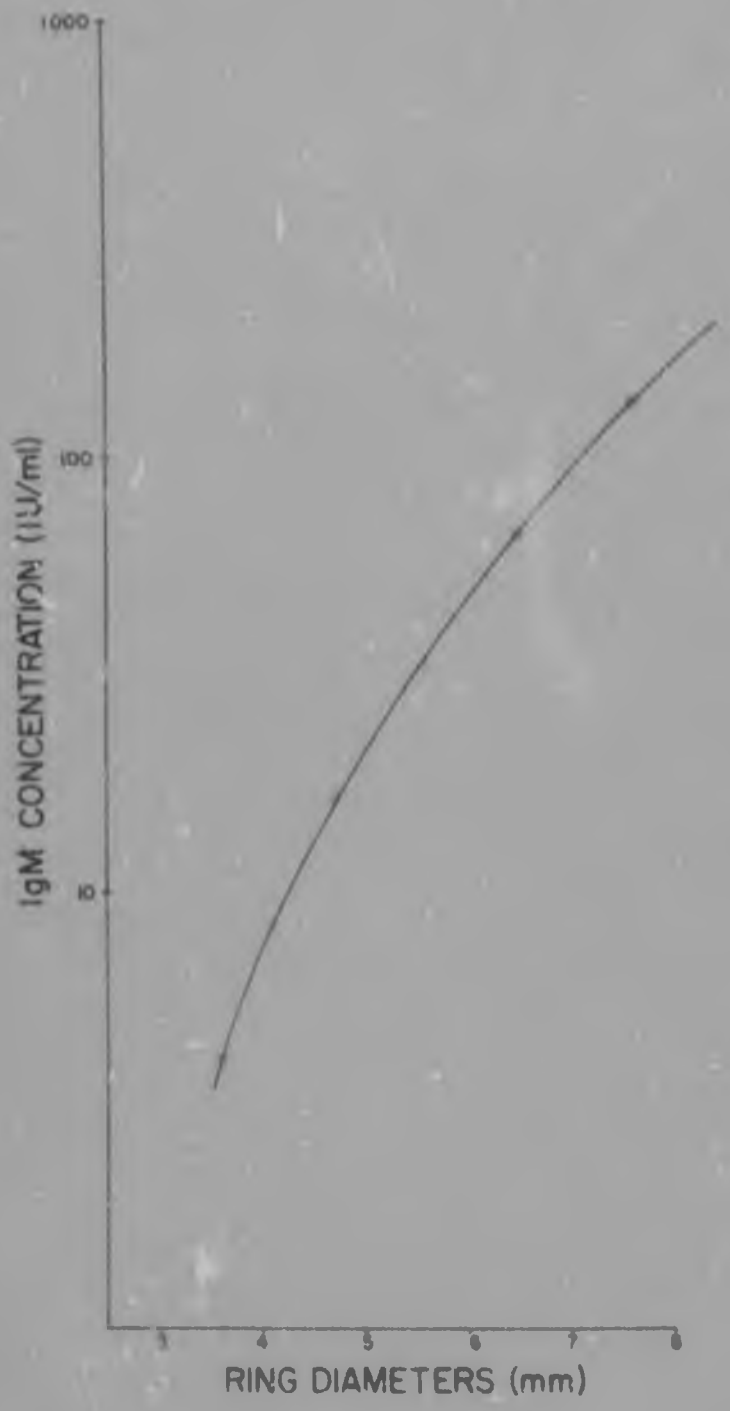


Fig. 4.8. Cord blood total IgM level calibration curve.

Digestion was allowed for 2 hours at 37°C in a shaking apparatus in a water bath, and the suspension was then filtered through a double layer of gauze and centrifuged for 15 mins at 2 000 rpm. The supernatant was poured off and the sediment resuspended in PBS, recentrifuged, and finally suspended in 5 ml of PBS. Four mice were each inoculated intraperitoneally with 1 ml of the suspension.

Two mice from each group were bled and killed after a week and peritoneal smears stained with Giemsa stain for organisms. The other 2 mice were bled and killed after 6 to 8 weeks, and crush and smear brain preparations were examined for cysts, and brain, liver and spleen passaged into a further 2 mice per organ. Passaged animals were examined after 6 to 8 weeks and discarded if negative after the third passage. IFA tests were performed on all mouse sera as described in 4.1.1.

4.4. Lymph Node Examination

Lymph node sections were examined by routine haematoxylin and eosin staining and by direct immunofluorescence.

As lymph nodes had been formalin fixed, they were processed according to the method of Huang *et al.* (1976) as follows. Four µm sections were cut from paraffin blocks and placed on slides (the method described by Huang *et al.* used a resin to bind the section to the slide, but this was omitted as the resin was unobtainable). Sections were dried, deparafinized and rehydrated, and digested in trypsin solution for 2 hours (trypsin solution made up of 0.1% trypsin powder, 0.1% $CaCl_2$ in distilled water, pH adjusted to 7.8 with 0.1 N NaOH). After digestion, sections were washed in distilled water and then immersed in PBS for 30 mins.

Digested tissues were then encircled with teflon and direct anti-*Toxoplasma* conjugate (Wellcome Reagents) in 1:50 dilution was placed on the sections for 60 mins at 37°C in a humid chamber. Direct conjugate was obtained from Wellcome Reagents. A portion of this conjugate was absorbed out with organisms used in the IFA test to remove anti-*Toxoplasma* antibodies. 0.5 ml of the conjugate required absorption with 30 million organisms before absorption was complete. The absorbed conjugate was used in parallel with the conjugate, and direct fluorescence was only regarded as positive when fluorescence occurred with the conjugate and not with the absorbed conjugate. Conjugate and absorbed conjugate were controlled by application to antigen slides used for the IFA test, and fluorescence occurred with the conjugate up to 1:8000 dilution, but not in the absorbed conjugate at 1:10 dilution. Omission of the use of the resin in the method followed did not appear to affect the performance of the method, and the tissue sections remained on the slides.

5.0 PREVALENCE OF *TOXOPLASMA* ANTIBODIES IN SOUTHERN AFRICA

As the prevalence of *Toxoplasma* antibodies in Southern Africa had not been studied, information on seropositivity rates and on the interpretation of serology on individual patients was therefore not available. The only published study was a skin test survey in Blacks in the Transvaal conducted by Schneider *et al.* (1955), in which 31% of persons tested were found to be toxoplasmin positive.

In view of this almost complete lack of information on toxoplasmosis in Southern Africa, I undertook serological surveys for toxoplasmosis in 5 major areas of Southern Africa. These surveys were carried out in the Parasitology Department in collaboration with Dr PR Mason. The IFA technique was chosen for these surveys in view of its simplicity, whilst giving results similar to those obtained in the dye test (Fletcher, 1965).

These surveys, on populations representative of the different areas and ethnic groups in Southern Africa, were conducted during the period 1973 to 1977, and the results are shown in this Chapter. The studies undertaken surveyed 806 persons in the Transvaal in 1973, 3379 persons in the Cape, Natal and South West Africa during 1974 to 1976, and 6705 patients tested at delivery in the Transvaal during 1975 to 1978. The only Province in South Africa not surveyed was the Orange Free State as this was done by Brink *et al.* (1975) in 1974, and these results are used for comparative purposes.

5.1 Patients and Methods

Sera were obtained from healthy persons in the community during employment or antenatal screening, from blood donors at blood transfusion centres, from research surveys to South West Africa and Botswana, from cat show exhibitors and from medical students. Sera were stored at -20°C until tested, and were tested by the IFA method as described in Chapter 4. Differences in prevalence were compared using the χ^2 test of significance.

5.2 Results

5.2.1 Transvaal

In the original survey of 806 sera, 37% (296) were positive in the IFA test at a dilution of ≥1:16. The results were analysed according to ethnic group, age and sex, and are shown in Tables 5.1 to 5.4.

TABLE 5.1. *Toxoplasma* IFA titres in different ethnic groups in first Transvaal study

Titre	Black	White	Coloured	Indian	Total
1:16	36	62	92	18	208
1:64	26	13	14	6	59
1:128	11	4	8	1	24
1:256	1	0	2	0	3
1:512	1	0	1	0	2
Totals	75	79	117	25	296
Sample size	255	236	272	43	806
Percentage +ve	29	33	43	58	37

TABLE 5.2. *Toxoplasma* IFA titres for different age groups in first Transvaal study

Titre	Age (years) 0-20	21-30	31-40	41-50	>50	Unknown	Total
1:16	21	60	36	28	13	50	208
1:64	4	16	9	5	3	22	59
1:128	6	2	3	2	3	8	24
1:256	0	0	1	0	0	2	3
1:512	0	0	0	1	0	1	2
Totals	31	78	49	36	19	83	296
Sample size	102	225	128	79	54	218	806
Percentage +ve	30	35	38	46	35	38	37

TABLE 5.3. Prevalence of *Toxoplasma* antibodies in different ethnic groups in first Transvaal study - age differences

Age group	White	Black	Coloured	Indian
0-20	16% (13)*	17% (49)	43% (23)	79% (15)
21-30	31% (51)	26% (73)	42% (84)	47% (17)
31-40	48% (23)	18% (22)	39% (80)	100% (3)
41-50	44% (18)	14% (5)	47% (55)	- (1)
>50	27% (22)	17% (6)	43% (21)	60% (5)

* Sample size in brackets

TABLE 5.4. *Toxoplasma* IFA titres in first Transvaal study - sex differences

Titre	Female	Male	Unknown	Total
1:16	114	94	0	208
1:64	23	36	0	59
1:128	12	11	1	24
1:256	2	1	0	3
1:512	1	1	0	2
Totals	152	143	1	296
Sample size	439	364	3	806
Percentage +ve	35	39	-	37

In this survey, the highest prevalence was in Indians (58%), although the sample size was only 43. Prevalence in Blacks and Whites showed no significant differences, but significant differences were found between the prevalence in these 2 groups combined and the prevalence in Coloureds ($p < 0.005$) and in Indians ($p < 0.001$) (Table 5.1).

IFA titres in the different age groups showed an increase from 30% under the age of 20 years to 46% in the 41-50 year group. The over 50-year group had a prevalence of 35% (Table 5.2).

The prevalence in males (39%) was consistently but not significantly higher than in females (35%).

Twenty-nine sera from Whites were collected from cat-show exhibitors, and 16 (55%) were positive, but the numbers in this group were too small for meaningful analysis.

As part of the screening survey for congenital toxoplasmosis in 3 Johannesburg Hospitals, 6705 patients were tested at delivery. These patients consisted of 2001 Whites at the Queen Victoria Maternity Hospital, 3659 Blacks at the Baragwanath Maternity Hospital, and 901 Coloureds and 144 Indians at the Coronation Hospital. Twenty-two percent of these patients were seropositive (1473/6705) as shown in Table 5.5, and the age distribution in 5680 of these patients is shown in Table 5.6 and distribution of titres in Table 5.7.

The Black, Coloured and Indian groups had practically no differences in prevalence (25%), which was significantly higher than that found in Whites (14%) ($p < 0.001$). Prevalence in all groups rose with age.

TABLE 5.5. Ethnic distribution of maternity patients with positive IFA tests in 3 Transvaal hospitals

Race	No. Positive	Percentage Positive	Sample Size
White	286	14.3%	2001
Coloured	236	26.2%	901
Indian	36	25.0%	144
Black	915	25.0%	3659
TOTAL	1473	22.0%	6705

TABLE 5.6. Age distribution of maternity patients with positive IFA tests in 3 Transvaal hospitals

QUEEN VICTORIA HOSPITAL

Age (yrs)	No. Positive	Sample Size	Percentage Positive
< 20	77	672	11.5%
21-25	78	569	13.7%
26-30	59	311	19.0%
31-35	28	124	22.6%
> 35	15	57	26.3%
TOTAL	257	1733	14.8%

CORONATION HOSPITAL

< 20	51	249	20.5%
21-25	78	318	24.5%
26-30	52	181	28.7%
31-35	20	61	32.8%
> 35	19	54	35.2%
TOTAL	220	863	25.5%

BARAGWANATH HOSPITAL

< 20	171	859	19.9%
21-25	282	1180	23.9%
26-30	189	647	29.2%
31-35	71	217	32.7%
> 35	65	181	35.9%
TOTAL	778	3084	25.2%

TOTALS

< 20	299	1780	16.8%
21-25	438	2067	21.2%
26-30	300	1139	26.3%
31-35	119	402	29.6%
> 35	99	292	33.9%
TOTAL	1255	5680	22.1%

TABLE 5.7. Distribution of maternity patients with positive IFA tests according to titre in 3 Transvaal hospitals

IFA Titre	HOSPITAL						TOTAL	
	QUEEN VICTORIA		BARAGWANATH		CORONATION			
	No.positive	%	No.positive	%	No.positive	%	No.positive	%
1:16	87	4.3	378	10.3	95	9.0	560	8.4
1:32	99	5.0	200	5.5	55	5.3	354	5.3
1:64	70	3.5	118	3.2	49	4.7	237	3.5
1:128	21	1.0	98	2.7	33	3.2	152	2.3
1:256	7	0.35	66	1.8	22	2.1	95	1.4
1:512	1	0.05	30	0.8	9	0.9	40	0.6
1:1024	1	0.05	20	0.5	6	0.6	27	0.4
1:2048	-	-	2	0.05	2	0.2	4	0.06
1:4096	-	-	2	0.05	1	0.1	3	0.04
>1:4096	-	-	1	0.03	-	-	1	0.015
Total positives	286	14.3	915	25.0	272	26.0	1473	22.0
Sample size	2001	100	3659	100	1045	100	6705	100

The results of both Transvaal surveys were combined and are shown in Table 5.8 and Fig. 5.1 according to age and ethnic group. The difference in prevalence between Whites and the other ethnic groups combined was significantly different ($p < 0.001$), and between Blacks compared with Coloureds and Indians was also significantly different ($p < 0.001$). The percent annual seroconversion rate, using figures calculated by Frenkel (1973a) was 1%.

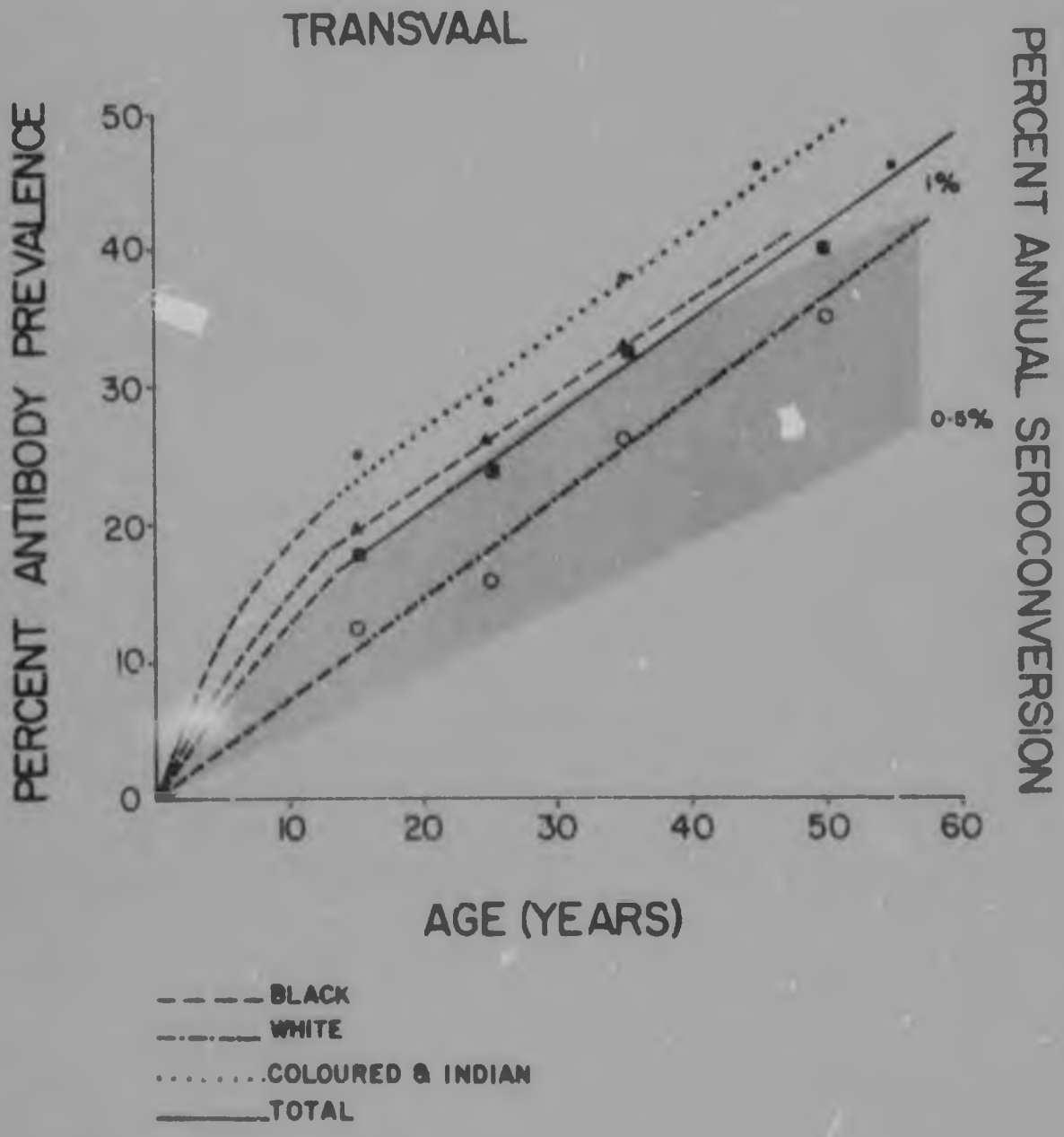


Fig. 5.1. Prevalence of *Toxoplasma* antibodies in the Transvaal. The shaded area indicates expected antibody prevalence at annual seroconversion rates of between 0.5 and 1%.

TABLE 5.8. Prevalence in the Transvaal of persons with positive IFA tests according to age and ethnic group - combined Transvaal studies.

Ethnic Group	Age (years) 0-20	21-30	31-40	41-50	>50	Total
White	12% (685)*	16% (931)	26% (204)	44% (18)	27% (22)	16% (1860)
Black	20% (908)	26% (1900)	33% (420)	14% (7)	17% (6)	25% (3241)
Coloureds & Indians	25% (287)	29% (600)	38% (198)	46% (56)	46% (26)	31% (1167)
TOTAL	18% (1880)	24% (3431)	33% (822)	43% (81)	35% (54)	23% (6268)

* Sample sizes given in brackets

5.2.2 Natal

In this survey, 30% of 635 sera were positive in the IFA test, and the ethnic and age group distribution of results are shown in Table 5.9 and Fig. 5.2. The difference between the prevalence in Whites compared to that in Blacks and Indians is significant ($p < 0.01$). The percentage annual seroconversion rate in Whites was about 0.75%, while in the other groups it was almost 2%.

TABLE 5.9. Prevalence of *Toxoplasma* antibodies in Natal by age and ethnic group

Ethnic Group	Age (years) 0-20	21-30	31-40	41-50	>50	Total
White	9% (23)*	24% (83)	19% (59)	21% (47)	40% (45)	24% (257)
Black	30% (110)	40% (68)	27% (30)	50% (8)	80% (5)	34% (221)
Indian	28% (43)	34% (74)	45% (31)	13% (8)	100% (1)	34% (157)
TOTAL	27% (176)	32% (225)	28% (120)	24% (63)	45% (51)	30% (635)

* Sample sizes given in brackets

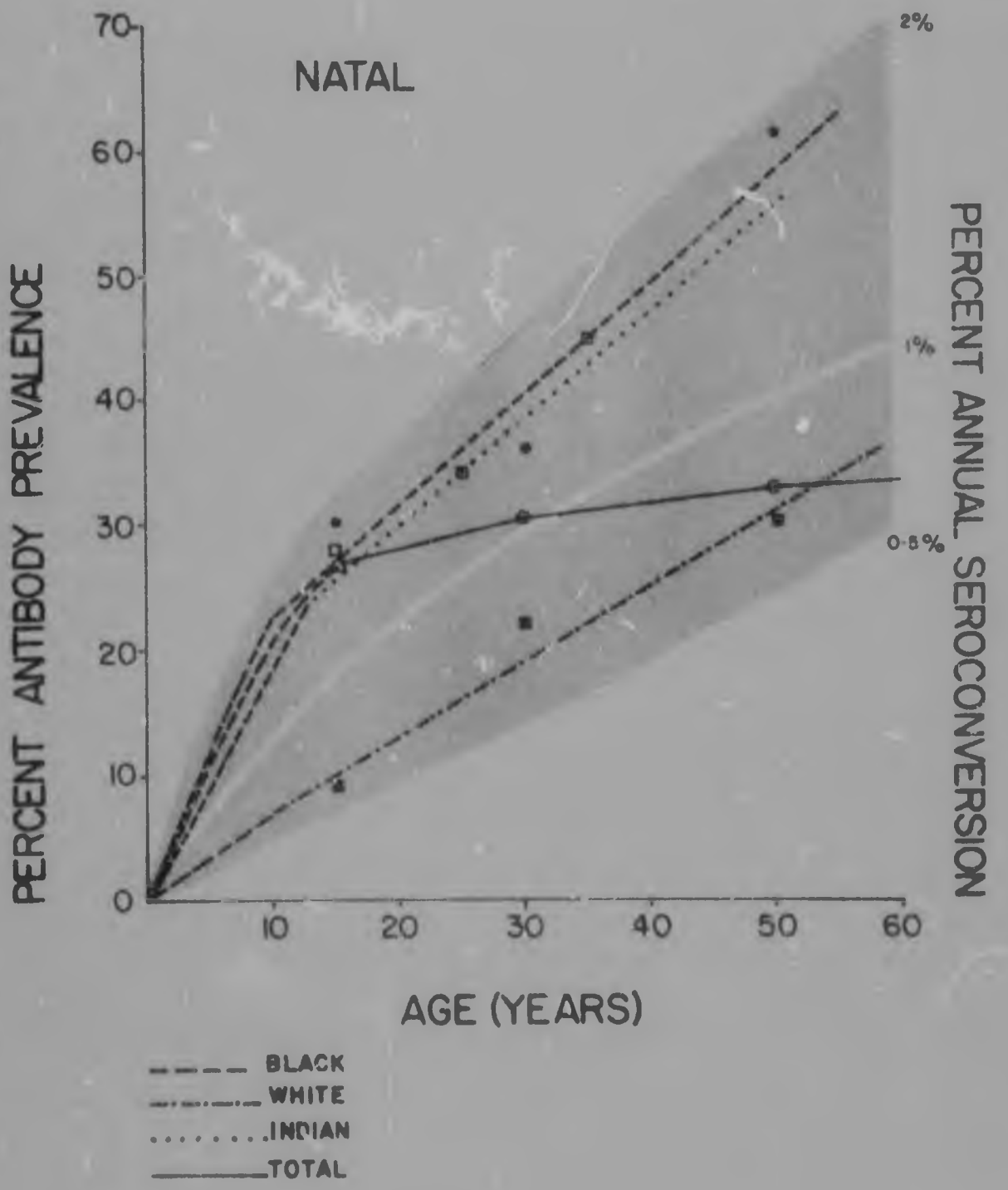


Fig. 5.2. Prevalence of *Toxoplasma* antibodies in Natal.

5.2.3 Eastern Cape

The overall prevalence in this region was 24% in the 970 sera tested, and results are shown in Table 5.10 and Fig. 5.3. Again the difference in prevalence between Whites and the other groups is significant ($p < 0.001$).

TABLE 5.10. Prevalence of *Toxoplasma* antibodies in the Eastern Cape by age and ethnic group

Ethnic Group	Age (years) 0-20	21-30	31-40	41-50	>50	Total
White	21% (24)*	12% (151)	14% (105)	21% (84)	31% (26)	17% (400)
Black	20% (55)	28% (97)	36% (64)	29% (31)	50% (18)	30% (265)
Coloured	45% (29)	23% (131)	30% (83)	35% (49)	23% (13)	29% (305)
TOTAL	27% (108)	20% (379)	25% (252)	27% (164)	34% (67)	24% (970)

* Sample sizes given in brackets

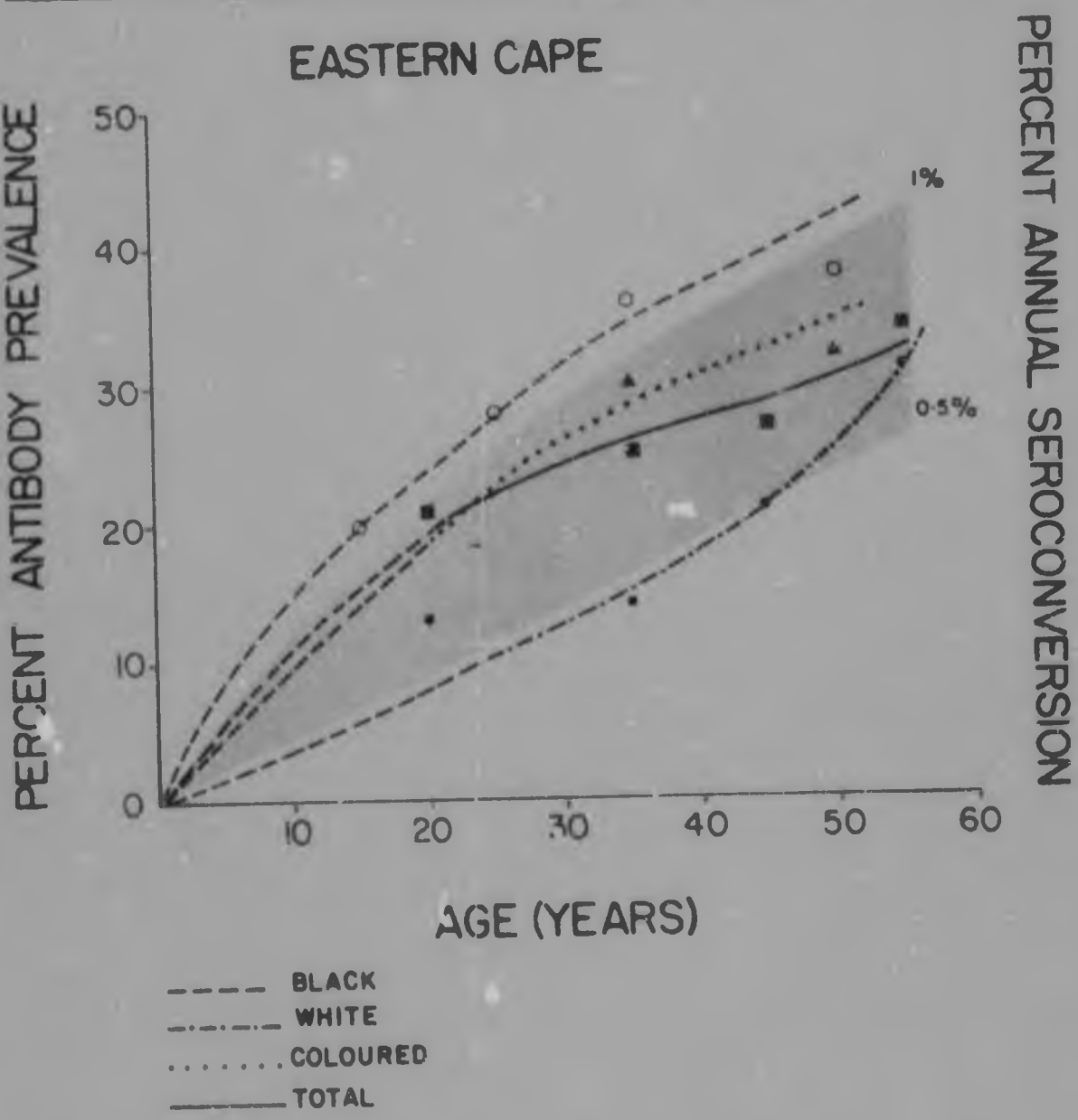


Fig. 5.3. Prevalence of *Toxoplasma* antibodies in the Eastern Cape.

5.2.4 Western Cape

The overall prevalence in this region, which has mainly Whites and Cape Coloureds, was 18% of 683 sera tested, and results are shown in Table 5.11 and Fig. 5.4. Again the prevalence in Whites is significantly lower than in Coloureds ($p < 0.001$), but the percentage annual seroconversion over the age of 20 years is extremely low, unlike the other regions where this continues to rise with age.

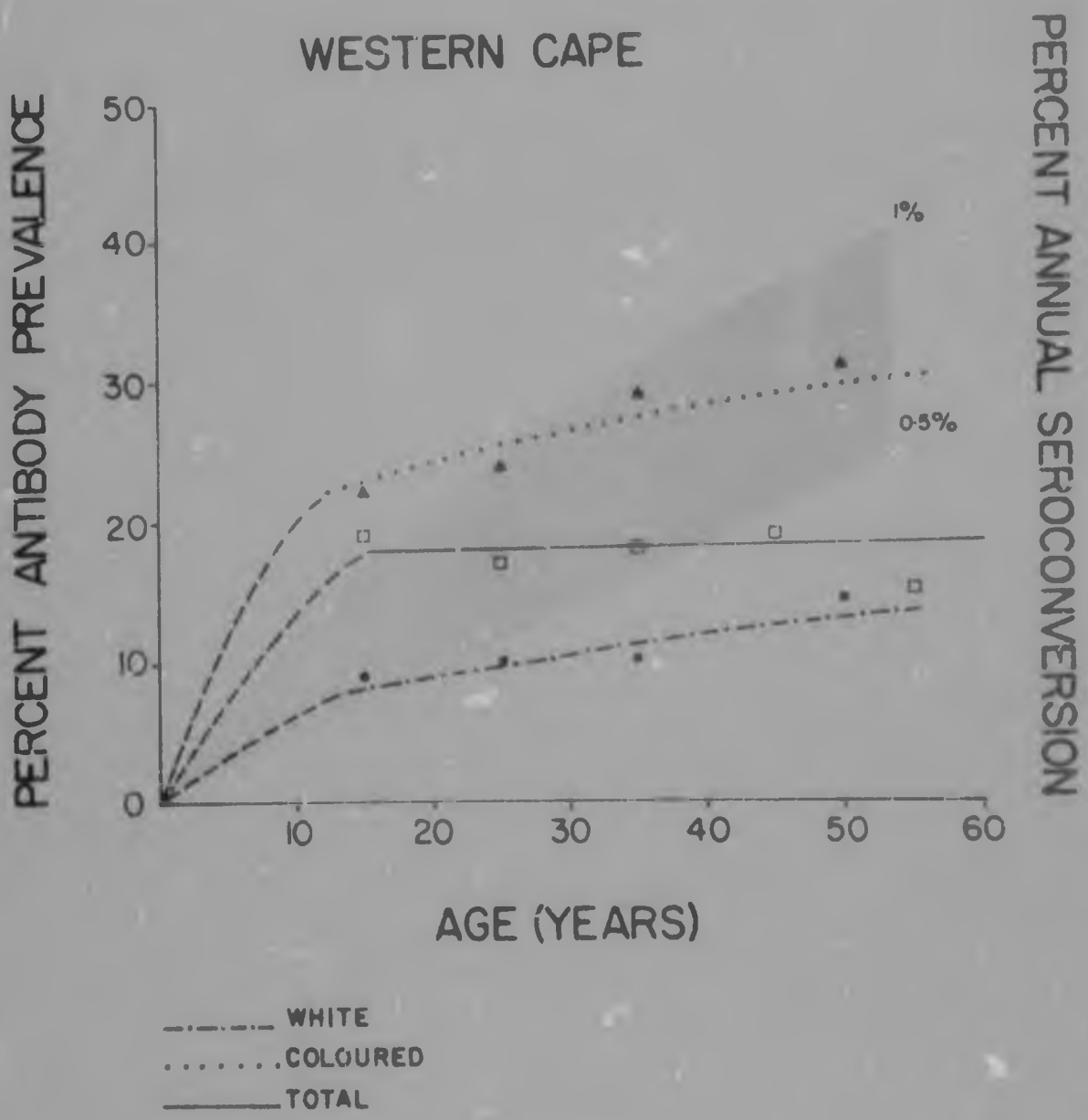


Fig. 5.4. Prevalence of *Toxoplasma* antibodies in the Western Cape.

TABLE 5.11. Prevalence of *Toxoplasma* antibodies in the Western Cape by age and ethnic group

Ethnic Group	Age (years) 0-20	21-30	31-40	41-50	>50	Total
White	9% (53)*	10% (134)	10% (62)	18% (33)	11% (36)	11% (318)
Coloured	22% (145)	24% (151)	29% (45)	21% (19)	40% (5)	24% (265)
TOTAL	19% (198)	17% (285)	18% (107)	19% (52)	15% (41)	18% (683)

* Sample sizes given in brackets

5.2.5 South West Africa and Botswana

Sera from persons in these regions were obtained from research surveys undertaken to study the San (Bushmen) and Dama (Negroid) of the Kalahari Desert by Professor T Jenkins. Sera from Whites were obtained from healthy residents of the Windhoek area. Results are shown in Tables 5.12 and 5.13 and Figs. 5.5 and 5.6. Prevalence in the San is similar to that in Whites, but in both is significantly lower than in the Dama ($p < 0.001$).

TABLE 5.12. Prevalence of *Toxoplasma* antibodies by ethnic group in South West Africa and Botswana

Ethnic Group	% Positive	Sample Size
San		
!Kung - Tsumkwe	6%	275
!Kung - Dobe	9%	186
!x$\ddot{o}$ and ǂhuã	9%	82
G/wi and G/ana	12%	182
Total San	9%	725
Dama	27%	77
White	12%	261
TOTAL	11%	1063

TABLE 5.13. Prevalence of *Toxoplasma* antibodies by age and ethnic group in South West Africa and Botswana (ages were available in 700 of the 1063 sera tested, and were not known for the Dama)

Ethnic Group	Age (years) 0-20	21-30	31-40	41-50	>50	Total
White	9% (35)*	9% (95)	9%(54)	12%(34)	23%(39)	12%(257)
San	5%(151)	7%(111)	10%(80)	16%(57)	14%(44)	9%(443)
TOTAL	6%(186)	8%(206)	10%(134)	14%(91)	18%(83)	10%(700)

* Sample sizes given in brackets

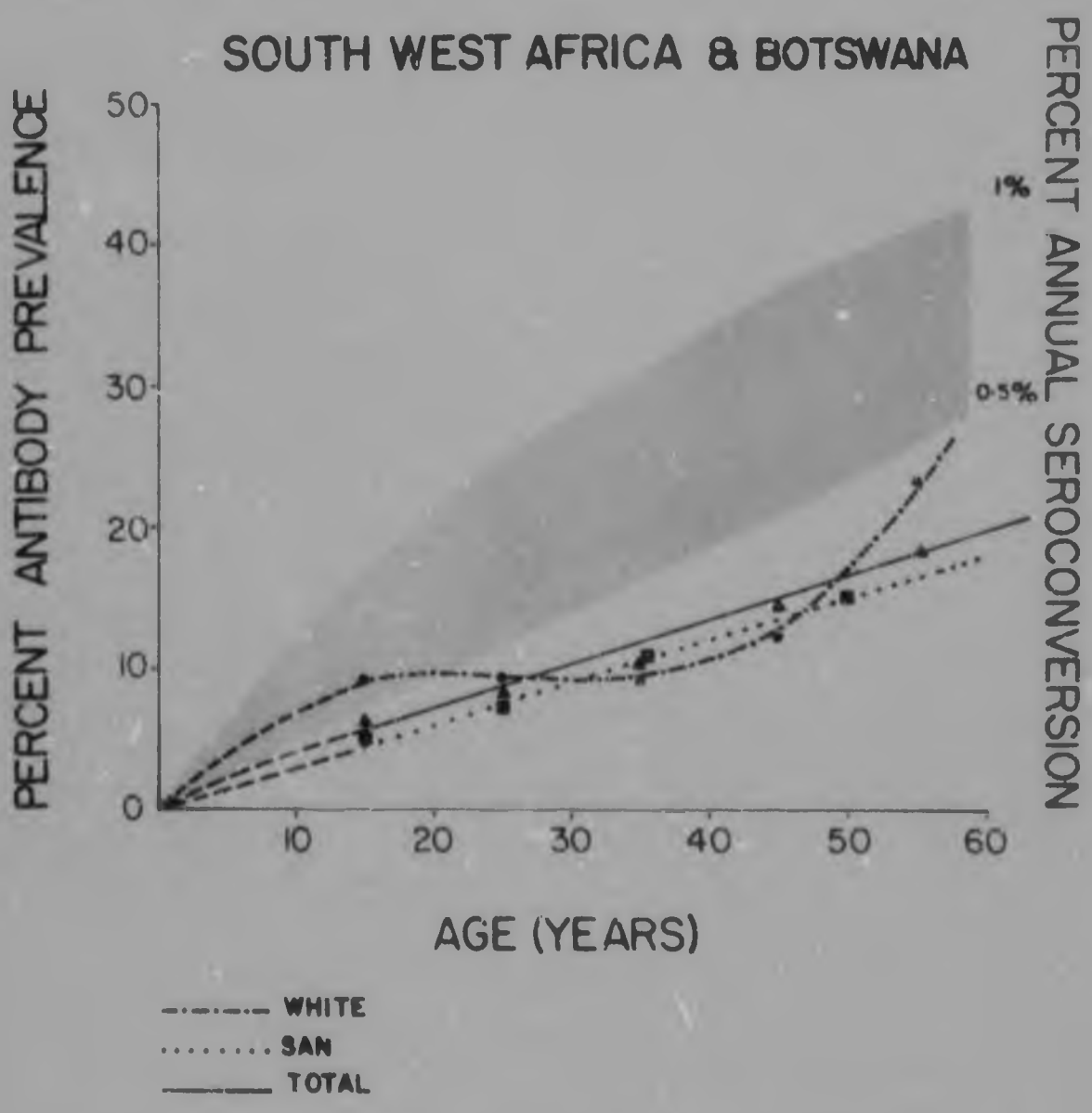


Fig. 5.5. Prevalence of *Toxoplasma* antibodies in South West Africa and Botswana.

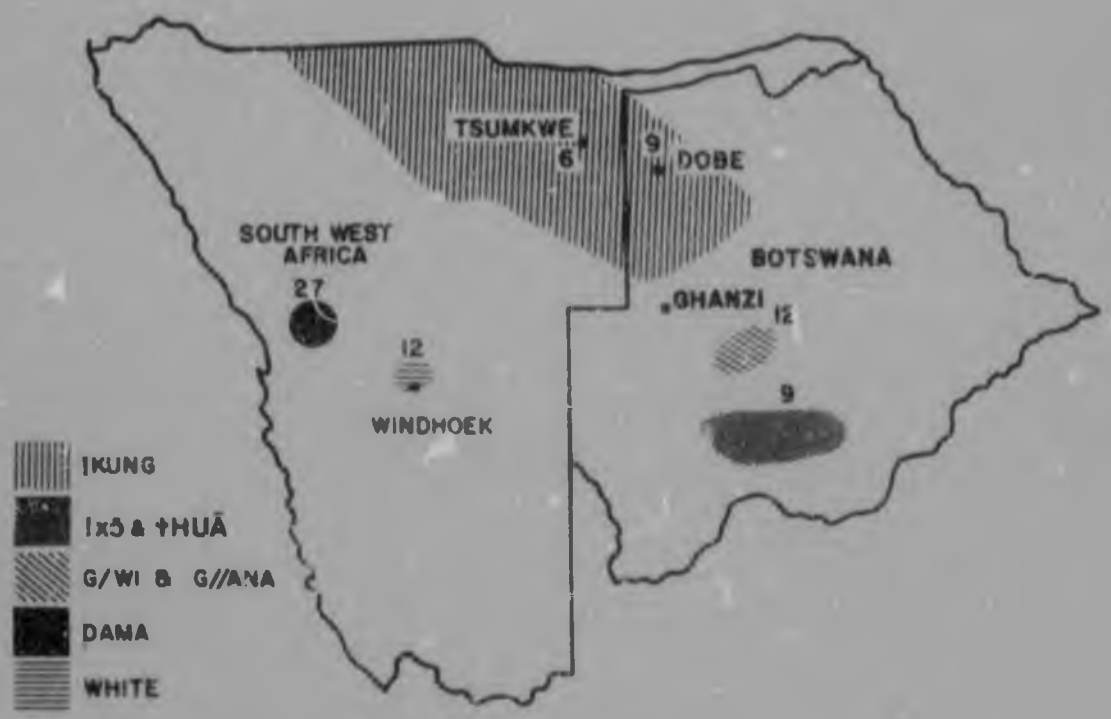


Fig. 5.6. Prevalence of *Toxoplasma* antibodies in South West Africa and Botswana. Figures show percentage seropositivity.

5.2.6 Orange Free State

This area has been surveyed by Brink *et al.* (1975) and their results are given to complete the picture in South Africa as they used similar methods. Table 5.14 and Fig. 5.7 show the prevalence according to age and ethnic group. Differences between the groups are not significant.

TABLE 5.14. Prevalence of *Toxoplasma* antibodies by age and ethnic group in the Orange Free State (as reported by Brink *et al.*, 1975)

Ethnic Group	Age (years) 0-20	21-30	31-40	>40	Total
White	8% (85)*	14% (95)	5% (22)	-	10% (202)
Black	4% (51)	6% (111)	16% (45)	-	8% (207)
Coloured	11% (57)	9% (107)	25% (36)	-	13% (200)
TOTAL	8% (193)	10% (313)	17% (103)	-	10% (609)

* Sample sizes given in brackets

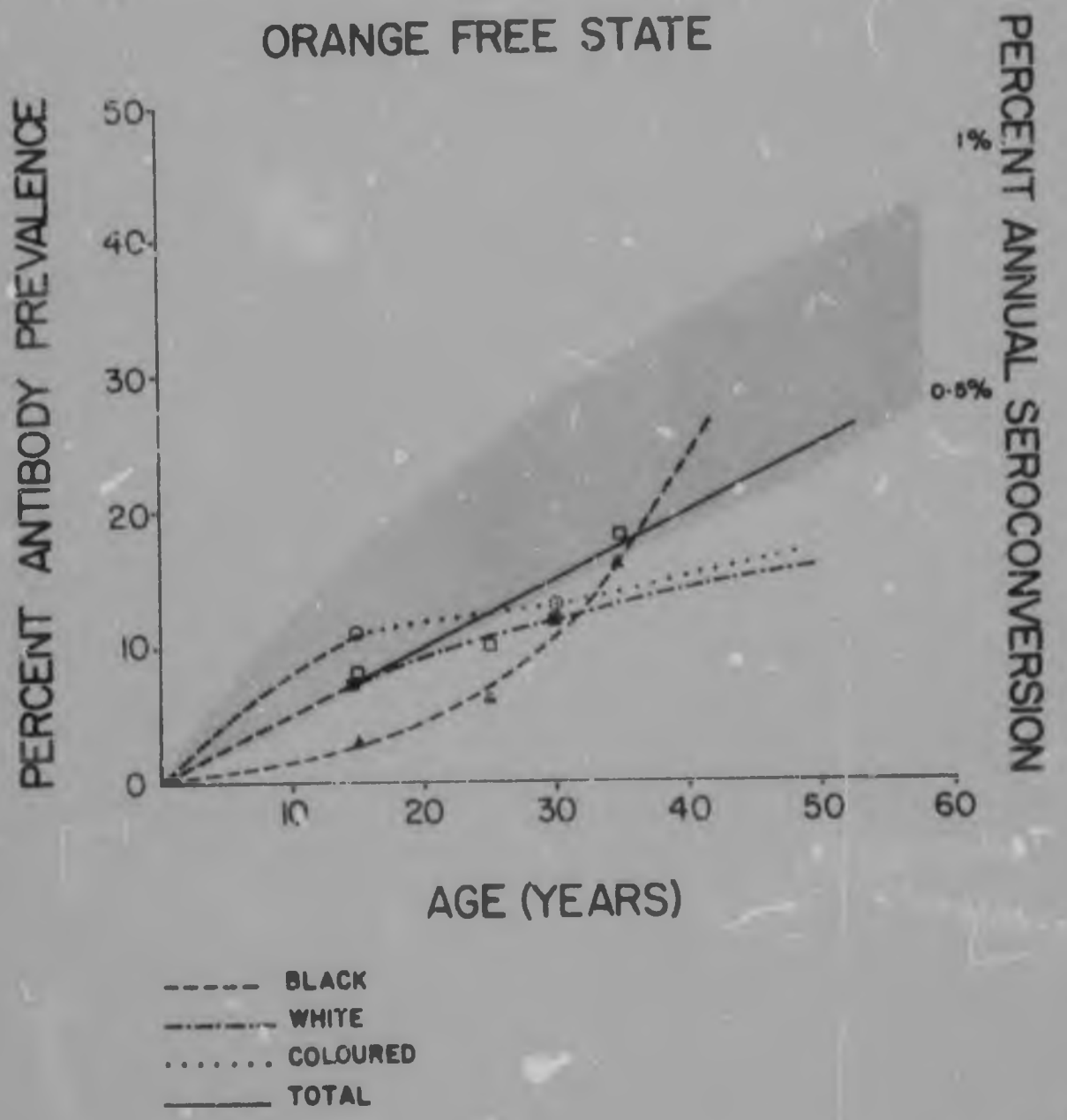


Fig. 5.7. Prevalence of *Toxoplasma* antibodies in the Orange Free State

5.2.7 Overall Pattern in Southern Africa

The overall picture in Southern Africa is shown in Tables 5.15 and 5.16 and Figs. 5.8 to 5.11. The overall prevalence is 21%, with the percentage annual seroconversion rate being about 1% up to the age of 25 years, and falling to 0.5% thereafter (Fig. 5.8). The overall prevalence

TABLE 5.15. Prevalence of *Toxoplasma* antibodies in Southern Africa according to region

Region	Percentage positive	Sample size	Percentage annual sero-conversion <20 yr group	>30 yr group
Natal	30%	635	2%	1%
Eastern Cape	2[illegible]%	970	0.8%	0.5%
Transvaal	23%	6268	1%	1%
Western Cape	18%	683	0.7%	0.1%
South West Africa and Botswana	11%	1063	0.5%	0.3%
Orange Free State*	10%	609	0.5%	0.5%
TOTAL	21%	10228	1%	0.5%

* Figures for the Orange Free State from Brink *et al.*, 1975.

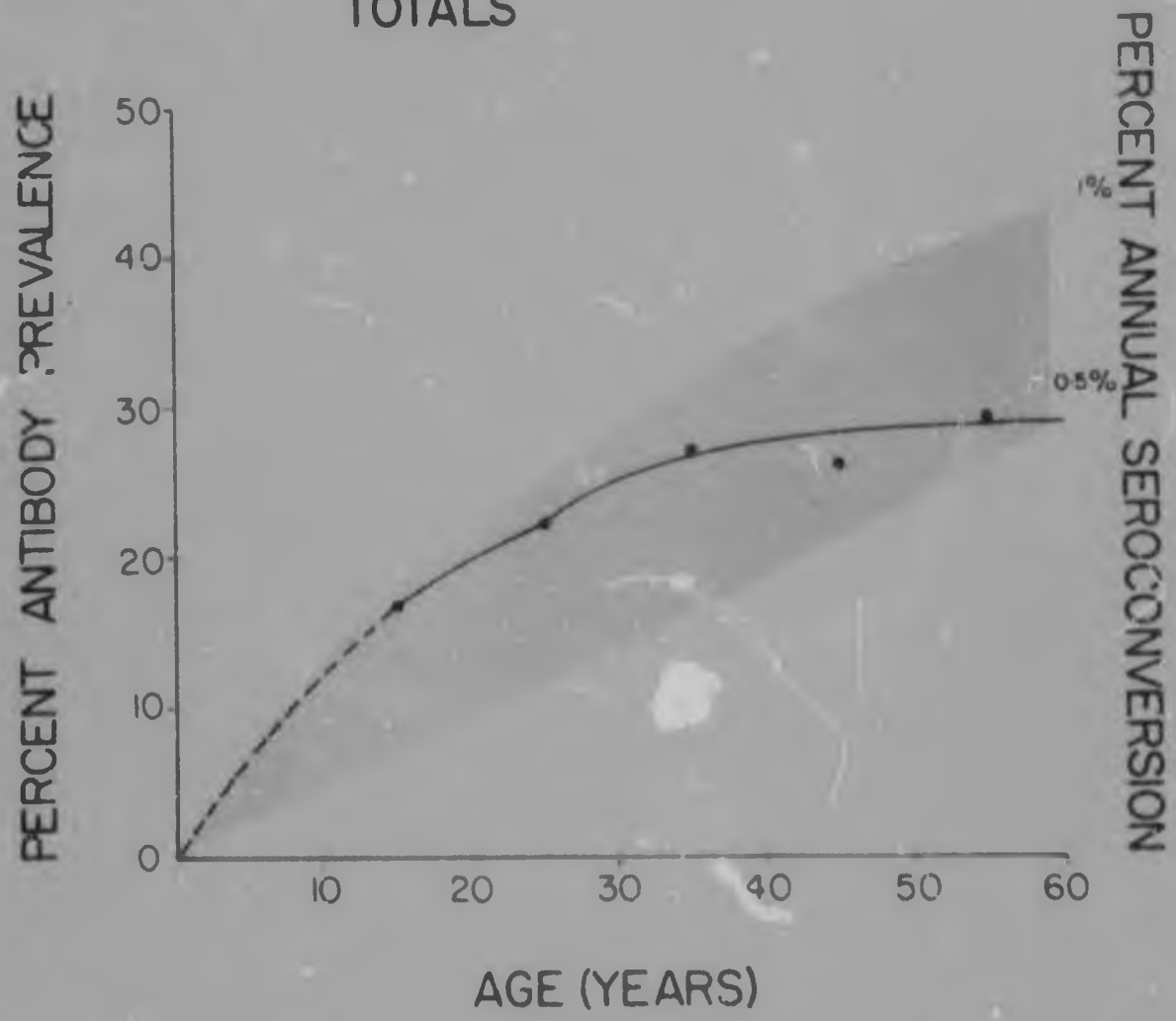


Fig. 5.8. Prevalence of *Toxoplasma* antibodies in Southern Africa - total all regions.

according to region varies from 10% in the Orange Free State to 30% in the Transvaal, with annual incidence rates varying from just below 0.5% to 1% (Fig. 5.9). Analysis according to ethnic group shows prevalence and incidence to be lowest in the San, intermediate in Whites and highest in Blacks, Coloureds and Indians (Fig. 5.10). Prevalences in Whites and San are significantly different from those in the other ethnic groups combined ($p < 0.001$).

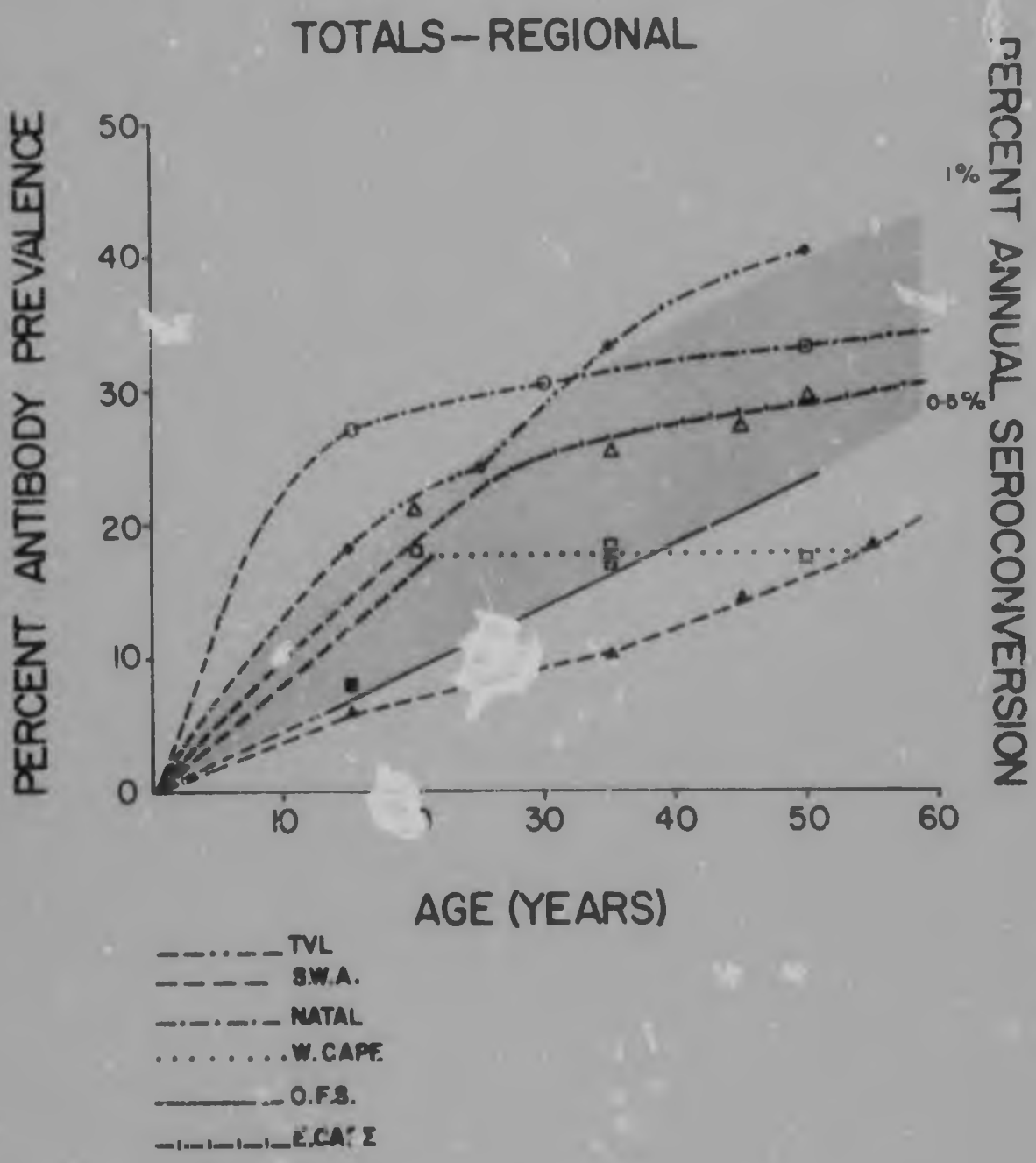


Fig. 5.9. Prevalence of *Toxoplasma* antibodies in Southern Africa according to region.

TABLE 5.16. Prevalence of *Toxoplasma* antibodies in Southern Africa according to ethnic group

Ethnic Group	Percentage positive	Sample size	Percentage annual sero-conversion
White	16%	3294	0.5%
Black	25%	4015	1%
Coloured & Indian	28%	2194	1%
San	9%	725	0.3%
TOTAL	21%	10228	0.5-1%

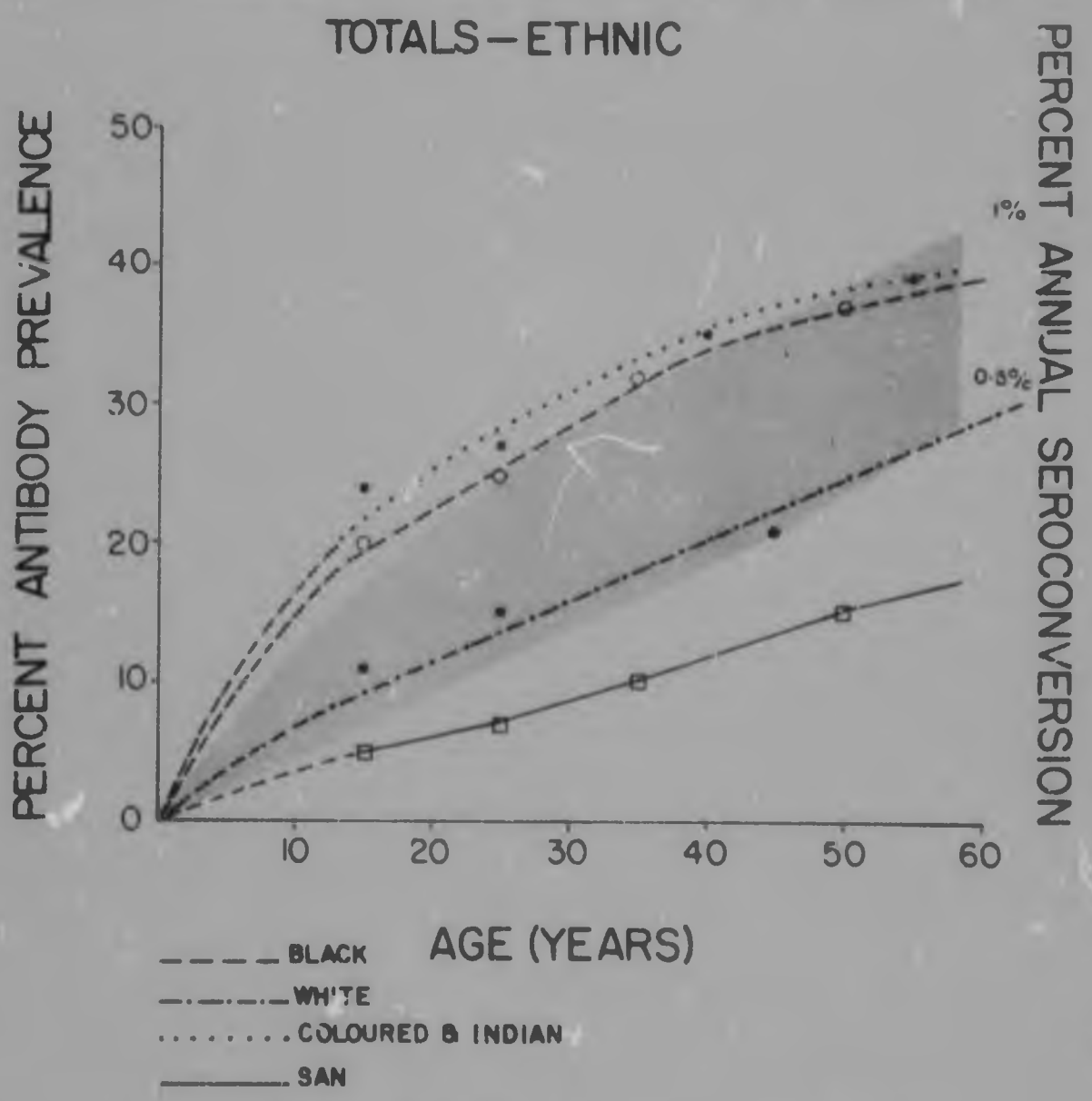


Fig. 5.10. Prevalence of *Toxoplasma* antibodies in Southern Africa according to ethnic group.

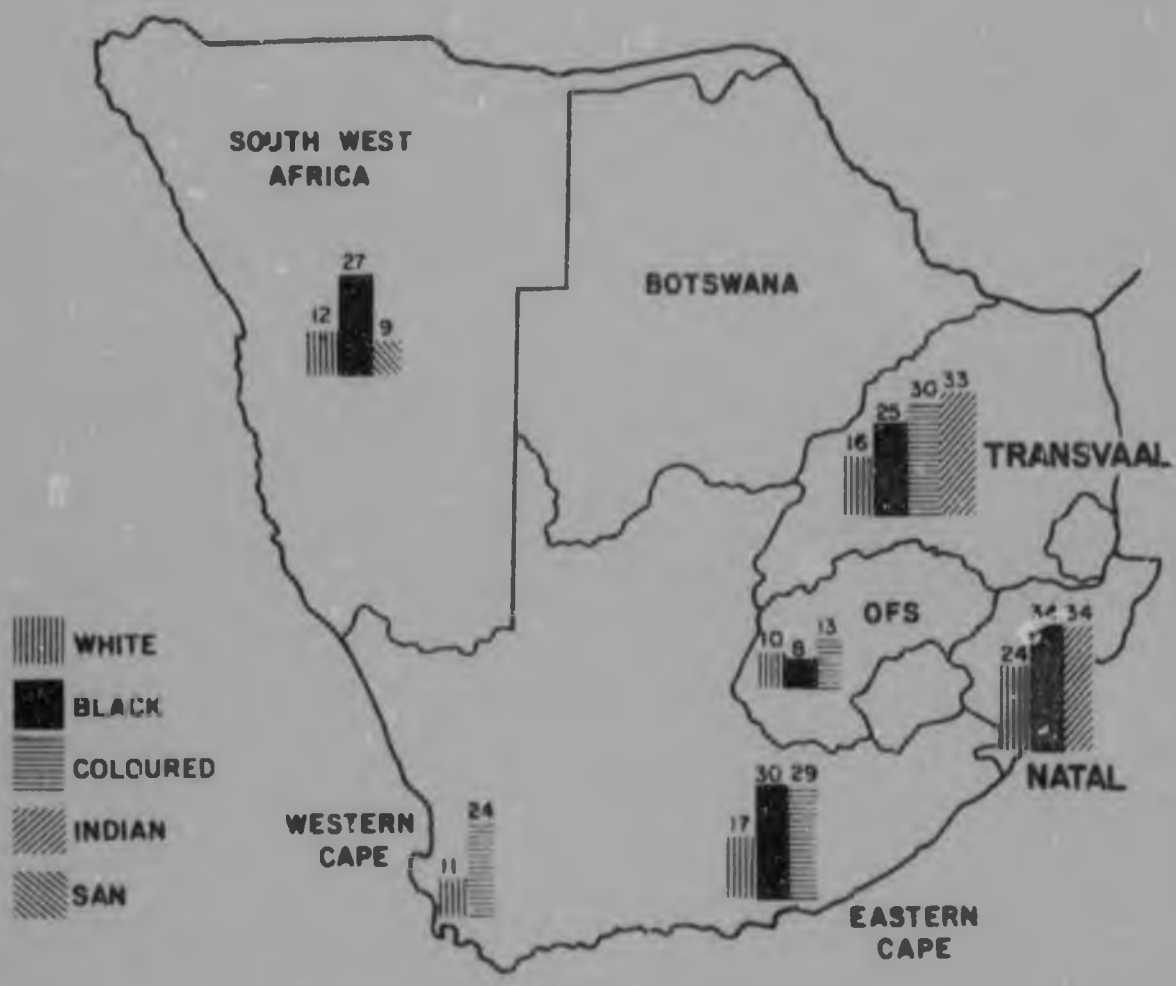


Fig. 5.11. Prevalence of *Toxoplasma* antibodies in Southern Africa according to ethnic group and region

5.3 Discussion

South Africa occupies the southernmost portion of the African continent, from latitudes 22°S to 35°N, and from longitudes 17°E to 33°E. The country has a surface area of 1 178 679 km² (456 000 square miles), and consists of 4 provinces, the Cape Province, Orange Free State, Natal and the Transvaal. Most of the country is subtropical except for the extreme north, which is in the tropics (South African Department of Information, 1978).

The surface of South Africa consists of an interior plateau, with a mean altitude of 1 200 m (4 000 ft), surrounded by a coastal margin.

Climatically, South Africa has a warm temperate climate, with rainfall occurring more abundantly on the east coast due to the warm east

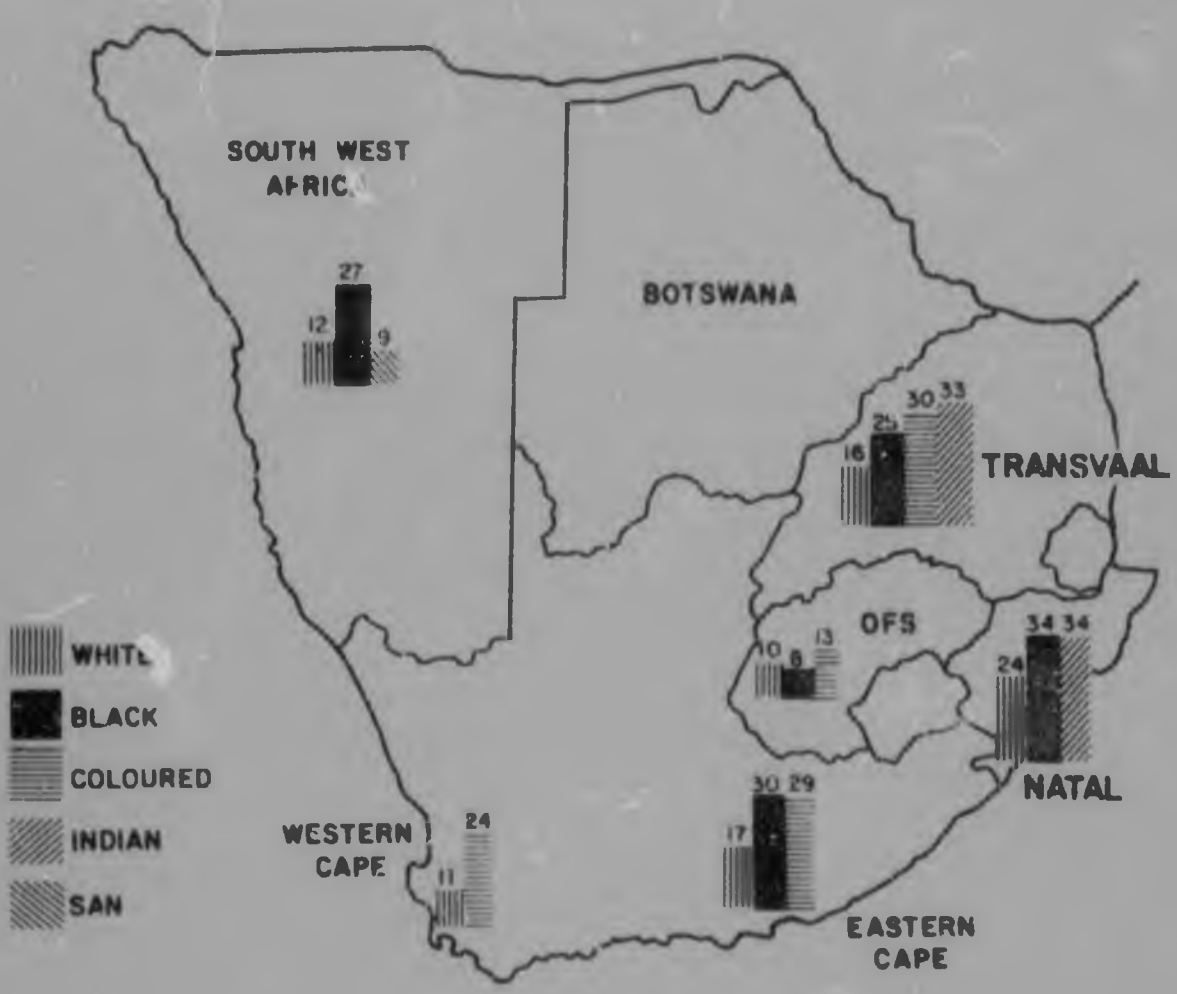


Fig. 5.11. Prevalence of *Toxoplasma* antibodies in Southern Africa according to ethnic group and region

5.3 Discussion

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Climatically, South Africa has a warm temperate climate, with rainfall occurring more abundantly on the east coast due to the warm east

coast Agulhas current and the cold west coast Benguela current. The elevated interior plateau results in lower mean temperatures than is normal for the latitude. Average annual rainfall for South Africa is 464 mm (world mean 857 mm), with 20% receiving less than 200 mm.

Mean annual temperatures are fairly uniform throughout the country, but can occasionally reach -12°C inland. Maximum temperatures of 32°C are common in summer along the Orange River Valley and the Transvaal Lowveld. There is, however, generally very little seasonal temperature change compared to that in Europe and North America.

Humidity is generally very low in the interior but is high over the coastal margin, particularly the east coast. The interior of the country is generally dry and there is a high rate of evaporation leading to very little discharge of water into rivers.

Five natural vegetation areas occur in South Africa. Desert or semi-desert is found along the west coast and most of the western interior (Karoo). Mediterranean vegetation occurs in the Cape Peninsula eastwards to Natal. Bushveld (savanna) covers large areas of the interior (Transvaal and Northern Cape) and temperate grassland covers large areas of the southern Transvaal and Orange Free State.

South West Africa (SWA) is a sparsely populated desert-like area to the south-west of South Africa, with an area of 824 269 km^2 (318 260 square miles). The country is dry and arid, with few perennial rivers, and most of the territory consists of desert or semi-desert. The Namib Desert constitutes about one fifth of SWA, and the Kalahari Desert covers the northern and eastern areas. There is virtually complete absence of surface water. Rainfall varies from less than 100 mm to 400 mm per year, and there is a very high rate of evaporation.

The population of South Africa (1976 estimate) is 23 761 000, consisting of 18% White, 69% Black, 10% Coloured and 3% Asian. Birth rates for Whites are about 19 per 1 000, and for the other ethnic groups about 28 per 1 000.

The population of South West Africa is 762 000 (1976 estimate), with 12% White, 80% Black, 2% Coloured and 3% San (Bushman).

The overall prevalence of *Toxoplasma* antibodies in Southern Africa was found to be 21%, varying from 30% in Natal to 10% in the Orange Free State (Table 5.15). The percentage annual seroconversion rate overall dropped from 1% in the under 20-year age group to 0.5% in the over 30-

year age group. Prevalence rose with age in all areas except for the Western Cape, where there was a much lower rate of increase over the age of 20 years than in the other areas. This occurred in both Whites and Coloureds, and appears to reflect a regional difference in acquisition of infection. The slow rate of increase could be caused by oocyst transmission being more important than meat, with resultant infections occurring mainly in childhood, and very little risk of infection during childbearing age.

Prevalence overall also varied with ethnic group, from 9% in the San to 28% in Coloureds and Indians. Annual seroconversion rates rose consistently with age in all ethnic groups, and the variations found on a regional basis are therefore probably due to regional rather than ethnic factors. In general, prevalence was lowest in the hottest, driest areas such as South West Africa and Botswana, and highest in the most humid and wettest areas such as Natal. However, in all areas, prevalence in Whites was always lower than that in Blacks, Coloureds and Indians, indicating greater exposure of these groups to infection. The reason for this difference is probably socio-economic, and could reflect greater exposure to oocysts shed by stray cats. Exposure to raw or undercooked meat would be expected to be higher in Whites, and this does not therefore seem to be a major factor in South Africa.

The overall pattern of toxoplasmosis in man in Southern Africa shows a fairly low prevalence and incidence of infection. Prevalence is generally highest in the hottest and wettest areas, and lowest in arid areas. Prevalence is also lower in Whites than in Blacks, Coloureds and Indians, probably due to socio-economic or cultural factors.

With annual birth rates of 19 per 1 000 for Whites and 28 per 1 000 for other ethnic groups, the estimated annual number of White births would be 81 260 and of other ethnic groups 545 530. The overall annual incidence of acute toxoplasmosis in the childbearing age group is about 1%. The expected number of mothers developing acute toxoplasmisis during pregnancy per year, using 0.75% incidence to cover the 9 months' gestation period, would therefore be just over 600 Whites and 4000 of other ethnic groups. If, as has been found in France, 40% of maternal infections resulted in foetal infection, and 6% in serious foetal disease, the expected annual numbers of congenital infections would be 240 in Whites and 1 600 in other groups, with 36 severe cases in Whites and 240 in other

groups. Very little information is available as to whether these estimates could be correct, but from the number of cases diagnosed and/or reported, it would seem that either the number of severe congenital cases is much lower than the numbers expected, or that the cases are not being correctly diagnosed.

At the Baragwanath Hospital, for example, only 2 cases of severe congenital toxoplasmosis have been diagnosed over a 3-year period, during which there were about 45 000 births at the hospital. The expected number of maternal infections would be 338, with 20 severe infections. As most patients with congenital infections are investigated fully with good laboratory services available, it is unlikely that severe cases would be consistently missed, and the possibility of local strains being of low virulence must be considered. Further evidence of the low virulence of local strains will be presented in Chapter 7.

6.0 REPORTS OF CASES OF CONGENITAL AND ACQUIRED TOXOPLASMOSIS

During the period in which prevalence studies were being conducted, I was consulted on many occasions regarding cases of suspected congenital or acquired toxoplasmosis. However, in most of these cases the diagnosis of toxoplasmosis was not substantiated, and only 6 definite *Toxoplasma* infections (2 congenital and 4 acquired) were seen.

6.1 Congenital cases

Two patients with symptomatic congenital toxoplasmosis have been seen at the Baragwanath Hospital.

Case 1

A Black female infant born in May 1975, birth weight 1.75 kg, presented at birth with prematurity (34 weeks) and hydrocephalus, and required 4 weeks' hospitalization. The mother had not attended antenatal clinic and gave no history of illness during pregnancy. An older sibling was well. After discharge, the mother did not return for follow-up visits, and only sought medical attention again 3 years later, in June 1978. At this time, the mother stated that the child had not been moving her right limbs as well as her left ones for some months. The child's milestones had been normal.

On examination, the child was alert, and both height and weight were below the third percentile. There was asymetrical hydrocephalus, with the right temporal area being very prominent, and the left temporal and occipital areas being flattened. There was bilateral microphthalmia, a left convergent strabismus, and nystagmus. The fundi showed bilateral optic atrophy with pigmented areas of retinochoroiditis. Estimated mental age on testing corresponded to that of a 2-year old. The child's right limbs showed muscular atrophy, increased tone and tendon reflexes, and weakness. Sensation and coordination were intact.

Investigations showed the following:

Cerebrospinal fluid - 3 lymphocytes/µl, protein 38 mg/100 ml, glucose 51 mg/100 ml.

Skull X-ray - bilateral periventricular calcification and copper-beaten appearance of raised intracranial pressure (Fig. 6.1).

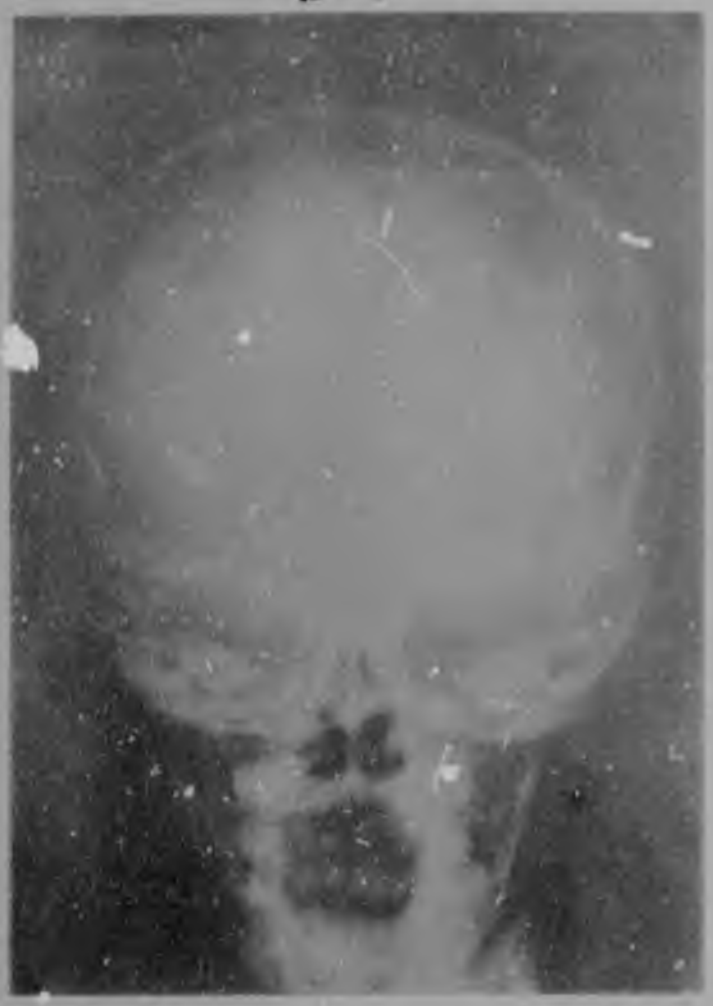

Fig. 6.1. Skull X-ray of case 1, showing bilateral periventricular calcification and copper-beaten appearance of raised intracranial pressure.

Fig. 6.2. Computed skull tomogram of case 1, showing hydrocephalus of the right lateral and the third ventricles, with a focus of calcification adjacent to the right lateral ventricle.

Computed skull tomogram - marked hydrocephalus of right lateral and third ventricles, with a focus of calcification adjacent to the right lateral ventricle (Fig. 6.2).

Serology

Toxoplasma - IFA 1:512
IgM-IFA 1:32
CFT 1:8

Cytomegalovirus, rubella and syphilis - all negative.

Maternal serology

Toxoplasma - IFA 1:512
IgM-IFA 1:32
CFT 1:16

In view of these results at the age of 3 years, the diagnosis of congenital toxoplasmosis was made. The classical triad of hydrocephalus, intracranial calcification and retinochoroiditis with serology indicative of recent infection in both mother and child makes the possibility of another cause highly unlikely.

Case 2

A Black male infant was born on 1 March 1977 at the Baragwanath Hospital at about 28 weeks' gestation with a birth weight of 1.14 kg. The mother had not attended antenatal clinic. The baby was in very poor condition at birth and died 8 hours later with features of respiratory distress. The baby was born during the period in which the survey for congenital toxoplasmosis described in Chapter 7 was being conducted and maternal and cord blood were submitted for *Toxoplasma* serology, and results were as follows:

Maternal: IFA 1:65536
IgM-IFA 1:16
CFT 1:16

Baby: IFA 1:32768
IgM-IFA 1:64
CFT 1:16

A specimen of placenta was not submitted for examination.

An autopsy was not performed when the baby died, and the child was buried. When the serology result was known on the next day, the diagnosis could therefore not be confirmed pathologically. However, in

view of the premature delivery, severe nature of the infant's disease and positive serology including positive cord blood IgM-IFA test, the diagnosis of congenital toxoplasmosis is extremely likely to be correct. Serology for cytomegalovirus, rubella and syphilis on maternal and cord sera were all negative.

6.2 Acquired cases

Four patients with *Toxoplasma* lymphadenitis have been seen over the period 1975 to 1978. In all cases, the diagnosis was made when lymph node biopsies were performed to exclude malignancies and the histologic features of *Toxoplasma* lymphadenitis were noted. The diagnoses were confirmed in all cases by positive serology and direct immunofluorescent staining of lymph node sections with conjugated anti-*Toxoplasma* serum as described in Chapter 4.

Case 1

A 25-year old White male, living in the Transvaal, presented in June 1975 with a single painless anterior cervical lymph node which had gradually enlarged in size over the previous 2 months. The node was excised and the diagnosis made on the histological appearance together with positive serology. The patient's dye test titre was 1:1024, IFA and IgM-IFA were both positive but end titres were not determined, and the CFT was negative. Direct immunofluorescence on node sections performed some time later was also positive. The patient was given a month's course of pyrimethamine (25 mg/day) and triple sulphonamides (4 g/day), and has been well since.

Case 2

A 30-year old Chinese male, living in Botswana, presented in mid-February 1977 with a history of noticing painless right cervical lymphadenopathy developing over the previous week. During the first few days of that month, the patient had prepared beef biltong from a freshly-slaughtered animal purchased from a local butcher. The meat had not been frozen and was handled by the patient but not by his wife.

An anterior cervical node was excised on 22 February to exclude malignancy, and *Toxoplasma* lymphadenitis was diagnosed histologically. Serology was then performed, and the results of repeat testing over the next 14 months are shown in Fig. 6.3. Peak IFA titre was 1:512000, peak IgM-IFA titre 1:2048 and peak CFT titre 1:32. Direct immunofluorescence on lymph node sections was also positive.

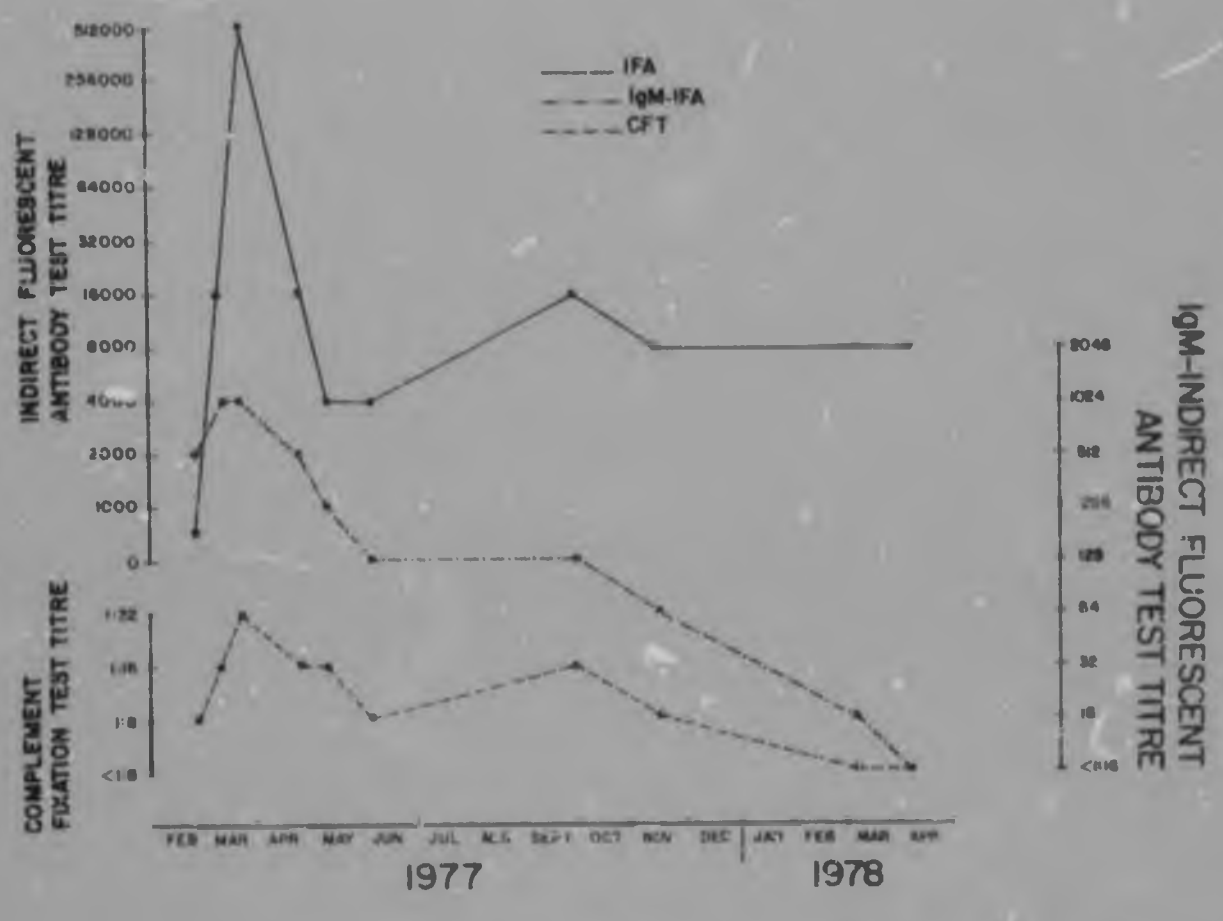

Fig. 6.3. Serology on case 2 of acquired *Toxoplasma* lymphadenitis. IFA, IgM-IFA and CFT titres were determined over a 14-month period after presentation.

The patient was given a 2-week course of pyrimethamine and triple sulphonamides, and has remained well during the follow-up period.

This patient may have contracted his infection from handling the beef he used to make biltong. After the meat had been salted and dried for several days, it was eaten by the patient and his wife and child. Only the patient developed acute infection, while his wife and child remained seronegative. Preparation of biltong would be expected to kill *Toxoplasma* cysts (Work, 1971), and the only time the patient had recently handled food was when he had prepared the biltong. Transmission therefore probably occurred during handling of the fresh meat as the other persons who ate the biltong did not become infected.

Case 3

A 30-year old White female, living in the Transvaal, presented in January 1978 with a 2 month history of bilateral painless posterior cervical lymphadenopathy. A lymph node biopsy was performed to exclude malignancy, and a histological diagnosis of toxoplasmosis was made. The patient's *Toxoplasma* serology was then performed - the IFA titre was 1:4096 and the IgM-IFA test was positive. Lymph node direct immunofluorescence was also positive. The patient was not treated and has been well on follow-up.

Case 4

A 19-year old White male, living in the Transvaal, presented in June 1978 with 2 painless lymph nodes in the right supraclavicular area, which had developed over the previous few weeks. The nodes persisted after a course of antibiotics, and a lymph node biopsy was performed. The histological report of the biopsy suggested toxoplasmosis as the diagnosis, and the patient's *Toxoplasma* serology was done. The IFA titre was 1:16000 with a positive IgM-IFA test. Four months later the IFA titre was 1:2000. Lymph node direct immunofluorescence was positive. The patient received a course of pyrimethamine and sulphonamides, and has remained well on follow-up.

These 4 cases illustrate typical acute toxoplasmic lymphadenitis presenting only with asymptomatic cervical lymphadenopathy (Table 6.1). The patients presented to different practitioners, and in none of these patients was the diagnosis considered before lymph node excision biopsies were performed. Serology for toxoplasmosis was not performed on any of these patients prior to biopsy, and requests to attempt isolation of the parasite were therefore not made. Awareness of *Toxoplasma* as a cause of lymphadenitis could have enabled the diagnosis to have been made prior to surgery, and surgery probably avoided in these cases. Three of the 4 cases were treated, although the necessity for treatment of such cases is doubtful (Remington, 1974).

The use of direct immunofluorescence has been useful in confirming the diagnosis in these cases, but care must be taken to ensure that false positive results are not obtained (Frenkel and Piekarski, 1978). This was done by absorbing out the direct conjugate as described in Chapter 4, and no fluorescence was obtained with the conjugate after absorption in these cases.

TABLE 6.1. Features of 4 patients with acquired *Toxoplasma* lymphadenitis

Patient No.	Age (yrs)	Sex	Ethnic group	Origin	Date of presentation	Clinical features	Histological diagnosis		Serology		Dye test	CFT
							H & E*	Immunofluorescent	IFA	IgM-IFA		
1	25	M	White	Transvaal	April 1975	Single anterior cervical node	*Toxoplasma* lymphadenitis	Positive	Positive†	Positive†	1:1024	Negative
2	30	M	Chinese	Botswana	February 1977	Right cervical lymphadenopathy	*Toxoplasma* lymphadenitis	Positive	1:512000§	1:2048§	ND¶	1:32§
3	30	F	White	Transvaal	January 1978	bilateral posterior cervical lymphadenopathy	*Toxoplasma* lymphadenitis	Positive	1:4000	Positive†	ND	ND
4	19	M	White	Transvaal	June 1978	Right supraclavicular lymphadenopathy	*Toxoplasma* lymphadenitis	Positive	1:16000§	Positive†	ND	ND

* H & E = haematoxylin and eosin stain

† Not titred to end-point

¶ ND = not done

§ Peak titre

7.0 TOXOPLASMOSIS DURING PREGNANCY IN SOUTH AFRICA

The complete lack of data on the incidence of toxoplasmosis during pregnancy and on congenital infection in South Africa prompted my investigation into the incidence of toxoplasmosis during pregnancy. From the fairly low prevalence of toxoplasmosis in the initial serological surveys, and the small number of congenital cases reported in the South African literature, toxoplasmosis should not be a major hazard during pregnancy in South Africa. However, no data were available to substantiate this assumption. The public health significance of definite data on this assumption is important in deciding whether screening for toxoplasmosis during pregnancy would be indicated.

To answer these questions, I undertook a study of the incidence of acute toxoplasmosis during pregnancy, and on foetal transmission, during the period 1975 to 1977 in 3 Johannesburg hospitals, covering all ethnic groups. For this study, I was awarded a 3-year grant by the South African Medical Research Council to cover the laboratory costs involved.

7.1 Patients and Methods

Patients were selected at random on hospital presentation for delivery, and specimens of maternal blood, cord blood, and placenta in antibiotic-containing saline were requested. Maternal blood specimens were obtained easily, but cord blood and placenta were not always submitted. Maternal and cord blood sera were tested by the IFA method on receipt, and if positive at ≥1:1024, placenta if submitted was inoculated into mice. If only maternal blood was submitted, a blood sample was obtained from the infant within 3 days of birth if the maternal IFA was ≥1:512. All sera positive in the IFA test were then tested by the CFT and all sera with IFA titres ≥1:512 or CFT positive at any titre were tested by the IgM-IFA test.

During the 6-month period before the study started at each hospital, sera from antenatal patients were submitted whenever routine venepunctures were performed and stored at -20°C. At delivery patients were checked for the presence of stored sera, and if available, stored sera were tested together with the delivery specimens. This system worked well at the Queen Victoria Hospital, where stored antenatal sera were available

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for 92% of patients with positive IFA test at delivery, but less well at Coronation Hospital (56%) and Baragwanath Hospital (1.%) as many patients did not attend antenatal clinic or antenatal sera were not submitted for storage. A further problem encountered at Baragwanath Hospital was that difficulty was experienced in obtaining staff co-operation in submitting cord blood and placenta specimens. However, in the few maternal sera without cord blood sera that had IFA titres of ≥1:512, blood was obtained from the neonates within 3 days of birth.

Methods used for the IFA test, IgM-IFA test, CFT and mouse inoculation are described in Chapter 4.

7.2 Results

Of the 6705 sera submitted from mothers at delivery, 1473 (22%) were positive at titres of ≥1:16 in the IFA test, as shown in Tables 7.1 and 7.2. Prevalence of IFA antibodies was lowest in Whites (14.3%) and similar in Blacks, Coloureds and Indians (25 to 26%). The annual seroconversion rate was 1% overall, being about 0.75% in Whites and 1.2% in the other ethnic groups (fig 7.1).

TABLE 7.1 Distribution of maternity patients with positive IFA tests at delivery

Hospital	Ethnic Group	No. Positive	Percentage positive	Sample size
Queen Victoria	White	286	14.3%	2001
Baragwanath	Black	91[illegible]	25.0%	3659
Coronation	Coloured	236	26.2%	901
Coronation	Indian	36	25.0%	144
TOTAL		1473	22.0%	6705

Results were classified according to IFA titre and presence of positive CFT and IgM-IFA tests. Criteria used (Table 7.3) were based on patients with chronic infection having IFA titres of up to 1:2048 with negative CFTs and IgM-IFAs ≤1:32, patients with recent infection having positive IFAs (titres of 1:32 to 1:512) with positive CFTs, and patients with evidence of definite or possible acute infection during pregnancy seroconverting or having high titre IFAs (≥1:1024) with positive

TABLE 7.2. Distribution of patients according to IFA test results

IFA titre	Queen Victoria Hospital		Baragwanath Hospital		Coronation Hospital		TOTAL	
	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
1:16	87	4.3%	378	10.3%	95	9.0%	560	8.4%
1:32	99	5.0%	200	5.5%	55	5.3%	354	5.3%
1:64	70	3.5%	118	3.2%	49	4.7%	237	3.5%
1:128	21	1.0%	98	2.7%	33	3.2%	152	2.3%
1:256	7	0.35%	66	1 8%	22	2.1%	95	1.4%
1:512	1	0.05%	30	0.8%	9	0.9%	40	0.6%
1:1024	1	0.05%	20	0.5%	6	0.6%	27	0.4%
1:2048	-	-	2	0.05%	2	0.2%	4	0.06%
1:4096	-	-	2	0.05%	1	0.1%	3	0.04%
>1:4096	-	-	1	0.03%	-	-	1	0.015%
Total IFA positives	286	14.3%	915	25.0%	272	26.0%	1473	22.0%
Total IFA negatives	1715	85.7%	2744	75.0%	773	74.0%	5232	78.0%
Sample size	2001		3659		1045		6705	

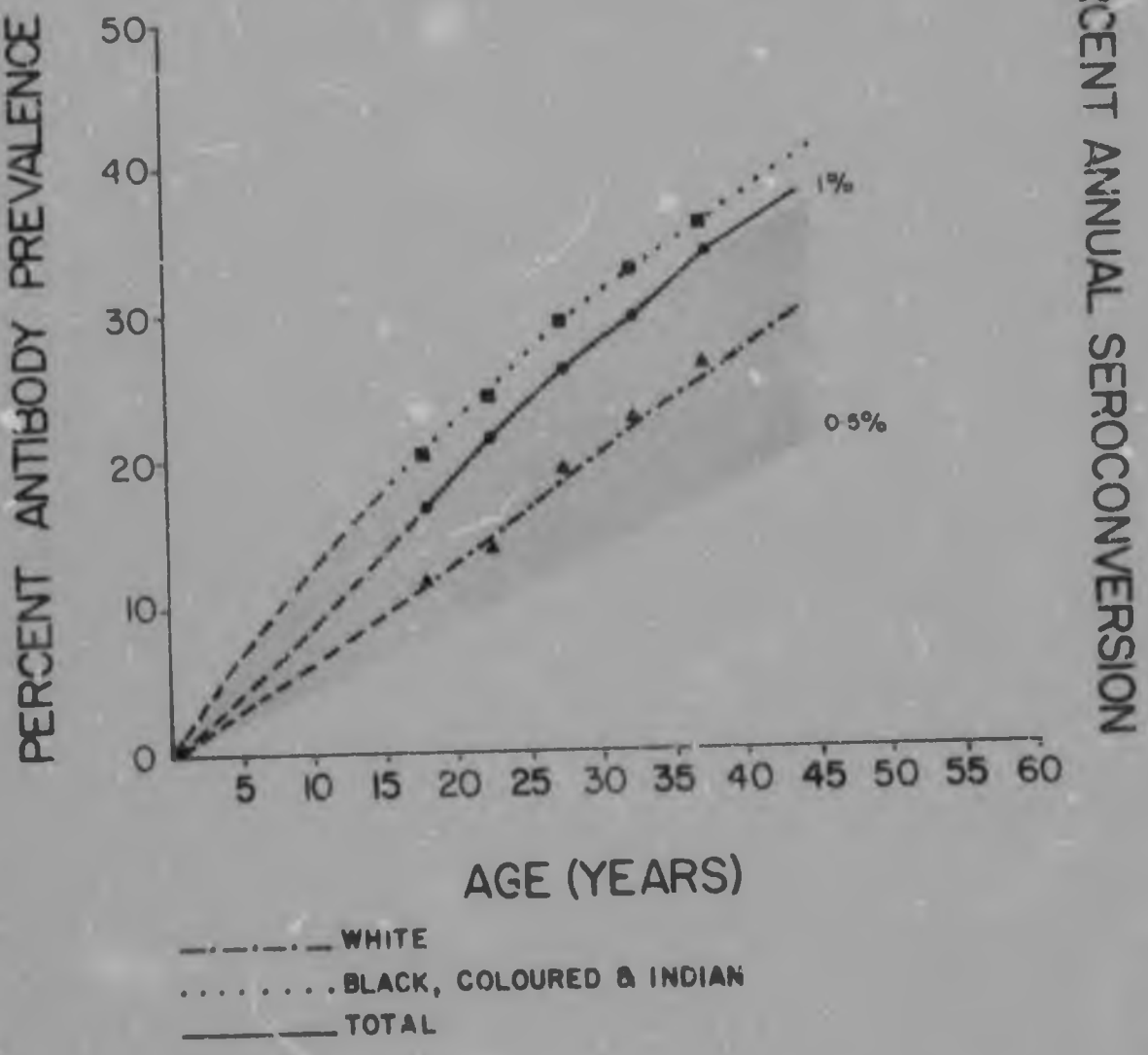

Fig. 7.1. Prevalence of *Toxoplasma* antibodies in maternity patients at delivery.

CFTs and IgM-IFAs. Patients with positive CFTs, classified as having recent infections (category 5), were assumed to have had their acute infection before their present pregnancy as titres were low (≤1:512 IFA and ≤1:32 IgM-IFA) and, where antenatal sera were available, remained unchanged during pregnancy. It is possible, however, that a few of these patients had early acute third trimester infections if antenatal sera were not available, but this group would be small and carry very little risk of symptomatic congenital infection.

TABLE 7.3 Criteria used to analyse patients with positive IFA tests and to define acute toxoplasmosis during pregnancy. Patients fulfilling criteria 1 to 3 were regarded as definite cases, and criterion 4 probable cases of acute infection

Criteria	No. of cases
1. Seroconversion from negative to positive IFA, IgM-IFA and CFT	3
2. Four-fold or greater rise in IFA titre in presence of or development of positive IgM-IFA and CFT	3
3. Single or peak titres of ≥1:2048 IFA, ≥1:64 IgM-IFA and ≥1:16 CFT	4
4. Single titres at delivery of 1:1024 IFA with ≥1:16 IgM-IFA and ≥1:8 CFT, regarded as probable cases	10
5. IFA ≤1:512 with CFT 1:8 or 1:16 and IgM-IFA ≤1:32 at delivery and no change in titre during pregnancy (if antenatal sera tested), indicative of recent infection (within past 2 to 4 years), but probably not during present pregnancy	89
6. IFA ≤1:2048 with CFT negative and IgM-IFA (if done) ≤1:32 indicative of past infection	1364

Results were analysed according to these criteria, and are shown in Tables 7.4 to 7.6 according to IFA, IgM-IFA and CFT results and criteria given in Table 7.3. Overall, 22% of patients (1473) had positive IFA tests, with 1.6% (109) having positive CFTs. Eighty-nine of the patients with positive CFTs were classified as having had recent infection but probably not during the present pregnancy, and 20 were classified as having evidence of definite or probable acute infection during the present pregnancy. Detailed results on all sero-positive patients are given in the appendices. Results on Whites from the Queen Victoria Hospital are given in appendix 1 (pages 119 to 129), on Blacks from the Baragwanath Hospital in appendix 2 (pages 130 to 154), and on Coloureds and Indians from the Coronation Hospital in appendix 3 (pages 155 to 164).

TABLE 7.4. Distribution of Patients with positive IFA tests according to IFA and CPT titres at delivery

Indirect fluorescent antibody test titre	Complement Fixation Test Titre						
	<1:8	1:8	1:16	1:32	1:64	1:128	TOTAL
1:16	560	-	-	-	-	-	560
1:32	346	8	-	-	-	-	354
1:64	196	37	4	-	-	-	237
1:128	131	18	3	-	-	-	152
1:256	77	14	4 (2)	-	-	-	95 (2)
1:512	35	2 (1)*	3 (1)	-	-	-	40 (2)
1:1024	16	5 (5)	6 (6)	-	-	-	27 (11)
1:2048	3	1 (1)	-	-	-	-	4 (1)
1:4096	-	-	1 (1)	1 (1)	-	1 (1)	3 (3)
>1:4096	-	-	1 (1)	-	-	-	1 (1)
TOTAL	1364	85 (7)	22 (11)	1 (1)	-	1 (1)	1473 (20)
Percentage	92.6%	5.8%	1.5%	0.07%	-	0.07%	100%

* Number in parentheses indicate patients with definite or probable acute toxoplasmosis during pregnancy

TABLE 7.5. Distribution of patients with positive IFA and CFTs and all positive IFAs with titres of ≥1:512

IFA titre	IgM-I.A titre													
	Acute infection (20 cases)							Recent infection (89 cases)			Past infection† (53 cases)			TOTAL
	<1:16	1:16	1:32	1:64	1:128	1:256	1:512	<1:16	1:16	1:32	<1:16	1:16	1:32	
1:32	-	-	-	-	-	-	-	1	7	-	*	*	*	8
1:64	-	-	-	-	-	-	-	21	12	8	*	*	*	41
1:128	-	-	-	-	-	-	-	12	4	5	*	*	*	21
1:256	-	-	1	1	-	-	-	6	6	4	*	*	*	18
1:512	-	2	-	-	-	-	-	1	-	2	17	9	7	38
1:1024	-	2	2	5	1	-	1	-	-	-	8	8	1	28
1:2048	-	-	-	1	-	-	-	-	-	-	-	2	1	4
1:4096	-	-	-	2	-	-	1	-	-	-	-	-	-	3
>1:4096	-	1	-	-	-	-	-	-	-	-	-	-	-	1
TOTAL	-	5	3	9	1	-	2	41	29	19	25	19	9	162

†Based on negative CFTs, but could have included some recent cases.

*Not done.

TABLE 7.6 Distribution of patients according to past, recent or acute infection

IFA titre	Queen Victoria Hospital			Baragwanath Hospital			Coronation Hospital			Total			Total of all IFA positives	
	1	2	3	1	2	3	1	2	3	1	2	3		
1:16	87	-	-	378	-	-	95	-	-	560	-	-	560	8.4%
1:32	94	5	-	197	3	-	55	-	-	346	8	-	354	5.3%
1:64	56	14	-	102	16	-	38	11	-	196	41	-	237	3.5%
1:128	14	7	-	86	12	-	31	2	-	131	21	-	152	2.3%
1:256	3	2	2	54	12	-	20	2	-	77	16	2	95	1.4%
1:512	1	-	-	25	3	1	8	-	1	34	3	2	39	0.6%
1:1024	-	-	1	13	-	8	4	-	2	17	-	11	28	0.4%
1:2048	-	-	-	2	-	-	1	-	1	3	-	1	4	0.06%
1:4096	-	-	-	-	-	2	-	-	1	-	-	3	3	0.04%
>1:4096	-	-	-	-	-	1	-	-	-	-	-	1	1	0.015%
TOTAL	255	28	3	857	46	12	252	15	5	1364	89	20	1473	
Percentage positive	12.8%	1.4%	0.15%	23.4%	1.3%	0.3%	24.1%	1.4%	0.5%	20.3%	1.3%	0.3%	22.0%	
Sample size	2001			3659			1045			6705			6705	

1. IFA positive, CFT negative, indicative of past infection (category 6)*
2. IFA positive, CFT positive, both constant and/or in low titre (IFA ≤1:512; CFT ≤1:16), indicative of recent infection (within past 2-4 years) (category 5)*
3. IFA positive, CFT positive, with evidence of definite or probable acute infection either by sero-conversion or if IFA, IgM-IFA and CFT are high (IFA ≥1:1024; IgM-IFA ≥1:16 and CFT ≥1:8) (categories 1-4)*

*See Table 7.3

TABLE 7.6 Distribution of patients according to past, recent or acute infection

IFA titre	Queen Victoria Hospital			Baragwanath Hospital			Coronation Hospital			Total			Total of all IFA positives	
	1	2	3	1	2	3	1	2	3	1	2	3		
1:16	87	-	-	378	-	-	95	-	-	560	-	-	560	8.4%
1:32	94	5	-	197	3	-	55	-	-	346	8	-	354	5.3%
1:64	56	14	-	102	16	-	38	11	-	196	41	-	237	3.5%
1:128	14	7	-	86	12	-	31	2	-	131	21	-	152	2.3%
1:256	3	2	2	54	12	-	20	2	-	77	16	2	95	1.4%
1:512	1	-	-	25	3	1	8	-	1	34	3	2	39	0.6%
1:1024	-	-	1	13	-	8	4	-	2	17	-	11	28	0.4%
1:2048	-	-	-	2	-	-	1	-	1	3	-	1	4	0.06%
1:4096	-	-	-	-	-	2	-	-	1	-	-	3	3	0.04%
>1:4096	-	-	-	-	-	1	-	-	-	-	-	1	1	0.015%
TOTAL	255	28	3	857	46	12	252	15	5	1364	89	20	1473	
Percentage positive	12.8%	1.4%	0.15%	23.4%	1.3%	0.3%	24.1%	1.4%	0.5%	20.3%	1.3%	0.3%	22.0%	
Sample size	2001			3659			1045			6705			6705	

1. IFA positive, CFT negative, indicative of past infection (category 6)*
2. IFA positive, CFT positive, both constant and/or in low titre (IFA ≤1:512; CFT ≤1:16), indicative of recent infection (within past 2-4 years) (category 5)*
3. IFA positive, CFT positive, with evidence of definite or probable acute infection either by seroconversion or if IFA, IgM-IFA and CFT are high (IFA ≥1:1024; IgM-IFA ≥1:16 and CFT ≥1:8) (categories 1-4)*

*See Table 7.3

Total IgM levels were measured in 2293 cord blood specimens from the Queen Victoria and Coronation Hospitals. IgM levels of <20 IU/ml are regarded as normal in Whites and <100 IU/ml in Blacks. Values in Coloureds and Indians have not been reliably established, but from the results obtained, seem to have a similar distribution to that in Whites (Table 7.7). Cord blood IgM levels were <30 IU/ml in the cord blood of babies of all the White, Coloured and Indian patients with definite or probable acute infection. Cord blood IgM levels were not determined in the remainder of specimens from White, Coloured or Indian patients, nor in any from Black patients.

TABLE 7.7. Cord blood total IgM levels

	IgM level (IU/ml)			
	<30	30-100	>30	TOTAL
Queen Victoria Hospital	1590 (86%)	236 (13%)	22 (1%)	848
Coronation Hospital	369 (83%)	67 (15%)	9 (2%)	445
TOTAL	1959 (85%)	303 (13)	31 (1.4%)	2293

Results on the 20 patients considered to have definite or probable acute infection during pregnancy are given in Tables 7.8 and 7.9. Patients A2, A3 and C3 fell into category 1 (seroconversion), patients A1, C1 and C2 into category 2 (four-fold rise in IFA titre), patients B1 to B4 into category 3 (single IFA titre of ≥1:2048 with positive CFT and IgM-IFA) and patients B5 to B12, and C4 and C5 into category 4 (single IFA titre of 1:1024 with positive CFT and IgM-IFA).

In White patients at the Queen Victoria Hospital, the highest IFA titre obtained was 1:1024 in patient A1. This patient acquired her infection during the first trimester of pregnancy, and probably had a higher peak between 12 and 24 weeks when sera were submitted. Four months post-natally however, this patient's CFT was negative, giving a maximum period of her CFT being positive of 10 months.

Patient A2 seroconverted at about 34-weeks' gestation, and at delivery her IFA titre was 1:256 and CFT 1:16, giving the impression of a titre peak between 34 weeks and term. At 3 months follow-up, the CFT was negative, giving the period of the CFT being positive as less than 6 months.

Patient A3 also seroconverted in the third trimester, and on follow-up at 3 months was still CFT positive, but with an IFA titre of only 1:256.

TABLE 7.8. Summary of serology in patients with evidence of definite or probable acute toxoplasmosis during pregnancy as defined in Table 7.3. Reciprocal titres are g[illegible]

	Patient No.	1st Trimester				2nd Trimester				3rd Trimester				Delivery Maternal			Cord			Post-delivery Mother				Post-delivery Child			
		Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
Definite cases	A1	12	32	-	-	24	512	32	16	36	1024	64	8	1024	128	8	512	-	-	4	128	16	-	-	-	-
	A2					20	-	-	-	28	-	-	-	256	64	16	128	-	8	5	256	-	-	16	-	-
										34	16	-	-													
	A3					26	-	-	-	32	-	-	-	256	32	8	128	-	8	3	256	16	8	16	-	-
	B1									28	2048	64	32	512	16	16	1024	-	16	20	512	-	-	-	-	-
	B2													65536	16	16	32768	64	16	20	256	-		Died at birth		
	B3													4096	512	128	2048	-	16	18	128	-	-	-	-	-
	B4													4096	64	32	4096	-	8	20	4096	16	8	-	-	-
	C1					21	32	-	-					512	16	8	512	-	-	18	128	-	-	-	-	-
	C2									36	512	16	16	4096	64	16	2048	-	8	20	256	32	-	-	-	-
	C3									30	-	-	-	2048	64	8	1024	-	-	20	512	32	-	-	-	-
Probable cases	B5													1024	64	8	512	-	8	Not traced						
	B6													1024	32	8	1024	-	-	20	256	64	-	-	-	-
	B7													1024	64	16	512	-	8	18	128	-	-	-	-	-
	B8													1024	64	16	1024	-	8	Not traced						
	B9													1024	32	8	1024	-	-	17	1024	-	-	-	-	-
	B10													1024	64	16	1024	-	-	20	256	32	8	-	-	-
	B11													1024	64	16	512	-	8	20	512	32	-	-	-	-
	B12													1024	512	16	1024	256	32	21	256	64	-	512	128	8
	C4													1024	16	16	1024	-	8	20	512	-	-	-	-	-
	C5													1024	16	8	10[illegible]	-	8	20	512	[illegible]	8	-	-	-

*-, negative test result at lowest dilution tested

TABLE 7.9. Clinical details of patients with definite or probable acute toxoplasmosis during pregnancy

	Patient No.	Age (yrs)	Obstetric history	Duration of present pregnancy	Illness during pregnancy	Criterion for acute infection*	Trimester infection acquired	Data on babies				
								Birth wt (kg)	Clinical condition and progress at birth	Follow-up Age (mths)	Serology	Clinical condition
Definite cases	A1	27	Normal	38 weeks	No	2	First	3.16	Normal	4	Negative	Normal
	A2	28	Normal	Full-term	'Flu-like illness with cervical lymphadenopathy at 32 weeks. Husband also had acute infection	1	Third	4.55	Normal	5	Negative	Normal
	A3	26	Normal	Full-term	No	1	Third	2.95	Normal	3	Negative	Normal
	B1	25	Normal	Full-term	No	3	Unknown	3.10	Normal	20	Negative	Normal
	B2	24	Premature labour	28 weeks	No	3	Unknown	1.14	Baby presented with respiratory distress and died 12 hrs after birth	-	-	-
	B3	30	Normal	Full-term	No	3	Unknown	2.78	Normal	18	Negative	Normal
	B4	21	Normal	Full-term	No	3	Unknown	3.05	Normal	20	Negative	Normal
	C1	35	Normal	3 weeks premature	No	2	Second	3.70	Normal	18	Negative	Normal
	C2	29	Normal	38 weeks	No	2	Third	2.80	Normal	20	Negative	Normal
	C3	22	Normal	36 weeks	No	1	Third	2.72	Normal	20	Negative	Normal
Probable cases	B5	17	Normal	36 weeks	No	4	Unknown	2.80	Normal	Not traced		
	B6	19	Normal	38 weeks	No	4	Unknown	2.92	Normal	20	Negative	Normal
	B7	22	Normal	Full-term	No	4	Unknown	2.65	Normal	18	Negative	Normal
	B8	32	Normal	Full-term	No	4	Unknown	3.05	Normal	Not traced		
	B9	20	Normal	38 weeks	No	4	Unknown	2.20	Normal	17	Negative	Normal
	B10	25	Normal	Full-term	No	4	Unknown	2.50	Normal	20	Negative	Normal
	B11	31	Normal	36 weeks	No	4	Unknown	1.68	Low birth weight	20	Negative	Normal
	B12	22	Normal	Full-term	No	4	Unknown	3.30	Cord around neck at birth. Respiratory distress at birth. Jaundiced on 3rd day. Discharged well aged 10 days	11	Positive	Normal
	C4	20	Normal	Full-term	No	4	Unknown	2.60	Mild jaundice	20	Negative	Normal
	C5	22	Normal	38 weeks	No	4	Unknown	3.25	Normal	20	Negative	Normal

* See Table 7.3.

These 3 patients (A1 and A3) had definite serologic evidence of toxoplasmosis during pregnancy, but peak IFA titre was 1:1024 and peak CFT titre was 1:16. These titres are surprisingly low, although titres could have risen considerably in between times when sera were tested.

Patient B1 had an IFA titre of 1:2048 and CFT titre of 1:32 at 32 weeks, indicating infection probably during the second trimester.

Patient B2 had not attended antenatal clinic and at delivery had an IFA titre of 1:65536 and CFT titre of 1:16. This patient delivered at an estimated 28-weeks of pregnancy, and the baby's cord blood had similar IFA and CFT titres with a positive IgM-IFA in a titre of 1:64. This child was the only case of symptomatic congenital infection detected in the pregnancy study, and further details of this child have been given in Chapter 6. In view of the severe foetal disease and comparatively low CFT, infection was considered to have been acquired in the first half of pregnancy.

Patients B3 and B4 had IFA titres of 1:4096 at delivery with CFTs of 1:128 and 1:32, indicating probable third trimester infections. Patients C1 and C2 both showed four-fold or greater rises in IFA titres with positive CFTs at delivery, and acquired their infections during the second and third trimesters respectively. Patient C3 seroconverted between 30 weeks and term (third trimester), and had an IFA titre of 1:2048 at delivery. None of the babies of these patients had evidence of congenital infection.

The cases considered to have evidence of probable acute infection during pregnancy (B5 to B12, C4 and C5) did not have antenatal sera available. They all had IFA titres of 1:1024, CFT titres of 1:8 or 1:16 and IgM-IFA titres of 1:16 to 1:512. Most patients with acute infection in other series had dye test titres of $\geq$1:1024, and this titre is considered significant by Desmonts and Couvreur (1974a), particularly if accompanied by a positive CFT and IgM-IFA. Congenital transmission occurred in 1 of the 10 probable acute maternal infections, and this baby was asymptomatic (B12).

All cases of acute infection were followed up and 18 of the 20 mothers were traced and mothers and babies retested between 3 and 20 months after delivery. Ten mothers with IFA titres of $\leq$1:512 and positive CFTs were also followed up, as well as 12 mothers with positive IFAs and negative CFTs at delivery (Tables 7.10 and 7.11). The only congenital cases

TABLE 7.10. Follow-up of 10 patients with IFA titres of ≥1:512 and positive CFTs at delivery. Reciprocal titres are given.

Patient No.	Antenatal												Delivery						Postnatal						
	1st trimester				2nd trimester				3rd trimester				Maternal			Cord				Mother			Child		
	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A7									32	512	128	8	256	32	8	256	-	8	2	Not done			64	-	-
A21	12	256	-*	8	36	256	-	8	34	128	-	8	64	-	8	128	-	8	4	128	-	8	-	-	-
A23					30	64	-	8	30	64	-	8	128	-	8	128	-	8	5	256	-	8	16	-	-
									36	128	-	8													
A24					16	128	-	8	28	256	-	8	128	-	8	128	-	8	5	256	-	8	-	-	-
									36	128	-	8													
A28									28	32	16	8	64	32	8	16	-	-	5	128	-		-	-	-
									36	64	16	8													
B30													256	32	8	128	-	8	14	256	-	-	-	-	-
B31					24	512	-	8					512		16	256	-	-	20	512	-	-	-	-	-
B36					23	256	16	8					256	16	8	128	-	-	14	256	-	-	-	-	-
C8					26	128	16	8					256	16	8	256	-	-	14	128	-	-	-	-	-
C9									33	128	64	8	128	32	8	64	-	-	14	128	-	-	-	-	-

*-, negative test result at lowest dilution tested

TABLE 7.11. Follow-up of 12 patients with positive IFA tests and negative CFTs at delivery.

Reciprocal titres are given

Patient No.	Antenatal									Delivery				Postnatal				
	1st trimester			2nd trimester			3rd trimester			Maternal		Cord		Mother			Child	
	Gestation (wks)	IFA	CFT	Gestation (wks)	IFA	CFT	Gestation (wks)	IFA	CFT	IFA	CFT	IFA	CFT	Months post-natal	IFA	CFT	IFA	CFT
A02				26	64	-	35	64	-	64	-	128	-	5	64	-	-	-
A21	10	256	-*	24	256	-	36	32	-	256	-	32	-	4	256	-	-	-
A221				21	32	-	31	16	-	32	-	16	-	3	32	-	-	-
				36	16	-												
A230				20	128	-	34	64	-	64	-	64	-	5	64	-	-	-
							38	64										
A232							32	32	-	32	-	32	-	4	64	-	16	-
							36	16	-									
A24(		30	64	-	32	128	-	32	-	32	-	32	-	4	128	-	16	-
A243					35	16	-	32	-	32	-	32	-	5	32	-	-	-
A255					30	64	-	32	-	32	-	32	-	5	32	-	-	-
					36	64	-											
B767										16	-	16	-	14	16	-	-	-
C45		22	128	-						512	-	256	-	14	128	-	-	-
C47					32	128	-	256	-	256	-	256	-	15	128	-	-	-
C124					35	1024	-	1024	-	512	-	512	-	15	64	-	-	-

*-, negative test result at lowest dilution tested

detected were the babies of patients B2 (which had died soon after birth) and ₹12, which was asymptomatic but seropositive. All the other babies traced had IFA titres of ≤1:16 and negative CFTs and IgM-IFAs. Two patients with probable acute infection during pregnancy could not be traced.

Only 1 mother (A2) had features suggestive of acute infection during pregnancy. She had an influenza-like illness with pyrexia, cervical lymphadenopathy and pharyngitis at 32 weeks' gestation while on holiday in Durban. This patient seroconverted at 34 weeks' gestation, and her husband also had serological evidence of acute infection, indicating a probable common source of infection.

Congenital transmission was therefore found in 2 out of 20 cases of acute infection during pregnancy (or 1 out of 10 definite and 1 out of 10 probable cases), giving a transmission rate of 10%.

The IgM-IFA results were useful in confirming IFA and CFT results indicative of acute infection, and titres were ≥1:16 in all definite or probable acute cases, and the highest titre seen was 1:512. The IgM-IFA was positive in cord blood of both babies with congenital infection, and negative in all other cord bloods tested. It was the most useful test for detecting congenital infection as the cord blood CFT was often positive, although being negative on follow-up.

In the 89 recent infections (IFA and CFT positive), the IgM-IFA was negative in 41 cases and positive (1:16 to 1:32) in the remainder. In patients regarded as having had past infection with IFA titres of ≥1:512, but negative CFTs, the IgM-IFA was positive (1:16 to 1:32) in 28 of the 53 sera (53%), and some of these patients may have had recent infections. The correlation between the CFT and the IgM-IFA results was poor, probably as the IgM-IFA was only regarded as positive at titres of ≥1:16. This may have limited the use of the IgM-IFA, although the IgM-IFA was positive in low titre in about half the patients with IFA titres of ≥1:512 but with negative CFTs. Improved standardization of the IgM-IFA should result in more reliable interpretation of this test.

7.3 Discussion

Frenkel (1973a) has drawn up charts of the relationship between incidence and prevalence of toxoplasmosis (Fig. 7.2) and the risk of maternal and foetal infection during pregnancy in the 20- to 30-year age group (Fig. 7.3 and Table 7.12). The maximum incidence of maternal infections occur in areas with annual incidence rates of 3% to 5%. Where the

incidence is less than 3%, infections during pregnancy occur less frequently, whereas if the incidence is over 5%, the majority of persons would already be infected by the time they reached childbearing age, and infection during pregnancy would therefore be less common.

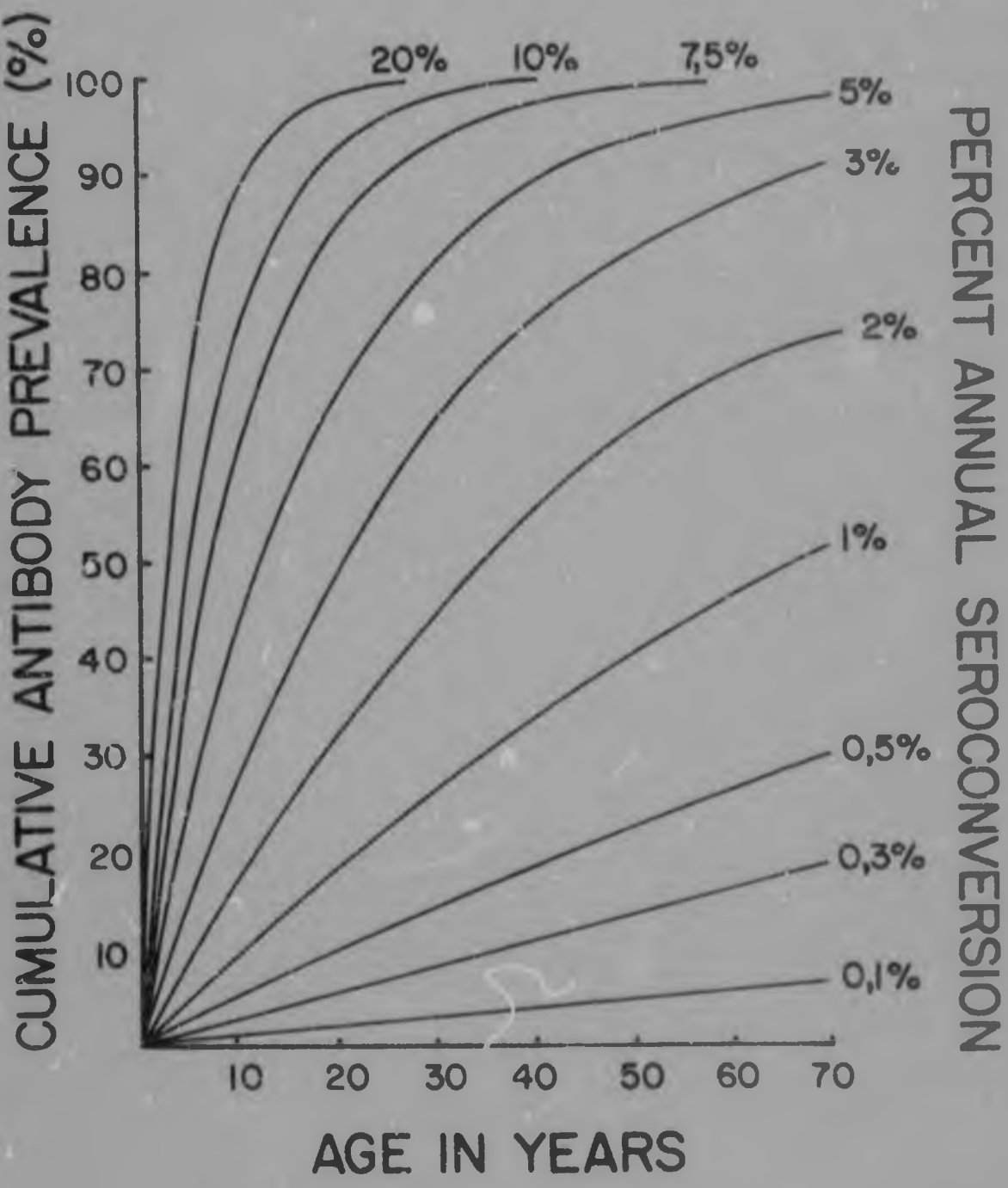

Fig. 7.2. Theoretical toxoplasmosis incidence rates in different age groups according to prevalence of antibodies (from Frenkel, 1973a).

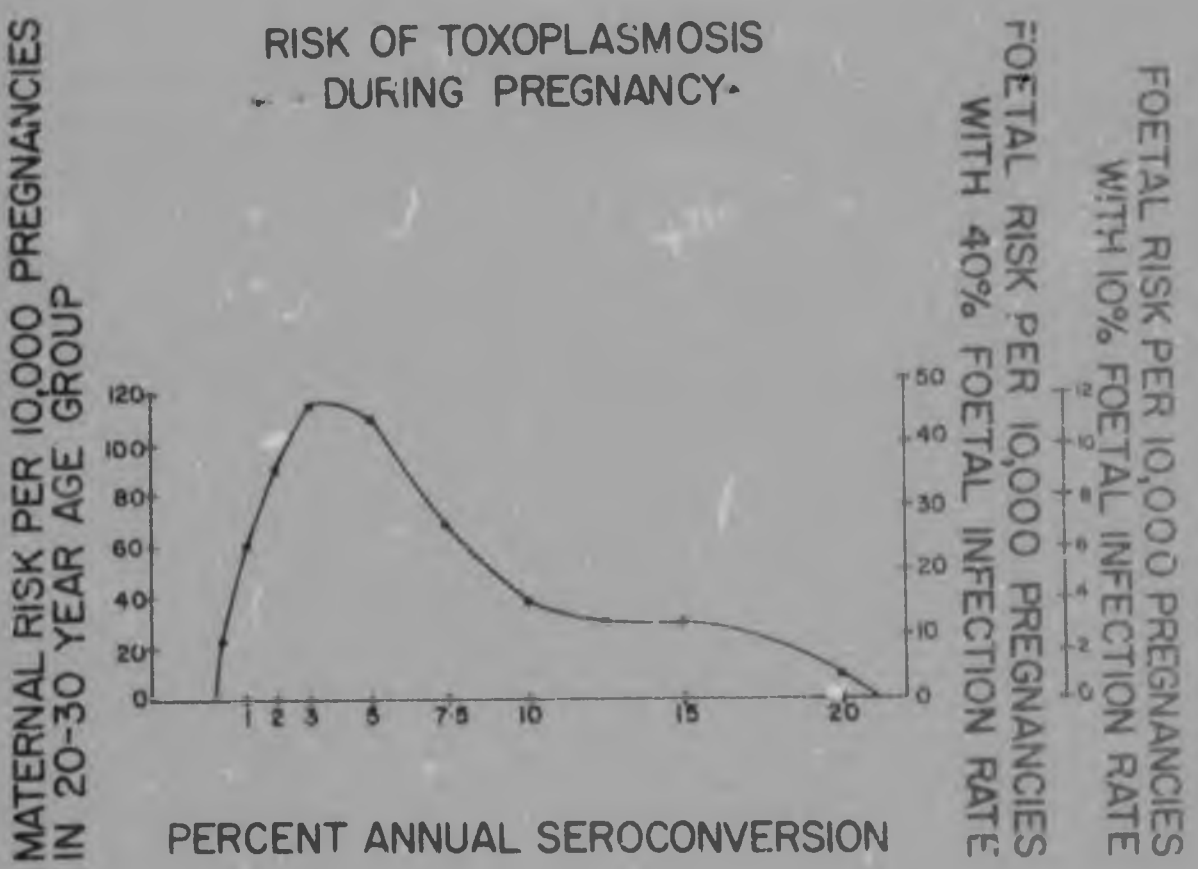

Fig. 7.3. Estimated maternal and foetal risk of infection in mothers between the ages of 20 and 30-years. Foetal transmission rates are indicated at 10% and 40% transmission rates (modified from Frenkel, 1973a).

TABLE 7.12. Theoretical calculated risk of maternal and foetal toxoplasmosis according to percentage annual seroconversion rate (modified from Frenkel, 1973a).

Percentage annual seroconversion	Maternal risk per 10 000 pregnancies	Foetal risk per 10 000 pregnancies with	
		40% foetal transmission	10% foetal transmission
0.25	20	8	2
0.5	40	16	4
1	60	24	6
2	90	36	9
3	115	46	12
5	110	44	11
7.5	70	28	7
10	40	16	4
15	25	10	3
20	10	4	1

This study has yielded a prevalence of *Toxoplasma* antibodies of 22%, with an approximate 1% annual seroconversion rate. Twenty maternal infections were detected with 2 congenital transmissions, giving a transmission rate of 10%.

Problems were encountered in the detection of all cases of maternal infection due to the lack of antenatal attendance, late antenatal presentation, difficulty in detection of infections in very early and very late pregnancy, and the apparent short period in which titres remained at high levels.

The IFA and IgM-IFA tests appeared to give good reproducible results, but the CFT gave many technical problems. With adequate standardization, the IgM-IFA should replace the CFT, although their results do not always coincide. The IgM-IFA was particularly useful in the early detection of congenital infection as the CFT in cord blood was often positive in the absence of congenital transmission.

Using the factors presented above and the results obtained in this study, with a 1% annual incidence, 60 new infections per 10 000 pregnancies would be expected in the populations studied. This study in general did not take abortions into account, and infections in early pregnancy could have been missed in patients only tested at delivery. Therefore assuming that evidence of infection during the second and third trimesters was detected, 40 infections per 10 000 pregnancies should be detected under the study conditions used. As 6705 patients were studied, the expected number of acute infections is 27, with 11 expected congenital infections at a 40% transmission rate.

The number of acute infections detected was 20, the difference probably being due to patients not tested antenatally being missed. Only 2 of the babies born to these 20 mothers had serological or clinical evidence of congenital infection at birth, and follow-up on 16 of the remaining 18 of these babies confirmed the absence of congenital infection. Follow-up examinations included serology on mothers and babies and physical examination of babies, including fundoscopy by an ophthalmologist.

These results have shown the number of maternal infections to be approximately that expected, but that the foetal transmission rate was much lower than expected (10% instead of 40% found in France). This transmission rate is particularly low as most of the infections detected were in the second and third trimesters, where transmission rates are known to

be even higher. It is particularly interesting, also, to note that only one asymptomatic case was detected, and that organisms could not be recovered from the placentas of 14 of the mothers with acute infection (the placentas of the congenital cases were unfortunately not submitted).

The results obtained in this study were compared with those obtained in other studies, as shown in Table 7.13. These studies are difficult to compare as most had the same problem as encountered in this study of inadequate serology during early pregnancy. This is clearly shown in the percentage of actual to expected acute maternal cases, which was 47% to 77%, except in the study by Desmonts and Couvreur (1974a), where the figure was 93%, apparently as in France women have to attend antenatal clinics by the end of the first trimester of pregnancy to obtain welfare benefits. However, the expected number of maternal infections in pregnancy calculated according to Frenkel's data (Figs. 7.2 and 7.3) appears to be remarkably accurate considering the techniques used and the actual number of cases detected. Although some of the estimates shown in Table 7.12 could be questioned, all the studies should have detected congenital infections with serologic evidence of infection at birth or on follow-up. Therefore the congenital infection rate per pregnancy should give the most reliable index of transmission rates during pregnancy. This rate was highest in France (0.4%), around 0.1% in North America and less than 0.05% in England and in the present study.

The transmission rate found in France of 40% has been assumed to be valid in other parts of the world. However, from this and the other studies considered, this transmission rate does not appear to be valid elsewhere. Actual transmission rates in other studies varied from 0 to 21%, and expected transmission rates from 0 to 18%. Transmission rates in the 3 North American studies were 13 to 21%. This study showed a transmission rate of 10% in South Africa.

From the above comparisons, definite differences in congenital infection and congenital transmission rates are found in different parts of the world, raising the question of the factors responsible for these differences. Differences in strain virulence in different parts of the world could explain these variations, with strains in England and South Africa apparently being of particularly low virulence. Factors such as infective dose, nutritional status, host genetic response to infection and host immunity do not appear to be responsible for these differences. The variations in

TABLE 7.13. Comparison of studies on maternal and foetal toxoplasmosis during pregnancy. Population seroconversion rates and expected values were calculated from those of Frenkel (1973a). Different criteria were used to define maternal infections during pregnancy and the best criterion for comparison is the congenital infection rate per pregnancy.

Author	No. of pregnancies studied	Population seroconversion rate[b]	No. of probable maternal infections[d] / No. expected[b]	No. of congenital infections: symptomatic/total (% symptomatic in brackets)	Congenital infection rate per maternal infection actual/expected[f]	Congenital infection rate per pregnancy
Kimball *et al.* (1971), New York	4048	1.5%	23/30 (77%)	1/3 (33%)	13%/10%	0.07%
Rouse & Bourne (1972), England	3187	1%	9/19 (47%)	0/0	0%/0%	0%
Desmonts & Couvreur (1974), France	16800[a]	3%	180/193 (93%)	24/70 (34%)	39%/36%	0.4%
Alford *et al.* (1974), Alabama, USA	7500	1.5%[c]	ND[e]/56	2/10 (20%)	- /18%	0.1%
Liems *et al.* (1977), Canada	4138	2%	19[g]/37 (51%)	0/4 (0%)	21%/11%	0.1%
Present study, South Africa	6705	1%	20/40 (50%)	1/2 (50%)	10%/5%	0.03%

a Estimated total sample size from data given (76% seronegative patients, with 180 converting at a rate of 6.3 per 100 pregnancies).

b Population seroconversion rates and expected maternal infections estimated from calculated data presented by Frenkel (1973a). Expected infections adjusted to number of patients studied.

c Assumed to be similar to that in New York as data not provided by authors.

d Number of probable maternal infections includes seroconverters and patients with high titres at delivery.

e ND = not done.

f These figures compare actual and expected foetal transmission rates, and does not take into account the assumption that the expected rate is 40% as found by Desmonts and Couvreur, and rates shown indicate these rates to vary from 0 - 40%.

g Number of probable maternal infections was not clearly stated by authors, and was taken to be patients with IFA titres ≥1:1000 (300 IU) with positive IgM-IFA tests.

virulence may affect the ability of organisms to cross the placenta, as symptomatic congenital infections, when they do occur, appear to cause as much damage to the foetus in areas of high and low foetal transmission rates. Comparison of *Toxoplasma* strains from these different areas may clarify the reasons for these differences.

8.0 TOXOPLASMOSIS IN SHEEP IN SOUTH AFRICA

Very little has been published on the prevalence of toxoplasmosis in domestic meat-producing animals in South Africa, and the only data available showed that all of 20 cattle, 16 of 20 pigs and 7 of 10 sheep were dye-test positive (SAIMR, 1959). When material from sheep became available during 1977 from the abattoir of the Onderstepoort Veterinary Institute, Pretoria, this was used to investigate the role of these animals in toxoplasmosis.

8.1 Materials and methods

Sheep, bred for slaughter on farms in the Transvaal, Orange Free State and Northern Cape, were received by the Onderstepoort Veterinary Research Institute, and slaughtered by severing the carotid and jugular vessels. The origin of individual animals could not be determined as they were pooled by stock agents prior to despatch to the abattoir. Blood was collected in a large vessel from the severed neck vessels, and serum separated and inactivated. From some animals, the diaphragm was removed and placed in antibiotic-containing saline. Serum was collected from 895 animals, and diaphragms from 147 of these animals.

Sera from animals from which diaphragm was available were immediately tested by the IHA and IFA methods as described in Chapter 3. Diaphragms from animals with high titres (≥1:512 IFA, with IHA usually positive as well) were inoculated into mice in view of the correlation between titre and success of isolation found by Work (1971). Sera from the remainder of the animals were frozen at -20°C until tested by the IFA technique.

8.2 Results

Of the 895 sera tested by the IFA method, 97% (868) were positive at a titre of ≥1:16. Titres of up to 1:16 384 were found. Distribution of results according to titre is shown in Table 8.1.

Of the 147 sera also tested by the IHA method, 97% (143) were positive in the IFA test and 30% (44) in the IHA test, with IHA titres of 1:16 to 1:1024 as shown in Table 8.2. The IHA method was used to obtain a rapid result to enable inoculation of diaphragm material into mice, but the method was not very successful as titres were generally lower than corresponding IFA titres, and end-points were very difficult to interpret as they tended to fade out rather than give a clear-cut end-point.

TABLE 8.1. Results of IFA tests on 895 sheep

IFA titre	Percentage	Sample size
Negative (<1:16)	3	27
1:16	8	75
1:32	24	215
1:64	26	233
1:128	24	218
1:256	6	54
1:512	3.5	31
1:1024	2.7	24
1:2048	1.5	13
1:4096	0.2	2
1:8192	0.2	2
1:16384	0.1	1
	100%	895

TABLE 8.2. Comparison of IFA and IHA results in sheep

	IHA titre					
IFA titre	Negative	1:16	1:64	1:256	1:1024	Total
Negative	4	-	-	-	-	4
1:16	24	2	-	-	-	26
1:32	30	10	-	-	-	40
1:64	13	5	1	-		19
1:128	23	2	3	-	-	28
1:256	6	4	1	-	-	11
1:512	3	1	4	2	-	10*
1:1024	-	1	2	3	1	7*
1:2048	-	-	-	-	2	2*
TOTAL	103	25	11	5	3	147

* Diaphragms from these sheep were inoculated into mice

Diaphragm, representing skeletal muscle from the 19 sheep with IFA titres of ≥1:512, was inoculated into mice after peptic digestion in artificial gastric juice as described in Chapter 4. However, no

isolates of *Toxoplasma* were obtained after 3 passages in mice, and all mice used remained seronegative. The only isolate obtained was an organism producing thick-walled brain cysts in the mice inoculated from one of the sheep. These cysts failed to fluoresce with direct anti-*Toxoplasma* conjugate, and may be cysts of *Besnoitia* species.

8.3 Discussion

The finding of 97% of sheep to be seropositive, and therefore to have evidence of chronic toxoplasmosis, was extremely interesting in assessing the possible role of these and other meat-producing animals in the spread of toxoplasmosis to man. A high seropositivity rate was also noted by Bigalke (1978), who stated that of many sheep tested by him by the dye test method, he had hardly ever seen a negative result. Representative high and low titre sera from sheep in this study were checked in the dye test, and dye test titres within one doubling dilution of the IFA method were obtained. Other coccidia such as *Besnoitia, Sarcocystis, Frenkelia* and *Hammondia* may cross-react in the IFA test, and may have affected the results obtained (Frenkel, J. Parasitol. 63:611-628, 1977).

Ovine abortion due to *Toxoplasma* has not been reported in the South African veterinary literature, probably as animals become infected before reaching maturity. Mutton and lamb are therefore almost always at risk of transmitting infection if eaten raw or undercooked. Fortunately, these meats are usually well-cooked, and probably do not represent a major hazard unless eaten raw.

Failure to isolate *Toxoplasma* from the diaphragms of the 19 high-titre seropositive animals was disappointing, but may have been due to the small number of diaphragms tested, the small amount of material used for digestion and inoculation, irregular distribution of cysts, or killing of cysts during the digestion process.

Investigation of other meat-producing animals may yield high seropositivity rates as well, and all fresh meat products in South Africa should be regarded as potentially containing *Toxoplasma* cysts.

9.0 CONCLUSIONS

Toxoplasma has been shown to be an extremely successful and versatile parasite throughout the world, with both sexual and asexual stages in its life cycle. This parasite is now known to be a sporozoan, with the cat as its definitive host, and it is capable of optional rather than obligatory changes between definitive and intermediate hosts (Frenkel, 1973a).

The findings presented here have shown that toxoplasmosis is present in Southern Africa, and that acute infection and congenital transmission occur as expected from experiences reported in other parts of the world.

9.1 Prevalence and incidence of toxoplasmosis in Southern Africa

The prevalence of *Toxoplasma* antibodies in over 10 000 sera, as shown in Chapter 5, is 21% overall, varying from 9% in the San of South West Africa and Botswana to 34% in Blacks and Indians in Natal. The overall annual seroconversion rate was 1% in the under 20-year age group, falling to 0.5% in the over 50-year group. This fall was particularly prominent in the Western Cape, where prevalence hardly rose over the age of 20 years. This suggests that oocyst transmission may be more important than transmission via meat as there are no major cultural or socio-economic differences between the Western Cape and the rest of the country, and meat supplied to the Western Cape comes from a wide area which also supplies the rest of the country. The finding of a lower prevalence in Whites than in Blacks, Coloureds and Indians supports oocyst transmission, as Whites, being of higher socio-economic status could be less exposed to oocysts but more exposed to rare meat.

The antibody prevalence found generally reflected climatic conditions, being lowest in the most arid areas and highest in the high-rainfall region of Natal, again suggesting oocyst transmission.

The groups studied in South West Africa and Botswana also yielded interesting contrasts between the San and the Negroid Dama. The Kalahari Desert supports a number of San communities whose members obtain their meat from hunting buck and small mammals. They will, if given the opportunity, hunt and kill lion and other predators. The Dama, on the other hand, are a settled pastoral people, keeping goats and sometimes

sheep and cattle in the northern parts of South West Africa. In both the San and the Dama, hygiene is very primitive, although the San consume raw meat more often than do the Dama. Both groups keep dogs, but domestic cats are virtually unknown. The seropositivity rate in the Dama (27%) was significantly higher than that in the San (9%).

9.2 Toxoplasmosis in pregnancy in South Africa

This was studied in 6705 patients in the Transvaal, and the results presented in Chapter 7. Twenty mothers with serological evidence of acute toxoplasmosis were detected (0.3%) and transmission occurred in 2 of the babies born to these mothers. One of the affected babies was severely affected and the other was asymptomatic. The congenital infection rate found was therefore 0.03% or 3 per 10 000 births, with a foetal transmission rate of 10%. With an estimated 627 000 births per year in South Africa, and 0.4% incidence of maternal infection per year (0.3% for 9 months' gestation), 2 500 maternal infections per year would be expected. With a 10% foetal transmission rate, 250 congenital infections would be expected. These figures are probably minimum estimates and may be as high as double these estimates (see Chapter 5). The number of congenital cases detected in this study was too small to give an estimate of the numbers of symptomatic and asymptomatic congenital infections, but if data from Desmonts and Couvreur (1973a) are applicable, 38 severe, 48 mild and 165 asymptomatic congenital infections would be expected annually in South Africa. The 38 severe cases would represent 1 severe congenital infection per 17 500 births or 0.006%.

9.3 Transmission of toxoplasmosis in Southern Africa

The evidence presented above has provided indirect data which indicate that oocyst transmission may be important in the younger age groups. Transmission of cysts via meat can certainly occur and probably accounts for a good proportion of infections, particularly in adults. However, as in other parts of the world, the role of the 2 methods of transmission, and the possibility of other methods existing, remains unresolved.

9.4 Virulence of South African *Toxoplasma* strains

Virulence in man is extremely difficult to evaluate, and the major difference shown in this study from the classic studies of Desmonts and Couvreur in France is the lower congenital transmission rate (Table 7.13). However, when congenital infections do occur, the affected babies appear to be as severely affected as those described elsewhere. Lower congenital

sheep and cattle in the northern parts of South West Africa. In both the San and the Dama, hygiene is very primitive, although the San consume raw meat more often than do the Dama. Both groups keep dogs, but domestic cats are virtually unknown. The seropositivity rate in the Dama (27%) was significantly higher than that in the San (9%).

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transmission rates than those reported in France were found in studies in North America and England, and strain differences could account for these variations. South African strains would be amongst those resulting in the lowest congenital transmission rates in the world.

No explanations for these differences in congenital transmission rates are available, other than that they may reflect differences in virulence.

9.5 Diagnosis of toxoplasmosis

Criteria for the serological diagnosis of toxoplasmosis in Southern Africa are similar to those described elsewhere. IFA seroconversion with positive IgM-IFA and CFTs are diagnostic in most cases, but problems can occur as these tests are not standardised in many laboratories. Persons incidentally found to have serological evidence of chronic infection are often diagnosed and treated for acute infections. Diagnostic criteria for acquired toxoplasmosis are presented in Table 9.1.

TABLE 9.1. Diagnostic criteria for acquired toxoplasmosis

Presentation	acute	recrudescent	chronic asymptomatic	chronic symptomatic
Clinical features	localized or generalized lymphadenopathy	encephalitis, myocarditis, and pneumonitis in compromised host	nil	retinochoroiditis
Serology*	IFA seroconversion, rise in titre, or single titre ≥1:1000 with positive IgM-IFA or CFT	IFA positive titre usually high, and IgM-IFA and CFT can be positive	IFA positive, titre 1:16 to 1:512, CFT and IgM-IFA negative	IFA positive but titres can be low, and IgM-IFA and CFT usually negative
Histopathology	*Toxoplasma* lymphadenitis	*Toxoplasma* encephalitis, etc.		*Toxoplasma* retinochoroiditis
Treatment	Nil if asymptomatic	Pyrimethamine and sulphadiazine	Nil	Pyrimethamine, sulphadiazine and steroids if active or progressive

* Dye test may be used instead of IFA test

The diagnosis of congenital infections depends on the demonstration of *Toxoplasma* IgM-IFA or CFT antibodies in babies' sera at birth, with these tests remaining positive after the age of 6 months, when maternal antibodies should have disappeared. Diagnostic criteria for congenital infection are presented in Table 9.2.

TABLE 9.2. Diagnostic criteria for congenital toxoplasmosis

Presentation	severe infection	mild infection	asymptomatic
Clinical features	hydrocephalus, intracranial calcification, retinochoroiditis, encephalitis, hepatosplenomegaly, etc.	retinochoroiditis	nil
Serology	IFA, IgM-IFA and CFT positive at birth, and remain positive past the age of 6 months. Maternal serology also positive.		
Treatment	Pyrimethamine and sulphonamides, even if asymptomatic at birth.		

9.6 Public health significance of toxoplasmosis in South Africa

The major public health role of toxoplasmosis is its causation of severe congenital infection. From the data presented in this study, 1 to 2 severe *Toxoplasma* infections per 16 500 births (0.006% to 0.012% of births) or 38 to 75 cases (including abortions) per year are estimated to occur in South Africa.

As there are at present no means such as immunization to prevent maternal infection as there are in rubella, the only possible control measure would be the serological diagnosis of maternal infections during pregnancy. As 79% of patients are seronegative on becoming pregnant, this would entail follow-up of about 500 000 women per year. Frequent serological testing would be required, and the only possible intervention would be treatment of mothers with spiramycin as therapeutic abortion would not be justified, or could not be done in late pregnancy.

As the serological techniques in toxoplasmosis are not nearly as simple as the reagin tests used to screen for syphilis in pregnancy, major laboratory facilities and trained staff would have to be provided for such a control programme.

From the practical point of view, control of toxoplasmosis is obviously impossible in view of the small number of severe congenital infections

that occurs, the problems associated with adequate diagnostic facilities, the late presentation of many patients for antenatal care if at all, and the higher priority for the control of diseases such as tuberculosis, measles, syphilis and malnutrition in South Africa. The only practical measure worth taking would be to publicise the measures advocated by Frenkel (1974), which include control of cats and avoidance of possible contact with cat faeces and raw meat during pregnancy (see 1.12).

As the prevalence and incidence of toxoplasmosis in Southern Africa are low, and the transmission rate during acute infections in pregnancy is also particularly low compared with that in other parts of the world, control measures would be of very little overall benefit to the community. Many severe infections result in abortion or neonatal death, and the cost of screening programmes during pregnancy would be prohibitive. Very little can, in any case, be done to prevent congenital transmission other than to avoid sources of infection during pregnancy.

Until vaccination becomes a reality, we will have to live with the small, although significant, threat of congenital toxoplasmosis. Screening for toxoplasmosis during pregnancy is not advocated for the reasons given above, although the risk of congenital infection can be reduced by simple measures such as education of pregnant women to wash their hands prior to touching their faces or eating.

APPENDIX 1.1. QUEEN VICTORIA HOSPITAL - IFA and CFT positives

Patient No.	Antenatal												Delivery						Postnatal						
	1st trimester				2nd trimester				3rd trimester				Maternal			Cord				Mother			Child		
	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A1	12	32	-	-	24	512	32	16	36	1024	64	8	1024	128	8	512	-	-	4	128	16	-	-	-	-
A2					20	-	-	-	28	-	-	-	256	64	16	128	-	8	5	256	-	-	16	-	-
									34	16	-	-													
A3					26	-	-	-	32	-	-	-	256	32	8	128	-	8	3	256	16	8	16	-	-
A4					22	128	-	8	36	64	-	8	64	-	8	64	-	8							
A5					22	64	32	8					32	16	8	64	-	8							
A6					20	32	-	8	38	64	-	8	64	-	8	32	-	-							
A7									32	512	128	8	256	32	8	256	-	8	2	Not done			64	-	-
A8					16	128	-	8	28	64	-	8	64	-	-	32	-	-							
									37	64	-	8													
A9					24	64	64	8	38	32	32	8	32	16	8	64	-	8							
A10									32	64	-	8	64	-	8	64	-	8							
									36	64	-	8													
A11					24	128	-	16	32	128	-	8	128	16	16	64	-	-							
A12					21	32	16	8	27	32	-	8	32	16	8	64	-	8							
A13									32	64	-	8	32	-	8	32	-	-							
									36	64	-	8													
A14					26	64	16	8	32	64	16	8	128	32	8	128	-	8							
A15					20	32	-	8	32	64	-	8	64	-	8	64	-	-							
									36	32	-	8													
A16					26	256	-	16	37	256	-	16	256	-	16	256	-	16							
A17									32	64	-	8	64	16	8	128	-	8							
A18					16	32	-	8	28	32	16	8	32	16	8	32	-	8							
A19					20	32	-	8	32	32	-	8	64	-	8	32	-	8							
A20					16	32	-	8	28	32	-	8	64	-	8	64	-	-							
									36	16	-	8													
A21					26	64	16	8	32	128	16	8	64	16	8	64	-	-							
A22	12	256	-	8	26	256	-	8	34	128	-	8	64	-	8	128	-	8	4	128	-	8	-	-	-

Patient No.	Antenatal												Delivery						Postnatal						
	1st trimester				2nd trimester				3rd trimester				Maternal			Cord			Mother				Child		
	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A23					20	64	-	8	30	64	-	8	128	-	8	128	-	8	5	128	-	8	16	-	-
									36	128	-	8													
A24					16	128	-	8	28	256	-	8	128	-	8	128	-	8	5	256	-	8	-	-	-
									36	128	-	8													
A25									30	128	-	16	64	-	16	64	-	-							
A26					20	64	16	8	28	256	32	16	64	16	8	64	-	8							
									34	128	16	16													
A27									34	128	64	8	128	32	8	128	-	8							
A28									28	32	16	8	64	32	8	16	-	-	5	128	-	-	-	-	-
									36	64	16	8													
A29									32	512	-	16	128	-	16	128	-	8							
A30									35	128	-	8	64	-	8	64	-	8							
A31									36	128	16	8	128	16	8	64	-	8							

APPENDIX 1.2 QUEEN VICTORIA HOSPITAL - IFA positive, [illegible]ive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A32									30	16		-	16		-	16		-
A33	9	64		-					33	64		-	128		-	128		-
A34	13	16		-					36	16		-	16		-	16		-
A35					20	32		-					32		-	32		-
A36					16	32		-	28	32		-	16		-	32		-
									36	16		-						
A37					26	64		-					128		-	64		-
A38													64		-	64		-
A39					26	16		-					32		-	32		-
A40					22	32		-					32		-	32		-
A41					14	32		-					32		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A42					21	64		-	30	128		-	128		-	64		-
									38	128		-						
A43					20	32		-	34	32		-	16		-	16		-
									38	32		-						
A44					18	16		-	36	32		-	32		-	32		-
A45									26	16		-	16		-	16		-
A46					22	64		-	38	64		-	32		-	32		-
A47									32	16		-	16		-	16		-
A48	12	16		-					29	16		-	16		-	-		-
									34	16		-						
A49					21	16		-					16		-	-		-
A50					16	64		-	32	64		-	64		-	128		-
A51									28	256		-	256		-	256		-
A52	10	64		-					38	32		-	32		-	32		-
A53									32	32		-	16		-	16		-
A54	12	16		-					30	32		-	16		-	32		-
A55									28	128		-	64		-	64		-
A56					24	64		-					128		-	64		-
A57					16	32		-	34	64		-	32		-	32		-
A58					26	64		-	38	128		-	64		-	64		-
A59					20	16		-	34	16		-	16		-	-		-
A60	12	32		-					32	32		-	32		-	32		-
A61									26	256		-	256		-	512		-
A62					26	64		-	35	64		-	64		-	128		-
A63									30	16		-	16		-	16		-
A64					24	128		-	36	16		-						
A65									28	128		-	64		-	128		-
A66									30	16		-	16		-	-		-
A67					26	256	-	-	34	16		-						
A68	13	64		-	26	32		-					32		-	32		-
A69					25	16		-	34	32		-	64		-	16		-
									32	32		-	16		-	-		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A70					19	16		-	36	16		-	16		-	32		-
					26	32		-										
A71									32	256		-	128		-	64		-
A72									32	16		-	16		-	-		-
A73									36	32		-	64		-	64		-
A74					24	16		-	32	16		-	16		-	16		-
A75					16	32		-	30	64		-	64		-	64		-
A76									34	16		-	32		-	16		-
A77									27	64		-	128		-	64		-
A78									36	64		-	32		-	32		-
A79					16	32		-	38	16		-	16		-	16		-
A80					16	16		-	30	32		-	16		-	32		-
A81					16	32		-					16		-	16		-
A82	9	32		-					27	16		-	32		-	32		-
A83					21	16		-	32	16		-	16		-	32		-
A84					18	64		-	28	32		-	64		-	64		-
A85					19	16		-	31	16		-	16		-	16		-
									36	16		-						
A86					19	16		-	32	16		-	16		-	16		-
A87					26	64		-	38	64		-	128		-	64		-
A88									28	16		-	16		-	16		-
A89									30	32		-	32		-	64		-
A90					26	16		-	36	16		-	16		-	-		-
A91	9			-	26	32		-	38	32		-	64		-	64		-
A92	8	64		-	26	32		-	36	64		-	64		-	128		-
A93					20	64		-	37	32		-	64		-	64		-
A94									34	32		-	16		-	-		-
A95					24	32		-					32		-	16		-
A96	10	32		-					28	32		-	16		-	16		-
A97									32	16		-	16		-	16		-
A98	7	32		-	26	32		-	38	32		-	32		-	16		-
A99					18	32		-	28	32		-	32		-	64		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A100					25	32		-	35	32		-	32		-	64		-
A101									34	16		-	16		-	-		-
A102					20	16		-	32	16		-	16		-	-		-
A103	7	32		-					37	16		-	32		-	64		-
A104									32	16		-	32		-	16		-
A105									28	16		-	16		-	32		-
A106					21	16		-	28	32		-	16		-	-		-
A107									35	32		-	32		-	16		-
A108					21	128		-	36	128		-	128		-	64		-
A109					26	64		FP*	35	64		FP	64		FP	128		FP
A110									28	16		-	16		-	-		-
A111									36	16		-	16		-	-		-
A112									38	16		-						
A113	13	16		-					32	16		-	16		-	16		-
A114									28	16		-	16		-	16		-
A115					22	32		-	36	32		-	32		-	32		-
A116					16	16		-	35	16		-	32		-	16		-
A117									29	16		-	16		-	-		-
A118	12	128		-					29	32		-	64		-	64		-
A119					26	64		-	28	128		-	64		-	64		-
A120					15	16		-	30	128		-	64		-	64		-
A121					14	64		-	27	16		-	32		-	-		-
A122	11	64		-					28	32		-	32		-	64		-
A123									36	64		-						
A124					26	32		-	30	32		-	32		-	32		-
A125					26	16		-	34	32		-	64		-	16		-
A126					19	64		-					32		-	16		-
A127	8	32		-					31	16		-	16		-	16		-
A128									30	32		-	32		-	32		-
A129									34	32		-	64		-	64		-
									35	32		-	16		-	-		-
									32	32		-	32		-	16		-
									36	64		-						

*FP = false positive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A100					25	32		-	35	32		-	32		-			-
A101									34	16		-	16		-	-		-
A102					20	16		-	32	16		-	16		-	-		-
A103	7	32		-					37	16		-	32		-	64		-
A104									32	16		-	32		-	16		-
A105									28	16		-	16		-	32		-
A106					21	16		-	28	32		-	16		-	-		-
A107									35	32		-	32		-	16		-
A108					21	128		-	36	128		-	128		-	64		-
A109					20	64		FP*	35	64		FP	64		FP	128		FP
A110									28	16		-	16		-	-		-
A111									36	16		-	16		-	-		-
A112									38	16		-						
A113	13	16		-					32	16		-	16		-	16		-
A114									28	16		-	16		-	16		-
A115									36	32		-	32		-	32		-
A116					22	32		-	35	16		-	32		-	16		-
A117					16	16		-	29	16		-	16		-	-		-
A118	12	128		-					29	32		-	64		-	64		-
A119									28	128		-	64		-	64		-
A120					26	64		-	30	128		-	64		-	64		-
A121					15	16		-	27	16		-	32		-	-		-
					14	64		-	28	32		-	32		-	64		-
A122	11	64		-					36	64		-						
A123									30	32		-	32		-	32		-
A124									34	32		-	64		-	16		-
A125					20	32		-					32		-	16		-
A126					26	16		-	31	16		-	16		-	16		-
A127	8	32		-	19	64		-	30	32		-	32		-	32		-
A128									34	32		-	64		-	64		-
A129									35	32		-	16		-	-		-
									32	32		-	32		-	16		-
									36	64		-						

*FP = false positive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A130					18	32		-	27	16		-	16		-	-		-
A131					17	64		-	32	128		-	64		-	64		-
A132					24	128		-					64		-	32		-
A133									35	32		-	32		-	32		-
A134									33	16		-	[illegible]		-	-		-
A135									32	16		-	[illegible]		-	16		-
									36	16		-						
A136					16	32		-					[illegible]		-	64		-
A137					26	16		-	32	16		-	16		-	-		-
A138					20	32		-	28	16		-	32		-	16		-
A139									36	16		-	16		-	16		-
A140									36	16		-	16		-	-		-
A141					26	64		-	35	32		-	[illegible]		-	64		-
A142									32	32		-	32		-	64		-
A143									29	16		-	32		-	-		-
									35	16		-						
A144	7	32		-	25	16		-	37	32		-	32		-	32		-
A145					19	16		-	36	16		-	16		-	-		-
A146									28	16		-	16		-	-		-
A147					25	64		-	32	32		-	32		-	64		-
A148					18	128		-	32	64		-	32		-	64		-
									36	32		-						
A149					26	64		-	32	64		-	64		-	64		-
A150					20	64		-	28	32		-	32		-	32		-
A151					21	64		-	29	32		-	64		-	32		-
A152					23	32		-	32	32		-	64		-	64		-
									38	64		-						
A153					20	128		-	30	64		-	64		-	16		-
A154					21	64		-	38	32		-	16		-	-		-
A155									33	32		-	32		-	64		-
A156					25	16		-	34	16		-	16		-	16		-
A157					16	16		-	29	16		-	32		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A158	9	64		-					38	32		-	32		-	32		-
A159					15	16		-	32	16		-	16		-	-		-
A160					18	64		-	34	32		-	64		-	64		-
A161									38	16		-	16		-	-		-
A162	11	32		-					36	32		-	32		-	32		-
A163					18	16		-	30	16		-	32		-	-		-
A164	8	16		-	26	16		-	35	16		-	16		-	-		-
A165					19	64		-	29	64		-	64		-	64		-
A166					20	32		-	30 34	32 32		- -	32		-	32		-
A167					18	128		-	28	64		-	64		-	64		-
A168					16	16		-	28	16		-	16		-	-		-
A169					25	32		-	35	16		-	32		-	64		-
A170					22	64		-	30 36	32 64		- -	64		-	32		-
A171					26	32		-	36	32		-	32		-	32		-
A172					25	32		-	32	64		-	32		-	32		-
A173					26	16		-					32		-	16		-
A174					19	64		-	27	64		-	64		-	32		-
A175					21	32		-	29	64		-	64		-	64		-
A176					23	64		-	31 36	64 32		- -	32		-	16		-
A177	13	64		-	20	64		-	38	32		-	64		-	32		-
A178									28 36	16 16		- -	32		-	32		-
A179	12	64		-	26	32		-	35	32		-	64		-	64		-
A180					19	16		-	28 37	16 64		- -	16		-	16		-
A181	8	64		-					31 34	64 64		- -	128		-	64		-
A182	13	32		-	26	32		-	36	32		-	32		-	32		-
A183	7	32		-	25	16		-	34	16		-	32		-	64		-
A184					20	64		-	30 38	32 64		- -	32		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A185									32	16		-	16		-	-		-
A186	13	16		-	26	16		-	32	16		-	16		-	32		-
A187					16	64		-	34	64		-	64		-	64		-
					25	64		-										
A188					17	16		-	29	16		-	16		-	16		-
									36	16		-						
A189									28	32		-	32		-	32		-
A190					26	64		-	36	128		-	64		-	32		-
A191					22	16		-	32	16		-	16		-	16		-
A192									35	32		-	64		-	32		-
A193					20	16		-	34	16		-	32		-	32		-
A194									32	16		-	32		-	32		-
A195	12	32		-	16	64		-	34	64		-	64		-	32		-
A196									32	16		-	32		-	16		-
A197	11	32		-	26	32		-	38	32		-	16		-	32		-
A198					18	64		-	31	128		-	32		-	64		-
									37	6		-						
A199									30	32		-	32		-	16		-
									38	64		-						
A200									35	16		-	32		-	16		-
A201					26	16		-	32	32		-	32		-	16		-
A202	12	32		-	25	64		-	32	64		-	32		-	32		-
A203									36	32		-	32		-	16		-
A204	6	16		-	26	32		-	34	32		-	64		-	32		-
A205					17	32		-	28	32		-	32		-	32		-
A206	11	32		-	24	32		-					32		-	32		-
A207	7	16		-					28	16		-	16		-	-		-
A208	10	64		-	26	64		-	34	32		-	64		-	64		-
A209					19	16		-	28	16		-	32		-	32		-
A210	13	16		-	26	16		-	36	16		-	16		-	16		-
A211					20	16		-	34	16		-	16		-	16		-
					26	32		-										
A212	8	64		-	22	64		-	30	32		-	32		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A213					21	16		-	35	32		-	32		-	32		-
A214									34	64		-	64		-	128		-
A215									28	16		-	32		-	32		-
A216					26	128		-	31	64		-	128		-	64		-
A217	10	256		-	24	256		-	36	512		-	256		-	32		-
A218	8	128		-	26	64		-	35	64		-	128		-	64		-
A219	12	16		-	26	16		-	35	16		-	16		-	-		-
A220					25	16		-	36	16		-	16		-	-		-
A22[illegible]					21	32		-	31	16		-	32		-	16		-
					26	16		-										
A222					20	32		-					32		-	32		-
A223									30	16		-	16		-	32		-
									35	16		-						
A224									36	16		-	32		-	16		-
A225									36	32		-	16		-	16		-
A226					26	16		-	34	16		-	16		-	32		-
A227					25	32		-	36	16		-	64		-	32		-
A228									34	32		-	32		-	-		-
A229									32	64		-	32		-	32		-
									37	32		-						
A230					20	128		-	34	64		-	64		-	64		-
									38	64		-						
A231					22	128		-	30	64		-	128		-	32		-
A232									32	32		-	32		-	32		-
									36	16		-						
A233					24	32		-	32	32		-	64		-	32		-
A234					20	32		-	28	32		-	16		-	16		-
A235	10	16		FP*	26	32		FP	34	16		FP	32		FP	16		FP
A236					26	32		-	33	32		-	64		-	16		-
									38	64		-						
A237					16	16		-	30	16		-	16		-	-		-
A238					26	32		-	35	32		-	64		-	64		-
A239					20	16		-	32	16		-	16		-	-		-
					25	16		-										

*FP = false positive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery							
													Maternal				Cord			
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT		IFA	IgM-IFA	CFT	
A240					22	16		-	28	16		-	16		-		16		-	
A241					20	64		-	32	128		-	32		-		32		-	
A242					25	64		-	29	64		-	32		-		32		-	
A243									33	16		-	32		-		32		-	
A244									32	64		-	32		-		16		-	
A245					14	64		-	31	128		-	64		-		32		-	
A246					24	64		-	28	32		-	32		-		32		-	
A247					18	16		-					16		-		16		-	
A248					18	64		-	28	64		-	32		-		32		-	
									34	32		-								
A249					19	32		-	33	64		-	32		-		16		-	
A250					25	16		-	30	32		-	16		-		16		-	
									34	16		-								
A251					16	64		-	28	128		-	128		-		64		-	
A252									34	32		-	32		-		64		-	
A253									32	64		-	64		-		64		-	
A254									29	16		-	32		-		32		-	
A255									30	64		-	32		-		32		-	
									36	64		-								
A256					15	16		-					16		-		-		-	
A257									28	32		-	32		-		32		-	
A258					22	32		-					16		-		16		-	
A259									28	16		-	32		-		16		-	
A260					20	32		-	28	64		-	32		-		32		-	
A261									28	16		-	16		-		-		-	
A262					25	512	-	-	31	256	-	-	64	-	-		64	-	-	
									37	256	-	-								
A263									34	32		-	64		-		64		-	
A264					25	64		-	29	64		-	128		-		64		-	
A265													16		-		16		-	
A266													16		-		16		-	
A267													64		-		16		-	
A268													16		-		-		-	

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
A269													16		-	-		-
A270													32		-	32		-
A271													16		-	-		-
A272													16		-	-		-
A273													64		-	64		-
A274													16		-	-		-
A275													16		-	-		-
A276													4		-	64		-
A277													16		-	-		-
A278													32		-	16		-
A279													32		-	16		-
A280													64		-	64		-
A281													64		-	64		-
A282													32		-	32		-
A283													32		-	32		-
A284													16		-	-		-
A285													16		-	-		-
A286													32		-	16		-

APPENDIX 2.1 BARAGNANATH HOSPITAL - IFA and CFT positives

Patient No.	Antenatal												Delivery							Postnatal					
	1st trimester				2nd trimester				3rd trimester				Maternal			Cord				Mother			Child		
	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B1									28	2048	64	32	512	16	16	1024	-	16	20	512	-	-	-	-	-
B2													65536	16	16	32768	64	16	20	256	-	-	Died at birth		
B3													4096	512	128	2048	-	16	18	128	-	-	-	-	-
B4													4096	64	32	4096	-	8	20	4096	16	8	-	-	-
B5													1024	64	8	512	-	8		Not traced					
B6													1024	32	8	1024	-	-	20	256	64	-	-	-	-
B7													1024	64	16	512	-	8	18	128	-	-	-	-	-
B8													1024	64	16	1024	-	8		Not traced					
B9													1024	32	8	1024	-	-	17	1024	-	-	-	-	-
B10													1024	64	16	1024	-	-	20	256	32	8	-	-	-
B11													1024	64	16	512	-	8	20	512	32	-	-	-	-
B12													1024	512	16	1024	256	32	11	256	64	-	512	128	8
B13									34	64	-	8	64	-	8	64	-	-	14	128	-	-	-	-	-
B14													64	32	8	64	-	-							
B15													64	-	8	32	-	8							
B16													128	-	8	64	-	-							
B17													64	32	8	64	-	8							
B18													32	16	8	-	-	-							
B19													64	32	8	64	-	8							
B20													128	-	8	64	-	-							
B21													64	-	8	64	-	8							
B22													32	16	8	32	-	-							
B23													64	-	8	16	-	-							
B24													128	32	8	64	-	8							
B25													64	16	8	32	-	-							
B26													64	-	8	32	-	-							
B27													64	16	16	32	-	8							
B28													128	32	8	64	-	-							
B29													128	-	8	128	-	8							
B30													256	32	8	128	-	8	14	256	-	-	-	-	-

Patient No.	Antenatal												Delivery						Postnatal						
	1st trimester				2nd trimester				3rd trimester				Maternal			Cord				Mother			Child		
	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months postnatal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B31					24	512	-	8					512	-	16	256	-	-	20	512	-	-	-	-	-
B32									33	128	-	16	128	-	8	32	-	-							
B33									36	128	16	8	61	32	8	64	-	-							
B34													64	16	8	64	-	8							
B35									30	256	-	8	256	-	8	256	-	8							
B36					23	256	16	8					256	16	8	128	-	-	14	256	-	-	-	-	-
B37													64	-	8	32	-	-							
B38													256	-	8	64	-	8							
B39													32	16	8	16	-	-							
B40													256	32	8	128	-	8							
B41													256	16	8	128	-	-							
B42													256	32	8	256	-	8							
B43													128	-	8	128	-	-							
B44													128	16	8	128	-	-							
B45													64	-	8	64	-	8							
B46													512	32	8	256	-	8							
B47													128	16	8	128	-	-							
B48													256	16	16	256	-	8							
B49													512	32	16	256	-	8							
B50													128	-	8	128	-	-							
B51													256	-	8	128	-	8							
B52													128	-	8	64	-	-							
B53													256	16	8	64	-	-							
B54													64	-	8	32	-	-							
B55													256	16	8	128	-	8							
B56													128	-	8	128	-	-							
B57													64	-	8	64	-	-							
B58													256	16	8	128	-	8							

APPENDIX 2.I. BARAGWANATH HOSPITAL - IFA POSITIVE, CFT NEGATIVE, CORD BLOOD SUBMITTED

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B59													128		-	32		-
B60									38	32		-	64		-	32		-
B61									33	256		-	256		-	128		-
B62													16		-	-		-
B63									30	16		-	32		-	-		-
B64													32		-	16		-
B65					18	16		-					16		-	16		-
B66					25	16		-					16		-	16		-
B67									30	32		-	64		-	32		-
B68					25			-					32		-	16		-
B69									34	16		-	16		-	-		-
B70													32		-	16		-
B71					26	32		-					16		-	32		-
B72									34	16		-	16		-	16		-
B73													16		-	16		-
B74													16		-	-		-
B75									36	2048	32	-	2048	32	-	1024	-	-
B76									36	64		-	128		-	64		-
B77													32		-	32		-
B78									37	256	32	-	512	32	-	128	-	-
B79									34	64		-	128		-	128		-
B80					23	64		-					128		-	64		-
B81					26	32		-					32		-	-		-
B82													32		-	-		-
B83													16		-	-		-
B84													16		-	-		-
B85													64		-	64		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal			Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B86													16		-	-		-			
B87													64		-	32		-			
B88													64		-	32		-			
B89													32		-	-		-			
B90													512	32	-	512	-	-			
B91													32		-	32		-			
B92													16		-	32		-			
B93									34	16		-	16		-	-		-			
B94													32		-	-		-			
B95													16		-	-		-			
B96									36	16		-	16		-	-		-			
B97									30	16		-	16		-	-		-			
B98													16		-	-		-			
B99													32		-	64		-			
B100													32		-	16		-			
B101													16		-	-		-			
B102													32		-	-		-			
B103									32	128		-	32		-	64		-			
B104									34	64		-	32		-	32		-			
B105													16		-	-		-			
B106													16		-	16		-			
B107													64		-	64		-			
B108													16		-	-		-			
B109													16		-	-		-			
B110		12	32										64		-	32		-			
B111													32		-	32		-			
B112													16		-	16		-			
B113													32		-	32		-			

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B114													16		-	16		-
B115													32		-	32		-
B116													128		-	256		-
B117									32		64	-	16		-	16		-
B118													16		-	16		-
B119													16		-	-		-
B120													16		-	-		-
B121									33		64	-	16		-	-		-
B122									33		16	-	16		-	32		-
B123									33		16	-	16		-	16		-
B124													32		-	16		-
B125													64		-	32		-
B126													16		-	-		-
B127													16		-	16		-
B128													16		-	-		-
B129													16		-	-		-
B130													16		-	32		-
B131													16		-	-		-
B132					16		32	-					64		-	32		-
B133													16		-	16		-
B134													32		-	16		-
B135									30		16	-	32		-	-		-
B136									32		128	-	16		-	32		-
B137													16		-	-		-
B138													16		-	-		-
B139													16		-	-		-
B140													16		-	16		-
B141													64		-	64		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B142													16		-	32		-
B143													16		-	-		-
B144													16		-	-		-
B145													16		-	16		-
B146									31	16		-	16		-	16		-
B147					26	16		-					32		-	16		-
B148					24	64		-					32		-	32		-
B149													16		-	-		-
B150													32		-	32		-
B151													32		-	16		-
B152													16		-	-		-
B153					19	16		-					16		-	-		-
B154													16		-	-		-
B155													16		-	16		-
B156													128		-	128		-
B157													32		-	32		-
B158													16		-	-		-
B159													16		-	16		-
B160													64		-	32		-
B161													32		-	32		-
B162													16		-	-		-
B163													16		-	16		-
B164					25	16		-					16		-	32		-
B165													16		-	16		-
B166													64		-	32		-
B167					25	16		-					16		-	16		-
B168									30	16			16		-	-		-
B169													16		-	-		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B170													16		-	16		-
B171													32		-	16		-
B172													64		-	32		-
B173													32		-	16		-
B174	10	16		-									16		-	16		-
B175									34	32		-	32		-	32		-
B176									35	64		-	32		-	32		-
B177									35	256		-	256		-	128		-
B178									37	16		-	16		-	16		-
B179													64		-	64		-
B180													64		-	128		-
B181													64		-	16		-
B182													32		-	32		-
B183													32		-	16		-
B184													16		-	-		-
B185													128		-	64		-
B186													256		-	128		-
B187													128		-	128		-
B188													16		-	16		-
B189													128		-	64		-
B190													128		-	128		-
B191													16		-	-		-
B192													16		-	-		-
B193													64		-	32		-
B194													64		-	16		-
B195													16		-	16		-
B196													32		-	32		-
B197		20	32	-									32		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestat (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B198					24	16		-					16		-	16		-
B199													64		-	32		-
B200									28	128		-	256		-	256		-
B201													32		-	16		-
B202													16		-	-		-
B203									35	32		-	32		-	32		-
B204													16		-	-		-
B205													16		-	-		-
B206													32		-	16		-
B207													32		-	-		-
B208													16		-	-		-
B209									36	16		-	16		-	-		-
B210													16		-	-		-
B211					26	32		-					32		-	32		-
B212													32		-	-		-
B213													16		-	-		-
B214													128		-	64		-
B215													64		-	64		-
B216													32		-	32		-
B217													16		-	16		-
B218													32		-	16		-
B219									33	32		-	32		-	32		-
B220									35	64		-	64		-	32		-
B221					24	256		-					64		-	128		-
B222													16		-	-		-
B223									37	16		-	16		-	-		-
B224													16		-	16		-
B225													32		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
BZ26													16		-	-		-
BZ27													16		-	-		-
BZ28													16		-	16		-
BZ29													16		-	32		-
BZ30													32		-	16		-
BZ31													64		-	32		-
BZ32					26		16	-					32		-	16		-
BZ33													16		-	-		-
BZ34													32		-	16		-
BZ35													16		-	-		-
BZ36													128		-	128		-
BZ37													16		-	-		-
BZ38													128		-	32		-
BZ39					23		64	-					32		-	32		-
BZ40					15		16	-					16		-	-		-
BZ41													16		-	-		-
BZ42													16		-	32		-
BZ43													16		-	-		-
BZ44													16		-	16		-
BZ45													16		-	-		-
BZ46													16		-	32		-
BZ47													32		-	-		-
BZ48													32		-	-		-
BZ49					23		256	-					128		-	128		-
BZ50									38		16		16		-	-		-
BZ51													16		-	16		-
BZ52													16		-	-		-
BZ53													16		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
BZ54													32		-	16		-
BZ55									28	16		-	16		-	16		-
BZ56													16		-	16		-
BZ57													16		-	16		-
BZ58					20	32		-					16		-	32		-
BZ59									37	32		-	16		-	-		-
BZ60													16		-	16		-
BZ61													16		-	32		-
BZ62													32		-	16		-
BZ63													16		-	32		-
BZ64									28	32		-	16		-	32		-
BZ65													16		-	32		-
BZ66													64		-	32		-
BZ67													64		-	64		-
BZ68													16		-	16		-
BZ69													16		-	-		-
BZ70													64		-	16(Twin 1)		-
BZ71																- (Twin 2)		-
BZ72													16		-	-		-
BZ73									28	64		-	16		-	16		-
BZ74									34	128		-	128		-	128		-
BZ75													256		-	128		-
BZ76													16		-	32		-
BZ77									33	128		-	128		-	64		-
BZ78													16		-	-		-
BZ79													256		-	128		-
BZ80													16		-	-		-

Patient No.	1st trimester			2nd trimester			3rd trimester			Delivery					
										Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	Gestation (weeks)	IFA	IgM-IFA	Gestation (weeks)	IFA	IgM-IFA	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B281										16		-	-		-
B282										16		-	-		-
B283										64		-	64		-
B284							28	16	-	16		-	16		-
B285										64		-	64		-
B286							28	256	-	32		-	64		-
B287							32	16	-	16		-	-		-
B288							35	32	-	64		-	32		-
B289										16		-	-		-
B290										64		-	64		-
B291										16		-	-		-
E292							36	16	-	16		-	-		-
B293										64		-	64		-
B294				23	32	-				16		-	16		-
B295				20	64	-				32		-	16		-
B296										32		-	16		-
B297										16		-	16		-
R298				20	16	-				64		-	16		-
B299	8	32								32		-	16		-
B300	12	128								256		-	256		-
B301							31	16	-	16		-	32		-
B302										16		-	32		-
B303										256		-	128		-
B304							28	32	-	16		-	16		-
B305							30	16	-	16		-	16		-
B306							32	128	-	64		-	64		-
B307							36	16	-	16		-	-		-
B308										64		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B309													16		-	-		-
B310													16		-	16		-
B311													16		-	-		-
B312													16		-	-		-
B313													32		-	16		-
B314													16		-	-		-
B315													16		-	-		-
B316													512	16	-	256	-	-
B317													16		-	-		-
B318													16		-	-		-
B319													1024	16	-	1024	-	-
B320									36		256	-	128		-	128		-
B321													16		-	-		-
B322													16		-	16		-
B323													512	16	-	256	-	-
B324													128		-	128		-
B325													16		-	-		-
B326									16		32	-	32		-	32		-
B327													32		-	32		-
B328													16		FP*	16		FP
B329													64		-	16		-
B330													128		-	128		-
B331													16		-	-		-
B332													16		-	-		-
B333													16		-	-		-
B334	8	16	-										16		-	-		-
B335													16		-	-		-
B336													16		-	-		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B337													16		-	-		-
B338													32		-	16		-
B339													16		-	16		-
B340													32		-	16		-
B341													16		-	-		-
B342									29		16	-	16		-	16		-
B343													16		-	-		-
B344													64		-	64		-
B345													64		-	32		-
B346													64		-	32		-
B347													64		-	16		-
B348													16		-	-		-
B349													64		-	64		-
B350													128		-	64		-
B351													512	16	-	256	-	-
B352													64		-	16		-
B353													16		-	-		-
B354					24	512	-	-					256	-	-	256(Twin 1) 256(Twin 2)		-
B355													128		-	128		-
B356													256		-	256		-
B357													16		-	-		-
B358													256		-	256		-
B359					24	16	-						16		-	-		-
B360													32		-	64		-
B361													64		-	32		-
B362									36	32	-		32		-	16		-
B363													16		-	-		-
B364													128		-	128		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B365													16		-	-		-
B366													16		-	16		-
B367													16		-	- (Twin 1)		-
																- (Twin 2)		-
B368													16		-	-		-
B369									30	128		-	256		-	128		-
B370													16		-	-		-
B371													16		-	-		-
B372													16		-	16		-
B373									30	32		-	16		-	32		-
B374													16		-	-		-
B375													16		-	-		-
B376													16		-	16		-
B377					25	16		-					16		-	-		-
B378					26	16		-					16		-	16		-
B379													16		-	-		-
B380									35	16		-	16		-	-		-
B381									34	16		-	16		-	16		-
B382									34	16		-	16		-	16		-
B383									36	32		-	32		-	16		-
B384													16		-	-		-
B385													16		-	-		-
B386													16		-	-		-
B387													32		-	16		-
B388													32		-	32		-
B389													16		-	-		-
B390					24	32		-					32		-	16		-
B391													32		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Child		
	Gestation (weeks)	IFA	IgM+ IFA	CFT	Gestation (weeks)	IFA	IgM+ IFA	CFT	Gestation (weeks)	IFA	IgM+ IFA	CFT	IFA	IgM+ IFA	CFT	IFA	IgM+ IFA	CFT
B392													32		-	-		-
B393									28	16		-	32		-	16		-
B394									35	16		-	16		-	16		-
B395													128		-	64		-
B396													16		-	-		-
B397									29	256		-	256		-	256		-
B398					22	64		-					64		-	32		-
B399									33	64		-	64		-	64		-
B400													16		-	16		-
B401													64		-	32		-
B402													64		-	32		-
B403													32		-	32		-
B404					19	128		-					256		-	128		-
B405													32		-	16		-
B406													16		-	16		-
B407													16		-	16		-
B408					24	64		-					32		-	-		-
B409													16		-	-		-
B410									27	128		-	256		-	128		-
B411									35	32		-	16		-	16		-
B412													32		-	16		-
B413													16		-	-		-
B414					24	64		-					128		-	64		-
B415									34	16		-	32		-	16		-
B416													16		-	-		-
B417					26	512	-	-					1024	-	-	512	-	-
B418													256		-	256		-
B419													32		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Child		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM IFA	CFT	IFA	IgM-IFA	CFT
B420													16		-	16		-
B421									37	16		-	16		-	-		-
B422													16		-	-		-
B423					26	1024	-	-					512	-	-	512	-	-
B424													16		-	-		-
B425													16		-	16		-
B426													1024	-	-	1024	-	-
B427													16		-	-		-
B428													512	-	-	256	-	-
B429													1024	-	-	512	-	-
B430									29	32		FP*	16		FP	-		-
B431									31	256		-	512	-	-	256	-	-
B432													512	16	-	256	-	-
B433													1024	16	-	512	-	-
B434													128		-	32		-
B435													16		-	-		-
B436									31	256		-	256		-	[illegible]		-
B437													16		-	-		-
B438									29	16		-	16		-	-		-
B439									30	16		-	16		-	16		-
B440									32	32		-	32		-	16		-
B441									33	16		-	16		-	-		-
B442									30	16		-	16		-	-		-
B443													16		-	-		-
B444									35	32		-	32		-	16		-
B445													16		-	-		-
B446													512	32	-	512	-	-
B447													16		-	-		-

* FP = false positive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Child		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B448									34	64		-	128		-	128		-
B449													16		-	-		-
B450													16		-	-		-
B451									27	16		-	16		-	-		-
B452									28	64		-	128		-	256		-
B453													[illegible]		-	32		-
B454													1024	-	-	128	-	-
B455													1024	16	-	1024	-	-
B456													1024	-	-	1024	-	-
B457									31	128		-	256		-	256		-
B458													16		-	16		-
B459													256		-	128		-
B460													256		-	256		-
B461													32		-	-		-
B462													16		-	-		-
B463									34	64		-	128		-	64		-
B464													32		-	-		-
B465									32	32		-	32		-	16		-
B466													32		-	-		-
B467													16		-	-		-
B468													16		-	16		-
B469													16		-	-		-
B470													16		-	-		-
B471													16		-	-		-
B472									31	64		FP*	128		FP	64		FP
B473													16		-	16		-
B474					24	32		-					64		-	64		-
B475													32		-	16		-
B476													16		-	-		-

* FP = false positive

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
													Maternal			Child		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B477													32		-	16		-
B478													1024	-	-	512	-	-
B479					26	16		-					16		-	-		-
B480									32	32		-	32		-	-		-
B481													1024	16	-	256	-	-
B482													32		-	32		-
B483													128		-	128		-
B484													16		-	-		-
B485													512	16	-	256	-	-
B486													16		-	16		-
B487													256		-	256		-
B488													256		-	128		-
B489													64		-	32		-
B490													256		-	128		-
B491					19	128		-					128		-	64		-
B492													16		-	16		-
B493									28	32		-	32		-	32		-
B494									32	128		-	256		-	256		-
B495													512	32	-	64	-	-
B496													32		-	16		-
B497									31	16		-	16		-	-		-
B498													128		-	128		-
B499													256		-	256		-
B500													16		-	-		-

APPENDIX 2.3. BARAGWANATH HOSPITAL, IFA ≥ 512, CFT negative, babies bled within 3 days of birth as cord blood not submitted

Patient No.	Delivery - Maternal			Neonatal		
	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
B501	2048	16	-	1024	-	-
B502	512	16	-	256	-	-
B503	512	-	-	512	-	-
B504	512	-	-	512	-	-
B505	512	16	-	256	-	-
B506	512	-	-	256	-	-
B507	512	32	-	1024	-	-
B508	512	-	-	512	-	-
B509	512	-	-	256	-	-
B510	512	-	-	512	-	-
B511	1024	16	-	512	-	-
B512	1024	-	-	1024	-	-
B513	1024	16	-	512	-	-
B514	512	-	-	1024	-	-
B515	512	-	-	256	-	-
B516	512	-	-	512	-	-

APPENDIX 2.4. BARAGWANATH HOSPITAL - IFA positive <1:512, CFT negative, cord blood not submitted

Patient No.	Delivery - Maternal IFA	CFT	Patient No.	Delivery - Maternal IFA	CFT
B517	16	-	B549	32	-
B518	32	-	B550	32	-
B519	32	-	B551	32	-
B520	64	-	B552	16	-
B521	16	-	B553	16	-
B522	128	-	B554	16	-
B523	128	-	B555	16	-
B524	32	-	B556	128	-
B525	128	-	B557	16	-
B526	128	-	B558	16	-
B527	64	-	B559	32	-
B528	64	-	B560	256	-
B529	32	-	B561	32	-
B530	32	-	B562	64	-
B531	128	-	B563	128	-
B532	32	-	B564	16	-
B533	32	-	B565	64	-
B534	64	-	B566	32	-
B535	16	-	B567	128	-
B536	256	-	B568	32	-
B537	16	-	B569	32	-
B538	64	-	B570	256	-
B539	256	-	B571	16	-
B540	128	-	B572	64	-
B541	128	-	B573	16	-
B542	16	-	B574	128	-
B543	32	-	B575	16	-
B544	16	-	B576	16	-
B545	16	-	B577	64	-
B546	32	-	B578	64	-
B547	32	-	B579	128	-
B548	16	-	B580	16	-

Patient No.	Delivery - Maternal IFA	CFT	Patient No.	Delivery - Maternal IFA	CFT
B581	16	-	B615	16	-
B582	64	-	B616	128	-
B583	16	-	B617	64	-
B584	16	-	B618	16	-
B585	16	-	B619	32	-
B586	16	-	B620	32	-
B587	16	-	B621	64	-
B588	16	-	B622	256	-
B589	64	-	B623	16	-
B590	64	-	B624	32	-
B591	32	-	B625	256	-
B592	128	-	B626	16	-
B593	128	-	B627	16	-
B594	256	-	B628	256	-
B595	128	-	B629	32	-
B596	16	-	B630	16	-
B597	64	-	B631	128	-
B598	64	-	B632	32	-
B599	32	-	B633	16	-
B600	16	-	B634	16	-
B601	32	-	B635	32	-
B602	16	-	B636	16	-
B603	16	-	B637	32	-
B604	256	-	B638	64	-
B605	64	-	B639	32	-
B606	32	-	B640	64	-
B607	32	-	B641	16	-
B608	16	-	B642	16	-
B609	128	-	B643	16	-
B610	128	-	B644	16	-
B611	32	-	B645	16	-
B612	256	-	B646	128	-
B613	128	-	B647	16	-
B614	16	-	B649	32	-

Patient No.	Delivery - Maternal IFA	CFT
B649	16	-
B650	16	-
B651	256	-
B652	128	-
B653	16	-
B654	16	-
B655	16	-
B656	16	-
B657	16	-
B658	16	-
B659	64	-
B660	128	-
B661	64	-
B662	32	-
B663	32	-
B664	64	-
B665	64	-
B666	16	-
B667	64	-
B668	16	-
B669	64	-
B670	32	-
B671	64	-
B672	16	-
B673	64	-
B674	128	-
B675	16	-
B676	16	-
B677	16	-
B678	32	
B679	16	-
B680	16	-
B681	512	-
B682	16	

Patient No.	Delivery - Maternal IFA	CFT
B683	128	-
B684	128	-
B685	64	-
B686	128	-
B687	256	-
B688	128	-
B689	16	-
B690	32	-
B691	64	-
B692	128	-
B693	16	-
B694	16	-
B695	128	-
B696	16	-
B697	16	-
B698	64	-
B699	16	-
B700	32	-
B701	16	-
B702	64	-
B703	32	-
B704	32	-
B705	128	-
B706	32	-
B707	16	-
B708	16	-
B709	128	-
B710	32	-
B711	16	-
B712	256	-
B713	32	-
B714	64	-
B715	64	-
B716	16	-

Patient No.	Delivery - Maternal IFA	CFT	Patient No.	Delivery - Maternal IFA	CFT
B717	256	-	B751	16	-
B718	16		B752	16	-
B719	16	-	B753	32	-
B720	32	-	B754	16	-
B721	128	-	B755	16	-
B722	16	-	B756	128	-
B723	32	-	B757	32	-
B724	16	-	B758	64	-
B725	16	-	B759	256	-
B726	256	-	B760	64	-
B727	16	-	B761	16	-
B728	256	-	B762	64	-
B729	16	-	B763	16	-
B730	128	-	B764	128	-
B731	64	-	B765	16	-
B732	128	-	B766	16	-
B733	256	-	B767	128	-
B734	256	-	B768	16	-
B735	32	-	B769	64	-
B736	32	-	B770	128	-
B737	64	-	B771	16	-
B738	16	-	B772	16	-
B739	32	-	B773	128	-
B740	128	-	B774	128	-
B741	32	-	B775	16	-
B742	32	-	B776	16	-
B743	32	-	B777	16	-
B744	64	-	B778	256	-
B745	16	-	B779	16	-
B746	16	-	B780	32	-
B747	32	-	B781	32	-
B748	64	-	B782	128	-
B749	32	-	B783	32	-
B750	256	-	B784	16	-

Patient No.	Delivery - Maternal IFA	CFT
B785	64	-
B786	256	-
B787	256	-
B788	16	-
B789	256	-
B790	128	-
B791	256	-
B792	16	-
B793	64	-
B794	128	-
B795	16	-
B796	64	-
B797	128	-
B798	256	-
B799	128	-
B800	16	-
B801	128	-
B802	16	-
B803	256	-
B804	128	-
B805	16	-
B806	32	-
B807	32	-
B808	128	-
B809	32	-
B810	32	-
B811	256	-
B812	32	-
B813	32	-
B814	64	-
B815	32	-
B816	16	-
B817	32	-
B818	16	-

Patient No.	Delivery - Maternal IFA	CFT
B819	32	-
B820	128	-
B821	32	-
B822	32	-
B823	16	-
B824	16	-
B825	32	-
B826	64	-
B827	256	-
B828	32	-
B829	16	-
B830	32	-
B831	32	-
B832	64	-
B833	16	-
B834	16	-
B835	32	-
B836	16	-
B837	32	-
B838	32	-
B839	128	-
B840	32	-
B841	32	-
B842	32	-
B843	64	-
B844	32	-
B845	16	-
B846	32	-
B847	32	-
B848	16	-
B849	16	-
B850	128	-
B851	32	-
B852	16	-

Patient No.	Delivery - Maternal IFA	CFT
B853	64	-
B854	32	-
B855	16	-
B856	128	-
B857	32	-
B858	16	-
B859	32	-
B860	16	-
B861	16	-
B862	32	-
B863	16	-
B864	16	-
B865	32	-
B866	32	-
B867	16	-
B868	32	-
B869	32	-
B870	64	-
B871	16	-
B872	32	-
B873	16	-
B874	16	-
B875	32	-
B876	16	-
B877	32	-
B878	16	-
B879	16	-
B880	16	-
B891	16	-
B882	16	-
B883	16	-
B884	32	-
B885	64	-
B886	32	-

Patient No.	Delivery - Maternal IFA	CFT
B887	32	-
B888	16	-
B889	32	-
B890	16	-
B891	16	-
B892	32	-
B893	32	-
B894	16	-
B895	16	-
B896	16	-
B897	32	-
B898	16	-
B899	16	-
B900	16	-
B901	16	-
B902	32	-
B903	32	-
B904	16	-
B905	32	-
B906	32	-
B907	16	-
B908	16	-
B909	32	-
B910	16	-
B911	16	-
B912	16	-
B913	16	-
B914	32	-
B915	64	-

APPENDIX 3.1. CORONATION HOSPITAL - IFA and CFT positives

Patient No.	Antenatal												Delivery						Postnatal							
	1st trimester			2nd trimester				3rd trimester					Maternal			Cord			Mother				Child			
	Gestation (wks)	IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT	Gestation (wks)	IFA	IgM-IFA	CFT		IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	Months post-natal	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	
C1				21	32	-	-						512	16	8	512	-	-	16	128	-	-	-	-	-	
C2								36	512	16	16		4096	64	16	2048	-	8	20	256	32	-	-	-	-	
C3								30	-	-	-		2048	64	8	1024	-	-	20	512	32	-	-	-	-	
C4													1024	16	16	1024	-	8	20	512	-	-	-	-	-	
C5													1024	16	8	1024	-	8	20	512	64	8	-	-	-	
C6				25	32	64	8						64	32	8	64	-	8								
C7				20	32	-	8						64	16	8	128	-	-								
C8				26	128	16	8						256	16	8	256	-	-	14	128	-	-	-	-	-	
C9								33	128	64	8		128	32	8	64	-	-	14	128	-	-	-	-	-	
C10				21	64	8	8						64	16	8	16	-	-								
C11								28	64	-	8		64	-	8	32	-	-								
C12								30	64	16	8		64	32	8	32	-	-								
C13													64	32	8	64	-	8								
C14													256	16	8	128	-	-								
C15													64	16	16	128	-									
C16													64	-	8	16	-	-								
C17													64	16	8	32	-	-								
C18													64	16	8	64	-	-								
C19													128	32	16	16	-	-								
C20													128	-	8	32	-	8								

APPENDIX 3.2. CORONATION HOSPITAL - IFA positive, CFT negative

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C21					20	16		-					16		-	-		-
C22	12	16		-									32		-	32		-
C23					20	32		-					64		-	64		-
C24					21	16		-					16		-	-		-
C25					20	64		-					64		-	64		-
C26					17	16		-					16		-	16		-
C27					20	32		-					64		-	64		-
C28									30	32		-	32		-	32		-
C29					25	32		-					32		-	-		-
C30					25	16		-					16		-	-		-
C31					21	16		-					16		-	-		-
C32					16	32		-					64		-	64		-
C33					16	32		-					32		-	-		-
C34					24	16		-					16		-	32		-
C35					22	16		-					32		-	-		-
C36					24	16		-					16		-	16		-
C37									30	32		-	64		-	32		-
C38									28	32		-	16		-	-		-
C39									28	16		-	16		-	32		-
C40					18	64		-					128		-	128		-
C41									28	16		-	16		-	-		-
C42									29	16		-	32		-	-		-
C43									30	32		-	32		-	-		-
C44					26	16		-					32		-	-		-
C45					22	128	-	-					512	-	-	256	-	-
C46									28	32		-	32		-	32		-
C47									32	128		-	256		-	256		-
C48	12	32		-									64		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C48					25	64		-					128		-	128		-
C50					23	16		-					16		-	32		-
C51					23	16		-					16		-	16		-
C52					15	16		-					32		-	-		-
C53									34	32		-	32		-	16		-
C54					23	32		-					32		-	-		-
C55									28	64		-	128		-	128		-
C56									29	16		-	16		-	-		-
C57									33	16		-	16		-	16		-
C58									34	64		-	64		-	32		-
C59					15	16		-					16		-	16		-
C60					22	128	16	-					512	16	-	512	-	-
C61					17	16		-					32		-	16		-
C62					17	64		-					64		-	16		-
C63					23	32		-					32		-	32		-
C64					18	16		-					16		-	-		-
C65					23	32		-					32		-	32		-
C66					19	64		-					128		-			-
C67					21	128		-								128		-
C68									36	16		-	16		-	-		-
C69									31	16		-	16		-	-		-
C70					18	32		-					16		-	-		-
C71									30	128		-	128		-	128		-
C72					19	16		-					16		-	32		-
C73									32	16		-	16		-	16		-
C74					25	16		-					32		-	16		-
C75					22	16							16		-	32		-
C76									37	16		-	16		-	16		-
C77					20	64		-					128		-	64		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C78					20	32		-					32		-	32		-
C79									27	16		-	16		-	-		-
C80					24	16		-					32		-	32		-
C81									32	16		-	16		-	-		-
C82					18	16		-					16		-	16		-
C83									37	16		-	16		-	-		-
C84									28	128		-	128		-	128		-
C85					26	256		-					128		-	128		-
C86					25	32		-					32		-	-		-
C87					24	16		-					16		-	-		-
C88					24	32		-					32		-	32		-
C89					22	16		-					16		-	32		-
C90					20	128		-					256		-	64		-
C91					18	16		-					16		-	-		-
C92					25	32		-					32		-	32		-
C93					16	32		-					16		-	-		-
C94					20	16		-					16		-	16		-
C95									27	16		-	16		-	-		-
C96									29	64		-	128		-	64		-
C97									37	32		-	32		-	-		-
C98					30	16		-					16		-	-		-
C99									38	128		-	128		-	128		-
C100					23	16		-					16		-	-		-
C101									33	16		-	16		-	-		-
C102					24	128		-					256		-	128		-
C103					25	64		-					128		-	128		-
C104									29	256		-	128		-	64		-
C105									28	128		-	128		-	64		-
C106					19	16		-					16		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C107									32	16		-	32		-	-		-
C108									34	16		-	16		-	16		-
C109									32	64		-	32		-	32		-
C110									29	16		-	16		-	16		-
C111									29	16		-	16		-	16		-
C112									28	64		-	128		-	128		-
C113									29	16		-	16		-	16		-
C114									31	32		-	32		-	32		-
C115					23	16		-					16		-	-		-
C116									31	64		-	32		-	32		-
C117					25	16		-					16		-	-		-
C118					24	128		-					256		-	256		-
C119					23	512		-					256		-	256		-
C120					21	1024	-	-					1024	-	-	1024	-	-
C121									29	16		-	32		-	32		-
C122									34	32		-	32		-	-		-
C123					26	16		-					16		-	-		-
C124									35	1024	16	-	1024	16	-	512	-	-
C125					21	32		-					64		-	64		-
C126									33	32		-	33		-	32		-
C127									34	16		-	16		-	16		-
C128									33	16		-	16		-	-		-
C129									32	32		-	16		-	-		-
C130									28	256		-	128		-	128		-
C131									29	16		-	16		-	-		-
C132									30	32		-	64		-	32		-
C133									35	64		-	64		-	32		-
C134									37	16		-	16		-	-		-
C135					26	64		-					32		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C136									36	64		-	64		-	16		-
C137									35	16		-	16		-	-		-
C138									34	16		-	16		-	-		-
C139					26	16		-					16		-	-		-
C140									28	256		-	512	16	-	-	-	-
C141									32	256		-	256		-	256		-
C142									33	128		-	128		-	256		
C143									31	16		-	16		-	16		
C144									30	16		-	32		-	-		-
C145									29	16		-	16		-	16		-
C146									29	32		-	32		-	32		-
C147									35	256	32	-	512	32	-	1024	-	-
C148									35	16		-	16		-	-		-
C149									35	256		-	128		-	128		-
C150									31	2048	16	-	2048	16	-	4096	-	-
C151									29	32		-	64		-	32		-
C152									36	128		-	28		-	128		-
C153									3.	16		-	16		-	-		-
C154									35	16		-	32		-	-		-
C155									31	32		-	32		-	16		-
C156									31	64		-	64		-	-		-
C157									31	128		-	256		-	256		-
C158									38	16		-	16		-	32		-
C159									36	256		-	128		-	128		-
C160									36	128		-	128		-	128		-
C161									37	128		-	64		-	64		-
C162									39	16		-	16		-	16		-
C163									38	256		-	256		-	256		-
C164													16		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal			Delivery Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C165													32		-	32		-
C166													16		-	16		-
C167													128		-	64		-
C168													64		-	64		-
C169													512	-	-	512	-	-
C170													64		-	64		-
C171													128		-	128		-
C172													32		-	16		-
C173													512	32	-	1024	-	-
C174													256		-	256		-
C175													64		-	32		-
C176													256		-	256		-
C177													16		-	-		-
C178													16		-	32		-
C179													256		-	256		-
C180													16		-	-		-
C181													32		-	-		-
C182													64		-	64		-
C183													32		-	32		-
C184													16		-	16		-
C185													16		-	16		-
C186													64		-	64		-
C187													64		-	16		-
C188													16		-	-		-
C189													1024	16	-	1024	-	-
C190													128		-	128		-
C191													32		-	32		-
C192													512	-	-	512	-	-
C193													64		-	-		

Patient No.	1st trimester				2nd trimester				3rd trimester				Maternal Delivery			Cord		
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT	IFA	IgM-IFA	CFT
C194													32		-	16		-
C195													16		-	-		-
C196													256		-	128		-
C197													128		-	128		-
C198													256	-	-	512	-	-
C199													16		-	32		-
C200													64		-	64		-
C201													64		-	64		-
C202													256		-	256		-
C203													32		-	-		-
C204													16		-	-		-
C205													16		-	-		-
C206													32		-	64		-
C207													32		-	-		-
C208													32		-	64(Twin 1) 32(Twin 2)		-
C209													16		-	32		-
C210													256		-	256		-
C211													16		-	32		-
C212													16		-	-		-
C213													16		-	-		-
C214													16		-	32		-
C215													64		-	64		-
C216													32		-	32		-
C217													64		-	64		-
C218													64		-	16		-
C219													32		-	32		-
C220													32		-	-		-
C221													16		-	32		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery					
	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Gestation (weeks)	IFA	IgM-IFA	CFT	Material IFA	Material IgM-IFA	Material CFT	Cord IFA	Cord IgM-IFA	Cord CFT
C222													256		-	512		-
C223													128		-	128		-
C224													16		-	-		-
C225													32		-	64		-
C226													32		-	32		-
C227													32		-	32		-
C228													16		-	-		-
C229													16		-	-		-
C230													16		-	-		-
C231													128		-	128		-
C232													64		-	32		-
C233													16		-	-		-
C234													64		-	64		-
15													16		-	-		-
C236													128		-	128		-
C237													16		-	-		-
C238													512	-	-	512	-	-
C239													1024	32	-	256	-	-
C240													64		-	256		-
C241													256		-	256		-
C242													32		-	32		-
C243													64		-	64		-
C244													32		-	-		-
C245													256		-	256		-
C246													128		-	128		-
C247													64		-	64		-
C248													16		-	16		-
C249													64		-	64		-
C250													32		-	16		-

Patient No.	1st trimester				2nd trimester				3rd trimester				Delivery			Cord		
	Gestation (weeks)	IFA	IgM+ IFA	CFT	Gestation (weeks)	IFA	IgM+ IFA	CFT	Gestation (weeks)	IFA	IgM+ IFA	CFT	Maternal IFA	Maternal IgM+ IFA	Maternal CFT	IFA	IgM+ IFA	CFT
C251													32		-	64		-
C252													16		-	-		-
C253													64		-	64		-
C254													128		-	128		-
C255													16		-	16		-
C256													32		-	32		-
C257													16		-	16		-
C258													16		-	-		-
C259													16		-	-		-
C260													16		-	16		-
C261													256		-	256		-
C262													64		-	16		-
C263													16		-	-		-
C264													16		-	16		-
C265													16		-	-		-
C266													64		-	32		-
C267													64		-	64		-
C268													16		-	-		-
C269													16		-	-		-
C270													128		-	128		-
C271													64		-	64		-
C272													256		-	256		-

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Author Jacobs M R
Name of thesis Toxoplasmosis in Southern Africa 1978

PUBLISHER:
University of the Witwatersrand, Johannesburg
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