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TOXOPLASMOSIS WITH SPECIAL REFERENCE TO PENINSULAR MALAYSIA AND SINGAPORE

VIJAYAMMA THOMAS PhD*

Toxoplasmosis is a zoonotic infection caused by ***Toxoplasma gondii,*** a protozoan belonging to family Toxoplasmidae and Class Toxoplasmida(1). *T. gondii* was described by Nicolle and Manceaux(2) in a small North African rodent *Ctenodactylus* ***gondii.*** It has a world-wide distribution, but frequency of infection in man and animals varies from country to country. In general, the prevalence rate of infection is greater in the tropics and subtropics than in colder regions. Only a single species of *Toxoplasma* has been described so far. However, a number of strains have been reported from different parts of the world by different authors. Different strains of ***Toxoplasma*** vary in their virulence and virulence of a strain is determined on the basis of its ability to kill white mice(3).

Toxoplasma gondii has little host specificity and it parasitises man and a large number of other mammals and birds. The organism is an obligatory intracellular parasite of nucleated cells, especially of the lymphoid macrophage system, muscles, the central nervous system and the retina. It may, however, circulate for short periods in the lymph and blood(1) or in the ventricular fluid as free endozoites(4).

T. gondii was first recognised in man by Wolf and Cowen(5). It was first suspected of causing human illness by Castellani(6). The case reported by Janku in Czechoslovakia, may have also been toxoplasmosis(7).

STAGES IN MAN

Only certain asexual stages of the parasite are recognised in man. Endozoites(8) were the first forms of the organisms described. Endozoites are small elongated forms measuring about 4 to 7 μm by 2–4 pm. One end of the parasite is rounded while the other end is narrower and pointed. The nucleus is typically central. This is an intracellular stage and multiplication occurs within the cytoplasm of the host cell by a process of internal budding or endogeny. During this process of endogeny, the original cell membrane of the parasite splits open freeing the progeny. It is now known that internal budding of the organisms may produce more than two daughter parasites. Therefore the process of multiplication is described as endopolygeny or endogeny(8).

By a continuous process of endogeny, the host cell becomes distended with 16 or more endozoites and is called a pseudocyst. The pseudocyst ruptures liberating the endozoites, each of which is capable of invading another host-cell. The parasites may spread locally or across the serous cavities as extracellular endozoites or as pseudocysts along the lympatics or the blood stream. Depending upon the virulence of the strain, the process of multiplication and invasion continues until the host succumbs to infection or more commonly, until it develops immunity. As the immunity develops the multiplication of endozoites within pseudocysts slows down, the extra-cellular parasites are destroyed and the acute proliferative phase comes to an end.

The acute stage is followed by formation of tissue cysts. Development of tissue cyst is slow and may take a few months. The tissue cysts which are normally spherical in shape may reach a size of about 100 pm in diameter, but in the muscle, these cysts may take any shape. The tissue cysts may contain thousands of parasites called cystozoites(8) which are also produced by endogeny. Cystozoites are generally smaller than endozoites. The endozoites and cystozoites are distinguishable by the position of the nucleus which is terminal in the cystozoites. The cyst wall is made up of parasitic tissue. Intact cysts do not provoke an inflammatory reaction but if they rupture local delayed hypersensitivity reaction may develop. Heavy pressure may rupture the cysts. The tissue cysts are commonly found in the brain, skeletal and heart muscles and in other tissues.

*Associate Professor, Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur (Address for reprint requests).

These asexual extraintestinal stages of ***Toxoplasma*** are formed in man and in a wide variety of animals including cats.

STAGES IN CAT

One of the most significant findings in the life cycle of *T. gondii* was the demonstration of an enteroepithelial cycle resulting in the formation of oocysts, a typical coccidian stage in cat faeces(9, 10). This finding was a great progressive step towards our understanding of the biology of ***Toxoplasma gondii.*** Cats are thus shown to be the definitive host in the intestine of which the parasite undergoes a cycle of development comparable to that of the genus ***Isospora.*** Only domestic cats and certain other members of the family Felidae have been shown to produce oocysts of ***Toxoplasma***(11–13). The stages observed in the intestinal epithelium of the cat are identical with those of the well-established endogenous cycles of coccidian parasites(9). The appearance of these stages as well as that of oocysts, show that this organism is a coccidian parasite closely related to the genus ***Isospora.***

Cysts, oocysts and pseudocysts are infective to cats(10). On ingestion, the parasites penetrate the epithelial cells of the cat's intestine. Within these cells, the parasite commences asexual schizogony producing merozoites. These merozoites either invade other adjacent epithelial cells or penetrate the mucous membrane. Those which penetrate, initiate a systemic infection and invade the extra-intestinal tissues of the host producing pseudocysts and later cysts while those which invade other surface cells develop either into more schizonts or into gametes. The schizogony cycle may continue for a few generations(13). Details of sequence of development, duration and location of various stages in cat as well as details of schizogony, gametogony and sporogony have been carefully worked out(13–15). In cats, both extra-intestinal and enteroepithelial cycles may occur concurrently.

Merozoites that would give rise to gametes undergo gametogony and differentiate into micro and macrogametes. Two gametes fuse to form a zygote. The zygote secretes a protective coat and is transformed into an oocyst. The oocysts are shed into the lumen of the gut and are passed out in the faeces, as unsporulated oocysts. By using cloned *T. gondii* for infection in cat, it has been demonstrated that every *T. gondii* parasite has the genetic potential for producing both micro and macro-gametes in the cat(16). As a result, infections in cats with a single cloned viable parasite can give rise to gametes and oocysts.

Freshly passed oocysts are oval or subspherical in shape measuring about 13 x 9 μm. The oocyst wall consists of a single colourless layer which is closely applied to the contents with only a small crescentic area separating the two(17). It has been demonstrated that the time required for shedding of oocysts depends on the initial infecting forms of the parasite. Oocysts appeared in cat faeces 3 to 5 days after cysts were given and 8 to 10 days after endozoites wereadministered but only 21 to 24 days after ingestion of oocysts from other cats(10).

Freshly shed unsporulated oocysts in cat faeces are not infective until sporulated. Sporulation is completed in 1 to 3 days at 23.8°C(10). In experiments conducted in Singapore, sporulation was completed in about 48 hours(17). Sporulated oocysts contain two sporocysts each with 4 sporozoites. If sporulated oocysts are ingested by mice or possibly other hosts, the sporozoites initiate the formation of pseudocysts and later resistant cysts in the tissues. If oocysts are ingested by the cat, the sporozoites released in the gut give rise to schizonts, gametes and oocysts in addition to the extra-intestinal stages.

Details of fine structure of various stages and life cycle of the parasite show that it is a coccidian parasite belonging to subphylum Apicomplexa.

TRANSMISSION

In man, transmission of toxoplasmosis may either be acquired or congenital. Man may acquire the infection in different ways. Infected cats shed large numbers of oocysts which sporulate and remain viable for months in moist soil(10) and may possibly be the main source of infection to a variety of animals and man. Chronically infected cats have been shown to reshed T. *gondii* oocysts in the absence of reinfections(18) increasing the chances of environmental contamination with oocysts from pet cats. Serological studies in Malaysia(19–21) have shown that there is a close relationship between cats

and prevalence of toxoplasmosis in various ethnii: groups. These studies have shown that ***Toxoplasma*** anti-bodies prevalence rates were highest among Malays who usually have closer contact with cats. Animals which are chronic carriers are infective to cats and man. Consumption of uncooked or undercooked meat of these animals results in infection. Serological studies in Singapore and Malaysia(22, 23) have shown that all types of domestic animals tested were seropositive to ***Toxoplasma*** antibodies (Table I) indicating that meat of these animals may play a role in the transmission of toxoplasmosis to man. However, the relative frequency of transmission by oocysts from cats and cysts in meat is not fully understood. As Malaysians in general eat well cooked meat, the role of contaminated meat may be less important than that in countries where raw meat or undercooked hamburgers are eaten(24). Climatic factors like high temperatures, humidity and heavy rain that may wash away oocysts and human habits of keeping pet cats as well as eating habits would play a role in the relative frequency of transmission by oocysts and contaminated meat. Cystozoites are resistant to pepsin and trypsin digestion in gastric juices unlike endozoites which are less resistant and usually do not survive gastric digestion(13). Therefore, oral infection by endozoites is less likely. Oocysts are resistant to 10 percent ammonia(25).

It is also known that ***Toxoplasma*** can enter the body by conjunctival, respiratory and cutaneous routes as well(26). Accidental laboratory infections have been reported from various laboratories(27–30). Laboratory workers have been reported to become seropositive after working with cat faeces containing ***T. gondii*** oocysts(31). Blood transfusion from normal donors do not carry an undue risk of ***Toxoplasma*** transmission(32). However, Lunde and

TABLE I

PREVALENCE OF ***TOXOPLASMA*** ANTIBODIES IN ANIMALS IN MALAYSIA AND SINGAPORE.

Country	*Authors and date*	*Animals tested*	*Number tested*	*Percent positive*	*Test used*
Malaysia	Singh ***et al.*** 1967	cat	5	40.0	IHA
		pig	48	12.5	IHA
		Oxen/ buffalo	98	11.2	IHA
		goat	273	9.5	IHA
		cattle	73	4.1	IHA
		horse	32	–	IHA
		dog	29	–	IHA
		chicken	122	–	IHA
Singapore	Singh ***et al,*** 1967	cat	29	20.7	IHA
		pig	202	27.7	IHA
		sheep	24	25.0	IHA
		cattle	70	35.7	IHA
		dog	28	–	IHA
		rat	87	28.7	IHA
		chicken	176	4.5	IHA
Singapore	Zaman ***et al,*** 1967	pig	130	26.0	IHA

Siegel(33) have shown that the parasite may be transmitted with the blood of patients with chronic myelogenous leukemia. Such cases of accidental innoculations are rare with no epidemiological significance.

Congenital transmission may be important in women who first contracted T. ***gondii*** infection during pregnancy. The risk of fetal infection has been shown to increase as the pregnancy progresses(34). About one third to half the number of babies born to women who acquired infection during pregnancy were found to be infected(35). There is no reliable record on the number of babies who are born with congenital infection in Malaysia. Infection in early life prior to first pregnancy may protect the babies from congenital transmission from mothers. Although maternal infection and resulting immunity protect the subsequent fetus, toxoplasmosis has been reported in two successive pregnancies in women (13, 36). The actual process by which the parasite traverses the placental barrier is believed to be either by the passage of infected leucocytes from the mother to the foetus or by the rupture of the cysts inside the uterine wall due to the stretching of the wall in pregnancy.

PATHOLOGY

T. gondii produces a wide spectrum of response in man ranging from an inapparent infection to serious disease and death in a few cases. As in most countries, ***Toxoplasma*** infection may be very common, but illhealth due to the parasite is relatively rare in Malaysia. Acquired infection may affect both children and adults and may pass unnoticed or mimic a viral syndrome with lymphadenopathy(37, 38). Lymphadenopathy may be localised or generalized with or without fever, headache and maculo-papular rashes. In its more severe forms, it may show complications of pericarditis and myocarditis(39), or simulate infectious mononucleosis(40). It is also recognized as a cause of acute dermatomyostitis(41) and chorioretinites(42). Death from acquired toxoplasmosis has occurred in patients with clinical illness simulating typhus fever group(43, 44) in laboratory workers accidentally innoculated(45) and in immunosuppressed patients(46). In Malaysia and Singapore, there are no known cases of complications or deaths due to acquired toxoplasmosis. However, a 20 year old male who attended the eye clinic of the University Hospital Kuala Lumpur, has been diagnosed as having classical fundus lesion of toxoplasmosis in right eye and when tested he was strongly seropositive(47). Histological evidence of acquired toxoplasmosis was present in sections of biopsy material from the enlarged cervical lymph nodes of two other patients(48) who were strongly sero-positive for IgG and IgM antibodies. The absence of significant numbers of confirmed cases inspite of the high prevalence of ***Toxoplasma*** antibodies indicates that toxoplasmosis is not an important cause of morbidity and mortality among those with acquired toxoplasmosis.

The congenital form of infection may also be asymptomatic or may be associated with one or more of the following: chorioretinitis, hydrocephaly, microcephaly, cerebral calcification and seizure disorders(49, 50). Encephalomyelitis, mental deficiency through cerebral involvement and convulsions were also reported(5). The pregnancy may terminate in an abortion, premature baby or in still birth. However, only about a third or less of congenitally infected infants show any clinical abnormalities at the time of birth(51). Information obtained from studies of children born to mothers who became sero-positive during early pregnancy shows that the risk of foetal infection is lowest during the first trimester, but most of those infected during this time either had evidence of congenital disease or died(34). On the other hand, it was noticed that mothers who acquired toxoplasmosis later in pregnancy posed a greater risk of foetal infection, but these usually results in asymptomatic infections only. Studies in Malaysia and Singapore have revealed seropositivity among a number of infants at the time of birth(21, 52). Although seropositive, none of them showed IgM specific antibodies indicating rarity of congenital toxoplasmosis in Malaysia(21). Nevertheless 3 cases of choroiditis in adults in Singapore were considered to be due to congenital infection(52). Congenital toxoplasmosis is more damaging to the host than is the acquired form which appears to be more common.

Pathogenesis depends upon the number of cells destroyed by the parasite, by hypersensitivity or by both(35). At the site of infection, *T. gondii* multiplies intracellularly destroying the

infected host cells. Disease manifestations in man may usually be due to host susceptibility.

PREVALENCE IN MALAYSIA

Serological surveys in Malaysia and Singapore have shown evidence of ***Toxoplasma*** infection in man(19–21, 53, 54) and in a number of domestic and wild animals(22, 23). A large number of tissue cysts have been isolated from the brains of pigs in Singapore(22). A strain of *T. gondii* has been isolated from Malaysian tree shrew(55). Most types of animals tested in Malaysia and Singapore showed seropositivity to *Toxoplasma* antigen. In general, animals from Singapore showed higher prevalence rates for ***Toxoplasma*** antibodies (Table I).

Studies on various groups of population have demonstrated the presence of specific antibodies in people of both sexes in all age groups including neonatals, infants, children and adults of all races. In all these studies, Malays showed the highest antibody prevalence followed by Indians, Orang Asli (aborigines) and Chinese (Table II). The pattern was the same in all age groups. Among various age groups, the prevalence rate was highest among children below 10 years of age indicating possible infection with oocysts from contaminated soil rather than from contaminated meat. The highest prevalence rate among Malays in all these studies also may indicate that oocysts from cats is the main source of infection. Serological studies using IFA technique have shown that a small percent of seropositive Malaysians are positive to IgM specific immunoglobulins(21). Deaths due to toxoplasmosis have not been reported in this region.

DIAGNOSIS

Except in very heavy infections, parasites are scarce in tissues or body fluids and are difficult to isolate. Furthermore, the parasite is not easily isolated with mouse innoculation of lymph node tissue or the buffy coat of peripheral blood during the acute stage of infection(56). Majority of cases are asymptomatic or when symptomatic may simulate viral or other diseases. A large proportion of normal population have toxoplasma specific antibodies indicating past or present infection. Therefore, diagnosis of toxoplasmosis is difficult and often depends on a combination of signs, symptoms, isolation of ***Toxoplasma,*** histopathology and serology(35). The organism may be isolated by inoculating mice with tissue or fluid from suspected cases.

Several serological techniques are in use to diagnose toxoplasmosis. These include Sabin Feldman or Methylene-blue dye (MBD) test(57), complement fixation (CF), indirect fluorescent antibody (IFA) and indirect haemagglutination tests (IHA). Other recent promising tests include enzyme linked immunosorbent assay (ELISA) and soluble antigen fluorescent antibody (SAFA) tests. The application and limitations of these tests for diagnosis of toxoplasmosis have been discussed at length(56). For diagnosis of individual cases, IFA and MBD tests are found to be the best techniques and these give almost identical results. The use of IFA techniques to measure specific IgM in the diagnosis of acute congenital and acquired toxoplasmosis from chronic infections has been demonstrated(58). In congenital toxoplasmosis the IgM titre would remain steady or increase for many days or weeks. Rheumatoid factors may however give false positive IgM results(59) complicating the interpretation of the results.

Indirect haemagglutination is an inexpensive, simple and rapid test which is very useful when a large number of specimens have to be tested. The titres are usually lower than those obtained for MBD and some discrepancy between IHA and MBD tests occur in early or congenital infections where the latter gives high titers and the IHA gives low titres or negative results(56). Complement fixation test has received limited acceptance for diagnosis of toxoplasmosis and it detects antibody later than does the dye test.

The value of high titres in these tests for diagnosis has been discussed(56). As the serological results change from negative to high titres in a few days after infection, rapid rise in titres is of great importance in interpreting the results. Similarly he has stressed that low titres probably indicate residual antibody rather than newly developed antibodies.

PREVENTION AND CONTROL

Since it is known that uncooked and undercooked meat can transmit ***Toxoplasma gondii,*** the meat should be thoroughly cooked especially if it is consumed by pregnant women.

In Malaysia, cats may be the main source of infection in man and therefore all persons

TABLE II

TOXOPLASMA ANTIBODY DISTRIBUTION IN SINGAPORE AND WEST MALAYSIA AMONG VARIOUS RACES

Country	*Author(s) and date*	*Donors*	*No. tested and percent positive among*					*Test used*
			Malays	*Indians*	*Chinese*	*Orang Asli*	*Total*	
Singapore	Zaman and Goh (1969)	Clinically suspected cases	92 39.1%	93 36.6%	569 21.8%	– –	25.7%	IHA
	Singh *et al* 1972)	Infants	43 18.6%	47 10.6%	69 11.6%	– –	13.2%	IHA
Malaysia	Tan & Zamen (1973)	All age groups	257 21.8%	251 13.5%	220 5.0%	– –	13.9%	IHA
	Bisseru & Lim (1974)	Gombak Hospital patients				44 4.6%		Methylene Blue Dye Test
	Cheah *et al* (1975)	Pregnant women	431 38.8%	373 25.2%	615 20.7%	– –	27.3%	IFA
	Thomas *et al* (in press)	All age groups and cord blood	118 33.9%	138 23.2%	212 14.6%	268 19.0%	20.9%	IFA

particularly pregnant women or children exposed to cats or contaminated soil should wash their hands before eating. Children should not be allowed to play in soil possibly contaminated by cat faeces.

TREATMENT

Treatment is unsatisfactory as no specific and effective drugs are currently available. The standard therapy in man consists of Pyrimethamine (Daraprim) and Sulphadiazine, used in high doses, and for a prolonged period. Pyrimethamine in doses of up to 50 mgs per day and Sulphadiazine in doses of 1–2 gms per day should be given for up to six months in severe cases of the disease and in intractable cases of uveitis(60). If treatment is stopped prematurely, a severe relapse may occur months later. The main hazard of treatment is toxic damage to the bone marrow. White blood cell counts should be estimated at frequent intervals and the drugs stopped if there are signs of marrow depression. Folinic Acid (5 mgs three times a day) should be given regularly throughout the treatment. It is desirable to interrupt chemotherapy for a week at a time during the course of the prolonged therapy. Pyrimethamine should not be used during the first trimester of pregnancy(61).

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